Neuroscience Letters 759 (2021) 136041

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Minireviews

Exploring the alternative: Fish, flies and worms as preclinical models for ALS

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ARTICLE INFO

Keywords:
Amyotrophic lateral sclerosis
Motor neurons
Rodents
Caenorhabditis elegans
Drosophila melanogaster
Danio rerio

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disorder characterized by the loss of upper and lower motor neurons. In general, patients succumb to respiratory insufficiency due to respiratory muscle weakness. Despite many promising therapeutic strategies primarily identified in rodent models, patient trials remain rather unsuccessful. There is a clear need for alternative approaches, which could provide directions towards the justified use of rodents and which increase the likelihood to identify new promising clinical candidates. In the last decades, the use of fast genetic approaches and the development of high-throughput screening platforms in the nematode *Caenorhabditis elegans*, in the fruit fly (*Drosophila melanogaster)* and in zebrafish (*Danio rerio*) have contributed to new insights into ALS pathomechanisms, disease modifiers and therapeutic targets. In this mini-review, we provide an overview of these alternative small animal studies, modeling the most common ALS genes and discuss the most recent preclinical discoveries. We conclude that small animal models will not replace rodent models, yet they clearly represent an important asset for preclinical studies.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease for which no cure is currently available. It is characterized by the death of upper and lower motor neurons in the motor cortex, brainstem and spinal cord, leading to muscle weakness [1]. ALS affects 1–2 individuals per 100,000 every year [2] and the lifetime risk is estimated at 1 in 400 [3]. This dreadful disease results in death on average 2 to 4 years post-diagnosis, mostly due to respiratory problems [4]. The clinical manifestation of ALS is characterized by a large variability, in terms of disease onset, progression and patient survival. This variability, present even within families with the same genetic cause, suggests the existence of crucial modifying factors [5].

ALS has a very diverse etiology, with both genetic and largely unknown environmental factors that could contribute to the risk of developing ALS. In 5 to 10% of cases, ALS is a familial disease, with an, in most cases, identified monogenetic cause [6]. However, the vast majority of patients suffer from the sporadic form of the disease. The so far identified mutations are mostly inherited in an autosomal dominant manner, and are present in more than 20 ALS-associated genes [7]. Approximately 40% of ALS patients in the Western world have a hexanucleotide repeat expansion in the *C9ORF72* (chromosome 9 open reading frame 72) gene [8,9]. Other common and widely studied ALS mutations are found in the *SOD1* (Superoxide Dismutase 1), *TARDBP* (Transactive Response DNA-binding protein 43) and *FUS* (Fused in Sarcoma) genes accounting for 2%, 0.9% and 0.7% of total ALS patients, respectively [7].

On a cellular level, axonal retraction and denervation of the muscle appears to be one of the first events, accompanied by microgliosis and astrogliosis, as well as proteinaceous inclusions in the remaining neurons [2]. A pathological hallmark of ALS, present in ~ 97% of all patients, are cytoplasmic inclusions of TDP-43, the protein encoded by the *TARDBP* gene [10].

Riluzole and Edaravone are the only two FDA-approved drugs used to treat ALS, which target excitotoxicity and oxidative stress, respectively [11]. Nonetheless, these drugs have a limited effect on disease progression and/or patient survival. As a consequence, symptom care, such as respiratory and nutritional support, is the main way to battle the disease. Hence, employing animal models in the laboratory to investigate and to identify new disease-causing mechanisms is necessary to

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https://doi.org/10.1016/j.neulet.2021.136041
Received 3 October 2020; Received in revised form 15 April 2021; Accepted 1 June 2021
Available online 10 June 2021

develop new and better therapeutic strategies.

2. Disease modeling in ALS

Elucidating ALS pathogenic mechanisms and developing new therapeutic strategies can only be achieved by modeling the disease. The discovery of genes which are involved in or causative for ALS paved the way for the development of different animal models, including rodents, fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*) and a nematode (*Caenorhabditis elegans*). By employing these models, it became evident that the molecular pathways involved in ALS are a lot more divergent than originally thought. Proposed disease mechanisms range from impaired RNA and protein metabolism to mitochondrial dysfunction, failure of DNA repair mechanisms and intracellular transport defects [2]. Mouse studies played a major role in providing these insights. Mice overexpressing mutant SOD1 exhibit motor neuron loss and recapitulate many aspects of the human disease [12–17]. Moreover, several rodent models overexpressing wild-type (WT) or mutant FUS or WT or mutant TDP-43 display phenotypes partially overlapping with the clinical presentation in ALS patients [16–20]. Of note, depletion of TDP-43 caused age-dependent progressive motor neuron degeneration in various mouse models as well [21–23], while FUS deficiency developed behavioral pathological abnormalities distinct from ALS [24,25]. In addition to ALS, the hexanucleotide repeat expansion in the *C9ORF72*

Table 1
Overview of genetic models in *Caenorhabditis elegans*. Studies modeling ALS-disease related genes *C9ORF72*, *SOD1*, *TARDBP*, *FUS* and others in *C. elegans*. For each model, the approach and transient or stable genetic manipulation technique are summarized. Cellular pathological hallmarks and, when present, motor phenotype (i.e. locomotion defects), neurodegenerative (i.e. GABAergic degeneration) and other phenotypes (e.g. reduced survival) are indicated. Legend: orange, present or affected; green, not present or not affected. Abbreviations: KD, knockdown; KO, knockout; Arg, arginine; MN, motor neuron; NMJ, neuromuscular junction; WT, wild-type. (*) EMS mutagenesis; ethyl methanesulfonate. (**) UV/TMP mutagenesis, trimethylpsoralen with ultraviolet light.

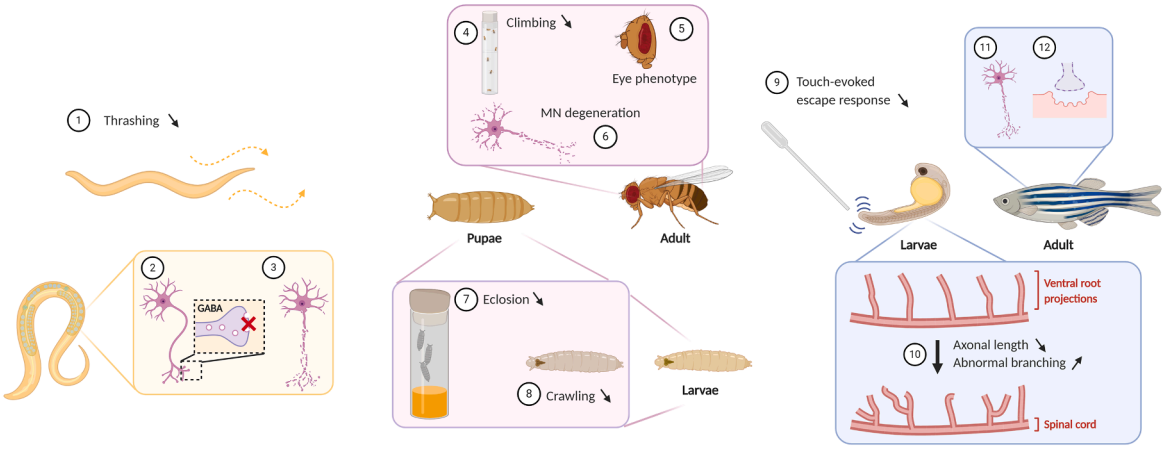
Caenorhabditis elegans								
Gene	**References**	**Approach (i.e. KD, overexpression…)**	**Pathological hallmarks (cellular changes, aggregates…)**	**Neurodegeneration and motor phenotype**				**Other phenotypes**
				Locomotion defects	Degeneration			
					GABA	*ACh*	*Glu*	
C9ORF72	(1,2) Therrien *et al.* 2013 (1) Corrionero *et al.* 2018	***C9ORF72 loss-of-function:*** (1) EMS-induced (*) null mutation of *alfa-1* (ok3062) (2) RNAi-mediated KD of *alfa-1*	- Cells sensitive to osmotic stress - Impaired autophagy					Defects in yolk storage and transport
	Wang *et al.* 2016	***C9ORF72 gain-of-function:*** Transgenic *C9ORF72* lines containing (1) 9 or (2) 29 repeats	- Impaired nucleocytoplasmic transport	(2)>(1)				Brood size and survival ↘
	Rudich *et al.* 2017	***C9ORF72 DPR toxicity:*** Transgenic lines containing GR, PR, PA or GA with repeat length 50		GR, PR	GR, PR			
SOD1	Oeda *et al.* 2001	Transgenic ***SOD1^{A4V}***, ***SOD1^{G37R}*** and ***SOD1^{G93A}*** *lines*	- Instability of mutant SOD1 proteins - Oxidative stress induces SOD1 aggregates in muscle					
	Wang *et al.* 2009	Transgenic ***SOD1^{G85R}*** line	- NMJ and synaptic vesicles ↘ - SOD1 aggregates in MNs - Axonal defects					Brood size , survival and rate of development ↘
	Gidalevitz *et al.* 2009	Transgenic lines ***SOD1^{G85R}***, ***SOD1^{G93A}***, ***SOD1^{127X}*** lines	- SOD1 aggregates in muscle	>> in G85R				
	Li *et al.* 2014	Stable overexpression of (1) **WT** or (2) ***SOD1^{G93A}***, specifically in MNs	- SOD1 aggregates in MNs	(2)>(1)	(2)>(1)			
	Baskoylu *et al.* 2018	CRISPR/Cas9-mediated introduction of a **single-copy** of ***SOD1^{A4V}***, ***SOD1^{H71Y}***, ***SOD1^{L84V}***, ***SOD1^{G85R}*** or ***SOD1^{G93A}***	- A4V, H71Y, G85R and G93A: SOD1 aggregates in MNs			ROS-induced	ROS-induced	
TARDBP	Liachko *et al.* 2010	Transgenic (1) **WT** and (2) ***TARDBP^{G290A}***, ***TARDBP^{A315T}***, ***TARDBP^{M337V}*** lines	- TDP-43 is highly phosphorylated in (2)	(2)>(1)	(2)			
	Ash *et al.* 2010	Transgenic **WT *TARDBP*** line	- Synaptic vesicles ↘					
	Zhang *et al.* 2011	Transgenic (1) **WT**, (2) ***TARDBP^{Q331K}***, ***TARDBP^{M337V}*** and (3) **C-terminal *TARDBP*** lines	- TDP-43 aggregates: (3) > (1,2)	(2)>(3)>(1)				Rate of development ↘
	Zhang *et al.* 2012	UV/TMP-induced (**) null mutations of ***tdp-1*** **(ok803, ok781)**	- Attenuates toxicity of aggregation-prone C-terminal fragments					Brood size and rate of development ↘
	Vaccaro *et al.* 2012	Transgenic (1) **WT** and (2) ***TARDBP^{A315T}*** lines	(2) TDP-43 aggregates and impaired GABAergic neurotransmission	(2)>(1)	(2)			
FUS	Murakami *et al.* 2012	Transgenic (1) **WT**, (2) ***FUS^{R514G}***, ***FUS^{R521G}***, ***FUS^{R522G}***, ***FUS^{P525L}*** and (3) C- terminal *FUS* (***FUS^{513}***, ***FUS^{501}***) lines	- R522G, P525L, 513, 501: FUS aggregates	R522G, P525L, 513, 501				R522G, P525L, 513, 501: survival ↘
	Vaccaro *et al.* 2012	Transgenic (1) **WT** and (2) ***FUS$^{S57\Delta}$*** lines	(2) FUS aggregates and impaired GABAergic neurotransmission	(2)>(1)	(2)>(1)			
	Markert *et al.* 2019	Transgenic C-terminal-truncated ***FUS^{501}*** line	- Abnormal NMJ - Synaptic transmission ↘ - FUS aggregates					Survival ↘
Others	Ikenaka *et al.* 2013 (*Dynactin1*)	shRNA-induced **KD of *dynactin1***	- Disrupted axonal transport - Autophagosome accumulation in MNs					
	Woo *et al.* 2017 (*CHCHD10*)	(1) Transgenic ***CHCHD10^{R15L}***, ***CHCHD10^{S59L}*** lines and (2) shRNA-induced **KD of *har-1***	- Mitochondrial and synaptic defects - TDP- 43 aggregates					Survival ↘

gene is associated with the neurodegenerative disease frontotemporal dementia (FTD) [26]. Both disorders are part of a complex disease spectrum [26]. *C9ORF72* mutations are characterized by clinical and pathological heterogeneity [5], which renders them the most challenging known ALS mutations to model in mice. Several attempts led to the generation of multiple C9ORF72 mouse models displaying pathological features (i.e. RNA foci and dipeptide repeat proteins (DPRs) generated by the non-ATG mediated translation of the repeats). However, most of these transgenic mice lack clinical ALS hallmarks, i.e. motor neuron degeneration and muscle wasting [27,28]. At least two groups seem to have successfully established C9 mouse models with degenerative phenotype [29,30], although there are some concerns regarding the reproducibility of the phenotypes in the C9-BAC mouse model [31]. Altogether, these attempts illustrate that developing relevant rodent models is not only labor-intensive and time-consuming, sometimes questions can also be raised about the validity of these rodent models. Hence, more and more researchers alter their view on the use of rodent models and start to pursue alternative approaches, not only to overcome modeling difficulties, but also for ethical reasons and to gain more comprehensive insights into pathways leading to ALS. In this review, we will discuss the most recent alternative small animal model developments in ALS. We specifically focus on advances in studies using *C. elegans, Drosophila melanogaster* and *Danio rerio*, which are presented in order of increasing complexity.

2.1. *Caenorhabditis elegans*

Due to the well-developed nervous system, the small nematode *C. elegans* is a widely used animal model in neurodegenerative disease studies [32,33]. Next to the ease of growth, the short life cycle (3.5 days) and life expectancy (around 3 weeks) make *C. elegans* worms interesting to utilize in genetic studies [32,33]. Its genome contains 60–80% genes that are homologous to humans with 42% of them comprising disease-causing genes [34,35]. The ease of modeling genetics is one of the greatest assets of *C. elegans*, which allows for the use of diverse genetic manipulation methods [36,37] and the deciphering of genetic interactions [32,38]. In this chapter, we list the most recent approaches and findings in *C. elegans* regarding the most frequently occurring ALS mutations (Table 1).

In the presented *C. elegans* models, motility was measured by observing the curling behavior and by using a "thrashing assay", which is based on counting body bends that worms make within a certain time frame (Fig. 1) [39]. The body movement of *C. elegans* is controlled by both excitatory (acetylcholine (ACH), glutamate) and inhibitory (gamma-aminobutyric acid (GABA)) inputs. However, predominantly GABAergic and cholinergic motor neurons, and to a lesser extent



	Caenorhabditis elegans	*Drosophila melanogaster*	*Danio rerio*
Homology to humans	80% (disease: 42%)	60% (disease: 75%)	70% (disease: 84%)
Life cycle	3.5 days	10 days	3 months
Life span	3 weeks	3 months	3.5 years
ALS phenotypes	locomotion defects, paralysis, GABAergic MN degeneration	locomotion defects (climbing, velocity, crawling), eclosion defects, MN degeneration, eye phenotype	axonal deficits, swimming abnormalities, MN degeneration
ALS genes			
C9ORF72	*alfa-1*	-	*C13H9orf72*
SOD1	*sod-1*	*dsod*	*sod1*
TARDBP	*tdp-1*	*TBPH*	*tardbp + tardbpl*
FUS	*fust-1*	*Cabeza (Caz)*	*fus*
DCTN1	*dnc-1*	*DCTN*	*dctn1a*
VCP	*cdc-48.1/2*	*ter94*	*vcp*
CHCHD10	*har-1*	*Chchd2*	*chchd10*

Fig. 1. Phenotypic and genetic aspects of alternative animal models *Caenorhabditis elegans, Drosophila melanogaster* and *Danio rerio*. In *C. elegans*, (1) a reduced amount of thrashes point to locomotion defects and (2) impaired GABAergic neurotransmission is often accompanied by (3) GABAergic neurodegeneration. In *Drosophila melanogaster*, the most commonly observed phenotypes in adults include (4) climbing defects, (5) eye degeneration and (6) motor neuron death. Additional phenotypes in ALS fly models include (7) reduced eclosion of sick pupae and (8) crawling defects of the larvae. In *Danio rerio* larval studies, the most used phenotypic readouts include (9) abnormal swimming behavior measured by touch-evoked escape response (TEER) and (10) an axonal phenotype with decreased axonal length and increased abnormal branching. Adult *Danio rerio* phenotypes comprise (11) motor neuron degeneration and (12) abnormal neuromuscular junctions. Important genetic and other facts of each model are shown as well, such as the homology percentage of each model to human in general and specific for disease-associated genes, the orthologs of human ALS-associated genes and information on the life cycle and lifespan. Abbreviations: MN, motor neuron.

glutamatergic neurons, are observed to assess neurodegeneration [40,41].

2.1.1. C9ORF72

The most common genetic cause of ALS is a GGGGCC hexanucleotide repeat expansion in the *C9ORF72* gene [8,9]. This repeat expansion could cause pathology by three non-mutually exclusive mechanisms, which are C9ORF72 loss-of-function [42], RNA toxicity [43–45] and DPR toxicity [46]. Modeling all three mechanisms was enabled by the existence of ALFA-1, a well-conserved homolog of C9ORF72 in *C. elegans* [47]. Interestingly, ALFA-1 was found to be a key player in endolysosomal pathways [48], confirming its functional similarity to human C9ORF72 [49,50]. Moreover, a recent study in *C. elegans* associated C9ORF72 with the activity of transcription factor EB (TFEB), regulating stress-sensitive metabolic homeostasis [51]. Upon ALFA-1 depletion in worm embryos, age-dependent locomotion defects, paralysis, and defects in yolk storage and transport were apparent [47,48]. As well as modeling C9ORF72 loss-of-function, introducing *C9ORF72* hexanucleotide repeats or DPRs also resulted in neurotoxic effects and dysregulated motor function [52,53]. Of the different overexpressed RNA and protein molecules, overexpression of larger repeat lengths and arginine-rich DPRs produced the strongest ALS-like phenotypes [52,53]. Chemical and genetic screens are a classical and a rapid tool to identify novel therapeutic targets. This is illustrated by the fact that the *C. elegans* ortholog of *VAMP-associated membrane protein B (VAPB)*, *F57A10.2*, and the enzyme *acid phosphatase 4 (acp-4)* were recently discovered as suppressor genes of the phenotypes in *C. elegans* models expressing 9 or 29 C9ORF72 hexanucleotide repeats [52]. In particular, loss-of-function of these proteins suppressed the neurodegenerative and motor phenotypes, which were accompanied by reduced brood sizes and survival rates [52]. As both C9ORF72 and VAP, the fly homolog of F57A10.2/VAPB, have been functionally linked to Rab proteins, the observed repeat-induced detrimental effects in *C. elegans* might be instigated by a dysregulated Rab pathway [52,54–57].

2.1.2. SOD1

SOD1, the first gene that was associated with ALS [58,59], has also been studied in *C. elegans*. Stable overexpression of mutant SOD1-G93A resulted in degeneration of GABAergic motor neurons [60], whereas CRISPR/Cas9-mediated expression of a single copy of the same mutation in the endogenous *sod-1* gene solely caused cholinergic and glutamatergic neurodegeneration upon oxidative stress induction [61]. Cytoplasmic aggregates and locomotion defects are a common theme in both WT and mutant SOD1 (i.e. A4V, G37R, G85R and G93A) *C. elegans* overexpression models [60–63]. Additionally, these studies resulted in the identification of some modifying factors. For instance, reducing the insulin-like growth factor receptor Daf-2 in the SOD1-G85R *C. elegans* model led to decreased SOD1 aggregates and significantly improved locomotion [64], suggesting that the DAF-2 Insulin/Insulin-like growth factor 1 signaling pathway could be a therapeutic target for ALS [65]. Later, Jablonski *et al.* identified RAD-23, a protein functional in autophagy and DNA repair, as modifier in both mutant SOD1 and TDP-43 *C. elegans* models [66]. More specifically, depletion of RAD-23 resulted in suppression of the locomotor phenotype, possibly mediated by the stabilization of aggregation-prone proteins [66]. Of note, an automated microfluidic-based screening platform for the monitoring of protein aggregation in *C. elegans* was developed to aid in the identification of pharmaceutical compounds specifically targeting aggregative propensities [67]. As the formation of aggregates is a common phenomenon in the ALS pathology [68], this high-throughput and multifunctional platform could be an interesting tool in ALS research [67].

2.1.3. TARDBP

Hyperphosphorylation, ubiquitination, truncation and aggregation of TDP-43, the protein encoded by *TARDBP*, is a well-studied phenomenon in *C. elegans*. Illustrating this, loss of endogenous tdp-1, the *C. elegans* equivalent of TDP-43, attenuated toxicity of aggregation-prone proteins, such as the C-terminal TDP-43 fragments [69]. Furthermore, phosphorylation of TDP-43 at serine residues 409/410 promoted the neurotoxic phenotype induced by overexpressing WT and mutant TDP-43 in *C. elegans* [70]. Notably, *C. elegans* studies also demonstrated that TDP-43 pathology is preceded by calcium upregulation and is aggravated upon cellular stress induction, suggesting that the stress response and regulation of intracellular calcium might be of importance [71,72]. The *TARDBP* mutations A315T, M337V, Q331K and G290A resulted in worse locomotion defects than WT overexpression and, interestingly, led to highly phosphorylated TDP-43 deposited in distinctive cytoplasmic aggregates [70,73,74]. Multiple therapeutic compounds, including different neuroleptic and antiepileptic drugs, have been identified by chemical screens performed in *C. elegans* [75–77]. As an illustration, α-methyl-α-phenylsuccinimide, the active metabolite of the approved antiepileptic drug methsuximide, ameliorated TDP-43 toxicity and, more specifically, reduced the number of neuronal breaks and cell body losses in GABAergic motor neurons [76].

2.1.4. FUS

Distinctive features of mutant FUS *C. elegans* models (i.e. R514G, R521G, R522G and P525L) comprise cytoplasmic mislocalization, formation of FUS aggregates, age-dependent motility defects, and paralysis as well as impaired GABAergic neurotransmission [74,78,79]. Recently, it was demonstrated that the FUS low-complexity domains drive phase separation and the formation of aggregates in *C. elegans* [80]. Of note, these aggregates were associated with the sequestration of important regulatory RNA-binding proteins [80]. Moreover, Markert *et al.* used innovative techniques to elucidate the impact of the FUS C-terminal pathological form (FUS501) on motor neuron health [78]. In particular, ultrastructural and electrophysiological approaches revealed reduced transmission from motor neurons to muscle tissue, suggesting a role for FUS in the organization of synaptic vesicles and neurotransmission [78]. In addition, the regulation of miRNA-mediated gene silencing was discovered to be involved in the functionality of FUS as well, and genetic analyses demonstrated that this function was well conserved for *fust-1,* the *C. elegans* ortholog of *FUS* [81]. Using a chemical genetic screening platform, Vaccaro *et al.* identified methylene blue as a potent modifier of both mutant FUS and mutant TDP-43 toxicity [82]. Moreover, treatment with methylene blue alleviated motor deficits and oxidative stress triggered by ALS mutations in both *C. elegans* and zebrafish [82]. These findings were validated in human cellular models in which methylene blue inhibited TDP-43 aggregation [83].

2.1.5. Other ALS genes

The effect of two additional genes associated with ALS were tested in *C. elegans* studies: the *coil-helix-coiled-coil-helix domain containing protein 10* (*CHCHD10*), involved in oxidative phosphorylation, and the motor protein-encoding *dynactin 1 (DCTN1)* [84–87]. First, shRNA-induced knockdown of the *DCTN1 C. elegans* ortholog, *dnc-1,* resulted in locomotion defects, cholinergic neurodegeneration and disrupted axonal transport [88]. Further support for the implication of this gene in ALS was provided by the recent zebrafish study displaying functional impairment of neuromuscular junctions and additional ALS-relevant phenotypes upon dctn1a depletion (see below) [89]. Next, Woo *et al.* showed that *C. elegans* modeling CHCHD10 loss-of-function mutations gave rise to mitochondrial and synaptic defects, paralysis, reduced survival and cytoplasmic TDP-43 accumulation, suggesting a link between mitochondrial and synaptic toxicity with TDP-43 pathology [90]. These findings were confirmed in mammalian cell lines, primary neurons and mouse brains [90]. Finally, silencing of *unc-13*, the ortholog of the recently identified ALS susceptibility gene *UNC13*A, suppressed motor neuron degeneration caused by mutant TDP-43 proteins in *C. elegans* [91,92].

2.2. Drosophila melanogaster

For more than a century now, fruit flies (*Drosophila melanogaster*) have been widely used in biological research as a major model organism, and have led to groundbreaking discoveries [93]. The advantages of *Drosophila* are numerous, including a short lifespan and generation time, allowing for fast progression of experimental work [94]. Moreover, fruit flies are easy and cheap to maintain in the lab, and large-scale experiments are favored since fruit flies can give rise to a large number of genetically identical progeny [95]. Finally, the complete fly genome has been sequenced, revealing that ~ 75% of human disease genes have a fly ortholog [96], and striking structural similarities exist between human and fly genes [97].

The tremendous amount of knowledge about *Drosophila* has led to the development and availability of a large repertoire of genetic tools, establishing flies as a prime model for ALS research and for the investigation of disease mechanisms of many ALS-associated genes (Table 2) [98]. Transgenic flies can be easily generated by either random insertion or, more importantly, by insertion of a human transgene in a 'safe harbour', which allows to evaluate the effects of overexpressed genes on a cellular and physiological level [99]. Moreover, RNA interference (RNAi) lines are readily available in public stock repositories and can be used to knock down specific ALS-related genes [99]. An extremely efficient technology is the UAS-GAL4 system, allowing for expression or knockdown of a gene in specific cell types [100,101], as well as CRISPR/Cas9 technology, for functional gene inactivation [102]. These technologies have led to the creation of multiple ALS-related *Drosophila* models, some of which are mentioned below (Table 2).

2.2.1. C9ORF72

Mechanisms of toxicity induced by the GGGGCC hexanucleotide repeat expansion in *C9ORF72* are being extensively studied in *Drosophila,* and as there is no known *C9ORF72* ortholog in flies, mainly the gain-of-function mechanisms are under investigation [103–105]. Hence, multiple transgenic flies have been generated showing expression of the expanded repeat and the presence of DPRs [106,107]. Interestingly, studies in *Drosophila* identified a direct relationship between the presence of hexanucleotide repeats and the TDP-43 proteinopathy, showing that the presence of these repeats could cause cytoplasmic TDP-43 mislocalization in salivary gland cells [108]. In addition, C9 ALS *Drosophila* models revealed that accumulated cytoplasmic TDP-43 caused Karyopherin pathology, including cytoplasmic mislocalization of Karyopherin-alpha (KPNA), thus relating C9 ALS to nucleocytoplasmic transport deficits [100,107,108]. Moreover, DPRs encoded by the *C9ORF72* expanded gene, were shown to directly bind to Nucleoporins in a C9 ALS *Drosophila* model [109], and knockdown of Karyopherin-alpha3 in flies enhanced DPR toxicity [110]. The C9-induced TDP-43 pathology, as well as the KPNA pathology were confirmed in *post-mortem* material from C9 ALS patients [100,111], suggesting that nucleocytoplasmic transport could be an interesting therapeutic target in ALS. Recently, a novel mechanism was identified using flies, suggesting a link between expression of the repeats of *C9ORF72* and tau accumulation [112]. It was demonstrated that downregulation of the *Drosophila* tau ortholog (dtau) rescued motor impairment and increased the lifespan of the C9 flies. Neurodegeneration in *C9ORF72* repeat-expressing flies was rescued as well. It was also discovered that the presence of expanded repeats increased tau accumulation and promoted pathology [112]. Of note, polyglutamine repeat expansions seem to play a role in the pathophysiology of ALS, since ATXN1 repeat expansions have been found to confer a risk for ALS, and cause cytoplasmic mislocalization of TDP-43 in a C9 ALS *Drosophila* model [113]. Moreover, a recent study found that arginine-rich DPRs reduced the number of Golgi outposts and dendritic branches in the fly neurons [114]. Last but not least, arginine-rich DPRs have been found to interact with ribosomal proteins, which led to translation inhibition, an event rescued upon expression of the eukaryotic translation initiation factor 1A (eIF1A) [115]. Altogether, findings in C9 ALS *Drosophila* models suggest that toxicity is especially linked to the expression of the arginine-rich DPRs, and not the other DPRs or hexanucleotide repeat RNA [106,114,115].

2.2.2. SOD1

In *Drosophila*, many attempts have been made to study SOD1 loss- and gain-of-function. For instance, SOD1 null mutation resulted in infertility of adult flies and reduced lifespan [116]. Overexpression of human SOD1 in adult motor neurons of *Drosophila*, did not only rescue the lifespan of a *Sod* null mutant, but also extended the normal lifespan of flies by up to 40% [117], suggesting that enhancement of the reactive oxygen metabolism can have beneficial results in fly aging and lifespan. Moreover, ubiquitous expression of SOD1 extended lifespan, but did not forestall age-related locomotor impairments (ARLI) in flies, whereas ubiquitous SOD1 knockdown accelerated ARLI and shortened lifespan [118]. These general effects of SOD1 expression compromised significantly the use of *Drosophila* as a model to study the effect of SOD1 mutations. There are a few exceptions. One study showed that expression of WT or mutant human SOD1 (A4V and G85R) in fly motor neurons caused climbing deficits in flies, progressing over time [119]. Moreover, defects were observed in the neural circuit accompanied by a stress-response in glial cells and focal accumulation of SOD1 in the motor neurons [119]. Another study used homologous recombination to genetically engineer four human SOD1 mutations (G85R, H71Y, H48R and G37R) in the endogenous fly SOD1 (dsod), and analyzed behavioral and biochemical phenotypes. Homozygous G85R, H71Y and H48R mutations resulted in reduced survival, eclosion defects, as well as larval and adult locomotor deficits due to axonal retraction from muscle, an important characteristic of ALS [120].

2.2.3. TARDBP

Mutations in the *TARDBP* gene have been identified in a subset of ALS patients. However, nuclear loss and cytoplasmic accumulation of TDP-43, is a pathological hallmark of ALS in general [10]. The TDP-43 fly ortholog is called *trans*-active response DNA-binding protein homologue (TBPH), and has been widely studied [121]. TBPH deletion caused lethality in larvae and reduction in the levels of histone deacetylase 6 (HDAC6), a cytoplasmic regulator of protein aggregation and degradation [122]. Moreover, inhibition of HDAC1, a crucial transcriptional regulator, ameliorated the WT or mutant TDP-43-induced toxicity in flies and cells [123]. Of note, hypomorphic TBPH alleles caused locomotor deficits and reduction in the flies' lifespan, mainly attributed to neuromuscular junction (NMJ) perturbations in the larval stage [124,125]. Diaper *et al.* discovered that TDP-43 pathology led to impairments of pre-synaptic transmission and defective motor control, as TBPH loss- or gain-of-function resulted in affected pre-synaptic efficacy in larvae and loss of neurons mediating motor functions in adults [126]. Recently, TDP-43 was found to promote neuromuscular synapse formation in flies by regulating expression of Disc-large (Dlg), an observation that was validated in iPSC-derived motor neurons from patients [127]. A particularly useful experimental technique to understand the level of neurodegeneration and toxicity induced after expression of a gene, is expression in the fly eyes using the GMR-gal4 driver [128]. Transgenic *Drosophila* selectively expressing human WT TDP-43 in the eye presented with notable ommatidia loss and neurodegeneration [128]. Furthermore, expression of hTDP-43 in motor neurons caused remarkable loss of axons and neuronal death [128]. In another TDP-43 fly model, the splicing factors SF2, Sf3b1 and Rbp1, were identified as modulators of TDP-43 production through autoregulation, establishing the TDP-43 autoregulatory system as a novel potential therapeutic target [129]. Due to the large heterogeneity that characterizes ALS, genetic modifiers have been suggested to play a crucial role in disease [130,131], and *Drosophila* is an ideal model to investigate this. In line with this, heterogeneous nuclear ribonucleoproteins (hnRNPs) were identified as modifiers of TDP-43 pathology in flies, which was also

confirmed in human neuronal cells [132]. Ataxin-2 was also identified as a modifier of TDP-43 toxicity in *Drosophila*, as the severe degenerative phenotype in the fly eye caused by TDP-43 expression was rescued by ataxin-2 null mutation and was further enhanced by ataxin-2 upregulation [133]. These results in flies seem to relate to the human situation, as ataxin 2 cytoplasmic inclusions were observed in ALS spinal neurons [133] and intermediate expansions in ataxin 2 conferred an increased susceptibility to ALS [134,135]. Finally, TDP-43 was shown to regulate stathmin-2 expression in human cells and tissues, and loss of stathmin-2 has been identified as a hallmark of TDP-43 pathology in flies as well [136]. Overall, there is no consistent evidence that the TDP-43 effects are mutant-specific, since expression of either WT or mutant TDP-43 often leads to ALS-related phenotypes in flies [128,137]. However, advanced techniques for fly work are constantly emerging, such as the recent optogenetic models of TDP-43 that were created [138]. Moreover, available engineering tools allow for targeted replacement of endogenous TBPH by the hTDP-43 gene (WT and mutant). As such, the issue of overexpression phenotypes in flies can be overcome by expressing the gene in normal levels under the native promoter [139].

2.2.4. *FUS*

FUS is structurally and functionally related to TDP-43 and common pathological mechanisms were suggested [140]. Similar to TDP-43, the *FUS* gene also regulated transmission in the NMJ of *Drosophila* [141,142] and deficits in synaptic transmission preceded the degeneration and loss of motor neurons [143]. The nucleocytoplasmic transport proteins Exportin-1 (XPO1) and Nucleoporin 154 were identified as modifiers of FUS toxicity, as their downregulation prevented FUS-induced toxicity and rescued the apoptosis of neurons in the ventral nerve cord [144]. Similarly, muscleblind has been identified as a modifier of FUS-mediated neurodegeneration in flies, where it regulates the mislocalization of mutant FUS in the cytoplasm and its accumulation into stress granules [145]. In addition, the molecular interactions of FUS domains can also be studied in flies. The prion-like N-terminal domain of FUS together with the C-terminal arginine-rich domains act synergistically to mediate FUS aggregation and maturation in stress granules [146]. Several unbiased genome-wide screens were performed using *Drosophila models* of FUS. In particular, several genes were identified as modifiers of both FUS-R521C and TDP-43-M337V fly models, suggesting that the phospholipase D pathway is an important mechanistic player in FUS and TDP-43 pathology, an argument further corroborated by mouse and human studies [147]. Unraveling a role of FUS in autophagy, Marrone and colleagues found that enhancement of autophagy reduced cytoplasmic FUS, restored homeostasis of RNA-binding proteins and rescued motor function in flies [148].

2.2.5. *Other ALS genes*

Fly models also exist for other ALS-associated genes, such as *Valosin-Containing Protein* (*VCP*), coding for a protein involved in proteasomal degradation [149]. Expression of human VCP rescued deficits induced after loss of fly VCP, connecting autophagy and lysosomal dysfunction to VCP-related disorders [150]. TBPH was also found to interact with VCP and to suppress VCP-induced degeneration in flies [151]. Moreover, mutation in *Senataxin* (*SETX*), a gene with RNA helicase activity, can cause a dominant form of ALS, called ALS4 [152]. An ALS4 *Drosophila* model showed that NMJ integrity and plasticity were modulated by SETX through the TGFβ pathway, paving the way towards a better understanding of this type of disease and to the development of novel therapeutic approaches [152]. ALS8, caused by a mutation in *VAPB* was modelled in flies as well [153]. Expression of the mutation dVAP33A led to impediment of BMP signaling pathways at the level of the NMJ, hence identifying a novel mechanism underlying ALS8 pathology [153]. More recently, a mechanistic interplay between SOD1, ROS and the mTOR pathway was found to regulate VAPB aggregation in a novel ALS8 fly model [154] and VAPB was also found to interact with Eph receptors [155].

2.3. *Danio rerio*

Over the last decade, the zebrafish (*Danio rerio*) has significantly gained popularity in biomedical research [156,157]. Next to being a vertebrate, its assets of translucency, ease of genetic manipulation and fast reproduction rate enable rapid screening of ALS disease-related genes [157,158]. Moreover, the ~70% homology between the human and zebrafish genome, and the fact that more than 80% of disease-relevant genes has at least one zebrafish ortholog, renders the use of this small animal model appealing for genetic ALS studies [157,158].

Moreover, the expanding variety of genetic strategies in zebrafish increases the possibilities for testing ALS hypotheses. In zebrafish, the genetic manipulation of a specific gene can be achieved in a transient or stable manner (Table 3) [159–162]. On one hand, morpholino-mediated knockdown or microinjection of DNA and RNA in zebrafish oocytes yields a short-term overexpression or downregulation, which allows fast genetic screenings [159,160]. On the other hand, (stable) transgenic zebrafish models can be created by chemical mutagenesis (e.g. ENU) or emerging targeted genetic manipulations, such as CRISPR/Cas9 and transposon-mediated BAC transgenesis [163–165]. These approaches have led to the generation of numerous zebrafish models presenting with ALS-relevant phenotypes (Fig. 1). Excitingly, many of these established models are currently being used for therapeutic compound screenings (see infra) [166].

2.3.1. *C9ORF72*

Similar to *C. elegans* and *Drosophila*, *Danio rerio* models were used to investigate the three possible C9 ALS-causing mechanisms (i.e. C9ORF72 loss-of-function, RNA toxicity and DPR toxicity). Interestingly, these zebrafish studies generally led to a patient-relevant neurodegenerative and motor phenotype. In particular, morpholino-mediated C9ORF72 knockdown [167] or the introduction of arginine-rich DPRs in zebrafish embryos [168,169] yielded a decreased length of ventral root projections (e.g. primary motor neuron axons) and behavioral abnormalities, including reduced swimming distance, lower average velocity and compromised overall mobility, measured in a touch-evoked escape response assay (TEER) [167–170]. Moreover, the relevance of pure RNA toxicity, without the interference of DPR toxicity, was demonstrated by the presence of an ALS-relevant axonal phenotype upon micro-injection of hexanucleotide repeat RNA in zebrafish oocytes [170], which is in contrast to findings in *Drosophila* [106]. Also interesting, the cardinal C9 ALS pathological hallmarks, including RNA foci and repeat-associated non-ATG (RAN)-translated DPR species were detected in zebrafish models [169–171], while TDP-43 pathology was absent or, in some models, not assessed [167,172]. To elucidate potential synergistic effects of different ALS-related genes, Ciura *et al.* showed that the toxicity caused by *C9ORF72* silencing was increased in the presence of ATXN2 with intermediate repeat lengths [173]. It is clear that the identification of disease modifiers in these models represents a promising path towards the development of novel therapeutic targets. For example, the RNA metabolism-regulator ELP3 and the RNA-binding protein Pur-alpha were found to rescue the axonal phenotype in the zebrafish model specific for RNA toxicity [170,174]. Additionally, chemical screens revealed C9ORF72 DPR toxicity-limiting compounds, which were shown to alleviate nucleolar stress induced by poly-PR and to restore nucleolus-related functions, including mRNA splicing and translation [175].

2.3.2. *SOD1*

Of the more than 150 identified missense mutations in the first discovered ALS-causative gene *SOD1* [58,59], at least four have been modeled in zebrafish in a transient and/or stable manner: G93A, G37R, A4V and T70I [176–179]. All these mutations are believed to cause a gain-of-function, resulting in toxic proteins, mitochondrial dysfunction and/or ER stress. The T70I point mutation, induced by TILLING chemical mutagenesis, provides mutant sod1 proteins at physiological levels

Table 2

Overview of genetic models in *Drosophila melanogaster*. Studies modeling ALS-disease related genes *C9ORF72*, *SOD1*, *TARDBP*, *FUS* and others in fruit flies. The specific approach for genetic manipulation is summarized for each model. Cellular pathological hallmarks and, when present, motor phenotypes (i.e. climbing and crawling defects), neurodegenerative (i.e. motor neuron loss) and other phenotypes (e.g. reduced survival, eye degeneration) are indicated. Legend: orange: present or affected; green: not present or not affected. Abbreviations: KD, knockdown; MN, motor neuron; SG, salivary glands; CNS, central nervous system; NMJ, neuromuscular junction; VNC, ventral nerve cord; CCAP, crustacean cardioactive peptide, AG, anterior ganglia.

Drosophila melanogaster							
Gene	**References**	**Approach (i.e. KD, overexpression…)**	**Pathological hallmarks (cellular changes, aggregates…)**	**Neurodegeneration and motor phenotype**			**Other phenotypes**
				Adult climbing defects	**Eclosion defects**	**Larval crawling defects**	
C9ORF72	Baldwin *et al.* 2016	***C9ORF72 DPR toxicity:*** Transgenic flies expressing 3 G4C2 repeats (1) or 36 G4C2 repeats (2)	- Axonal transport deficits due to DPRs (2)				
	Solomon *et al.* 2018	***C9ORF72 DPR toxicity:*** ***UAS-DsRed2-G4C2 (8,32,56,64)*** ***UAS-GA/GR/PR/PA (8,64)*** ***UAS-hTDP-43*** ***deltaNLS-TBPH***	- DPRs cause TBPH cytoplasmic mislocalization - Cytoplasmic TDP-43 enhances DPR toxicity - KPNA pathology				Locomotor impairment and eye degeneration
	West *et al.* 2020	***C9ORF72 DPR toxicity:*** UAS-Gal4-induced **DPR** expression ***UAS-(AP)1024, UAS-(GA)1020, UAS-(PR)1100, UAS-(GR)1136***	- DPR selective accumulation in the CNS - DPR-induced TDP-43 mislocalization - DPR-induced neurodegeneration and cell death - NMJ perturbations				Motor function ↘, altered lifespan, but not viability
	Freibaum *et al.* 2015	***C9ORF72 DPR toxicity:*** Transgenic stocks expressing G4C2 repeats by UAS-Gal4 binary system ***UAS-G4C2 (8,28,58)***	- NMJ perturbations - Nuclear envelope abnormalities in SG cells - Nuclear RNA accumulation				Rough eye phenotype, larval size ↘ and locomotor impairment
SOD1	De Rose *et al.* 2017	D42-Gal4-induced expression of **hSOD1** in MN					Muscle electrophysiological activity ↘ and impaired mitochondrial morphology in MNs
	Watson *et al.* 2008	UAS-Gal4-driven expression of **hSOD1** (**WT, A4V, G85R**)	- Partial loss of MN nuclei - G85R accumulation in retinal cells - hSOD1 foci in MN - Stress response in glia				Progressive motor dysfunction and age-dependent electrophysiological defects
	Gallart-Palau *et al.* 2016	UAS-Gal4-driven expression of **hSOD1** (**WT, G85R**)	- Mitochondrial deficits				Lifespan ↘ and impaired motor behavior
TARDBP	Estes *et al.* 2011	GMR-mediated overexpression of human **TDP-43** (**WT,A315T**) in fly eyes	- Cytoplasmic localization and axonal aggregates in larval imaginal discs - TBPH translocation and aggregation in cytoplasm - Abnormal NMJ - Impaired larval locomotion				Adult retina neurodegeneration
	Li *et al.* 2010	GMR-mediated expression of **hTDP-43** in eyes (1), mushroom bodies (2), motor neurons (3).	- Necrotic lessions in eyes - Formation of autophagic vacuoles (AV) and multivesicular bodies (MVB) (1) - Axonal loss and neuronal death (2) - Cytoplasmic aggregation, axonal swelling (3)				Ommatidia loss
	Baldwin *et al.* 2016	GOF and LOF of **TDP-43** (**WT, M337V**) (1) and **TBPH** (2)	- Disrupted transport of vesicles (1) - Disrupted mitochondrial transport (2)				
	Wang *et al.* 2011	Expression of **TBPH** or **TDP-43** by targeted transgenesis					Lifespan ↘, locomotion and velocity ↘ and no obvious developmental defects
	Feiguin *et al.* 2009	**TBPH LOF** by imprecise mobilization of $TBPH^{EY10530}$ transposon using Δ2−3 transposase	- NMJ perturbations				
	Otte *et al.* 2020	Optogenetic induction of **TDP-43** proteinopathy	- Hyperphosphorylated and ubiquitinated detergent-insoluble cytoplasmic optoTDP-43 aggregates				Wing phenotypes
FUS	Baldwin *et al.* 2016	GOF or LOF of **Caz (WT, P398L)** and **GOF of FUS (WT, P525L)**	- Axonal transport defects - **Disrupted transport of vesicles**				
	Wang *et al.* 2011	Expression of **Caz** or **FUS** by targeted transgenesis					Lifespan ↘, locomotion and velocity ↘ and developmental defects
	Xia *et al.* 2012	UAS-Gal4-induced expression of **Caz, hFUS, hFUS-R521G**	- NMJ perturbations - VNC-MNs disruption - Neurodegeneration and MN apoptosis				Wing defects, viability ↘, locomotion ↘ and retinal degeneration
	Steyaert *et al.* 2018	UAS-Gal4-induced expression of **hFUS (WT, R521G, R521H)**	- Loss of CCAP neurons - Degeneration of AG neurons				Pupal lethality
Others	Kim *et al.* 2018	UAS-Gal4-induced expression of **UBQLN2 (WT, P497H, 525S, P4X)**	- TDP-43 pathology - Interaction with G4C2 repeats - Protein aggregation in MNs - NMJ perturbations				Eye degeneration
	Baek *et al.* 2019	UAS-Gal4-induced expression of ***CHCHD10^{S59L}***	- Neuronal and muscle degeneration - CHCHD10 insoluble aggregates				Eye toxicity

in zebrafish [179]. This is advantageous and more patient-relevant, especially compared to the overexpression levels in rodents and other zebrafish models. Overall, the majority of the SOD1 zebrafish models showed both behavioral and structural deficits comparable to the C9 ALS zebrafish phenotypes [176–180]. Moreover, characteristic for these models, and analogous to patients, are the abnormal formation of neuromuscular junctions, sensitivity to oxidative stress and dysfunctional mitochondria [177–180]. It was discovered that, in addition to rescuing the C9 ALS zebrafish model, ELP3 had neuroprotective effects on the mutant SOD1-A4V zebrafish model as well [174]. Furthermore, genetic and pharmacological downregulation of EphA4, a receptor in the ephrin axonal guidance system, showed beneficial effects in SOD1 zebrafish bearing the mutations A4V, G93A or G37R, which was also confirmed in rodent models and patient samples [181]. A class of neuroleptics, identified in a *C. elegans* library screen, stabilized neuromuscular transmission, alleviated motility defects and prolonged survival in SOD1 transgenic zebrafish models, as well as in TDP-43 and FUS transgenic zebrafish models [75]. Additionally, a chemical screen identified the small molecule, TRVA242, which significantly improved defects in SOD1, but also in C9ORF72 and TDP-43 zebrafish models [182]. Interestingly, TRVA242 also stabilized deficits in a SOD1-G37R mouse model. Altogether, these interesting pharmacological findings increase the relevance of the zebrafish as an alternative animal model, as zebrafish data can be extrapolated to rodent models. This was further corroborated by a study showing that Riluzole alleviated the motor phenotype in mutant SOD1-G37R zebrafish embryos [183].

2.3.3. TARDBP

Both gain- and loss-of-function mechanisms are believed to play a role in TDP-43-related ALS [184–187]. However, to what extent each mechanism contributes to the disease is still unclear. In zebrafish, two orthologs for the human *TARDBP* were identified, i.e. *tardbp* and *tardbpl* [188]. Introducing *TARDBP* point mutations (i.e. A315T, G348C, A382T) or knocking down zebrafish tardbp resulted in similar swimming and degenerative phenotypes [189], reinforcing the possibility of synergistic mechanisms to be at play in TDP-43 ALS patients. Moreover, depleting both tardbp and tardbpl simultaneously caused a harsher zebrafish phenotype, comprising weight loss, reduced survival and muscle atrophy on top of the swimming and motor deficits, which were already present upon a tardbp-only KO [188,190,191]. Recently, zebrafish experiments showed that neurofilament splicing, essential for proper axonal transport and overall motor neuron health, was regulated by TDP-43, suggesting that both factors might be biologically and pathologically associated [192]. Further substantiation of zebrafish transgenic lines as assets for the investigation of TDP-43 ALS was found by performing comparative sequencing analyses. In particular, transcriptomic changes found in the G348C zebrafish model, were in line with transcriptomic data obtained from TDP-43 knock-in mouse models [187,193].

2.3.4. FUS

As mentioned before, the RNA-binding proteins TDP-43 and FUS have similar functions in RNA metabolism, suggesting that they could be involved in similar pathogenic mechanisms [194,195]. This idea was corroborated upon modeling both mutant FUS overexpression (i.e. gain-of-function) and depletion of endogenous fus (i.e. loss-of-function), which were in first instance similar to findings in the TDP-43 zebrafish models and also corresponded to aspects of the clinical and pathological presentation of ALS patients [196,197]. The *FUS* mutations (P525L, H517Q, R521G, R495X and G515X) in zebrafish overexpression models resulted in cytoplasmic mislocalization of the FUS protein [198,199]. The extent of mislocalization correlated with the clinical severity of the mutation in patients and was in some cases triggered upon heat shock [199]. Additionally, zebrafish overexpressing the human FUS mutant R521H or zebrafish depleted from endogenous fus showed both behavioral defects, decreased axonal length and abnormally branched axons. Apart from the thorough investigation of single disease-relevant genes, combinatorial effects of multiple ALS-related genes were analyzed as well. To illustrate this in the context of *FUS*, mutant SOD1 exacerbated the ALS phenotype when co-expressed with mutant FUS [196]. Moreover, the motor phenotype in zebrafish embryos caused by knockdown of tardbp was rescued upon overexpression of WT FUS, suggesting a common pathogenic pathway for these RNA-binding proteins [196]. Evidence for this association was also found in *Drosophila* and in iPSC-derived cellular models, which could further support the validity of zebrafish models in the context of ALS [200,201].

2.3.5. Other ALS genes

Models of less frequently occurring ALS-related genes have also been established. For example, the depletion of dynactin, a cofactor of the motor protein dynein, [89] and the endoplasmic reticulum ATPase VCP [202] resulted in ALS-associated phenotypes, including touch-evoked escape response deficits, axonal abnormalities and impairments of autophagy and neuromuscular junctions. The link between ALS and several other proteins implicated in the cytoskeleton formation and maintenance, such as the motor proteins KIF5A and KIF1Bβ, is currently being investigated in zebrafish [203,204]. This suggests that also axonal transport and protein homeostasis might play a crucial role in the ALS pathogenesis.

3. To be, or not to be alternative, that is the question

Several ALS studies suggest that not one, but a combination of multiple genetic variations and/or disease modifying factors could at least partially explain the lack of correlation between phenotypes in disease models and clinical observations [4,205]. Moreover, this helps to explain the diversity between patients, even those with the same genetic mutation [5]. Hence, combining several causative genes into a single model organism might be necessary to recapitulate better the human situation. This oligogenicity makes ALS modeling a difficult task. Especially in rodents, this is challenging. The lack of oligogenic models could be part of the reason why many treatments, effective in monogenic mouse models, failed to show benefit in patient studies [206]. Nevertheless, preclinical studies are necessary to provide directions for guiding patient trials. Rodent models fulfill this critical role to a reasonable extent.

On the other hand, the small-animal models described in this review seem to be very valuable to identify modifying factors in ALS. Large unbiased genetic screens already identified new ALS-related modifiers that could help to explain the heterogeneity and to dissect new pathogenic mechanisms [52,66]. A large repertoire of innovative tools is currently available to maximally exploit the capabilities of these small animal models. Automated high-throughput screening platforms are gaining in popularity, which is especially the case for studies using *C. elegans* [67]. In comparison to other models, flies have a complex enough brain to be relevant, but a small enough nervous system compared to rodents, to allow for easy and thorough analysis [207]. In flies, the ease of genetic manipulation and the state-of-the-art *in vivo* imaging techniques which have been developed during the last decade are a powerful research tool [208]. In zebrafish, emerging metabolomics tools [209], reversible optogenetic models [210], *in vivo* live imaging measuring nucleocytoplasmic transport [211], axonal transport [212] and excitatory postsynaptic potentials (EPSCs) using whole-cell patch clamp recordings [213] provided new insights into the pathogenesis of ALS. Overall, better insights into modifying factors, into disease pathways and into causative genes from several multicellular models is of utmost importance. Especially because of ethical reasons and the fast experimental turnover, small animal models can accomplish a large part of this requisite.

Nevertheless, several limiting factors indicate that *Danio rerio*, *Drosophila melanogaster* and *C. elegans* are not irrefutable as models for

Table 3
Overview of genetic models in *Danio rerio.* Studies modeling ALS-disease related genes *C9ORF72*, *SOD1*, *TARDBP*, *FUS* and others in zebrafish. The specific approach and transient or stable genetic manipulation technique are summarized for each model. Cellular pathological hallmarks and, when present, motor phenotype (i.e. swimming defects), neurodegenerative (i.e. axonal deficits) and other phenotypes (e.g. reduced survival) are indicated. Legend: orange, present or affected; green, not present or not affected. Abbreviations: MO, morpholino; KD, knockdown; KO, knockout; Arg, arginine; MN, motor neuron; NMJ, neuromuscular junction. (*) TILLING mutagenesis, targeting induced local lesions in genomes. (**) ZFN mutagenesis, zinc finger nucleases. (***) ENU mutagenesis, N-ethyl-N-nitroso-ureum.

Danio rerio							
Gene	**References**	**Approach (i.e. KD, overexpression…)**	**Pathological hallmarks (cellular changes, aggregates…)**	**Neurodegeneration and motor phenotype**			**Other phenotypes**
				Swimming defects	Axonal deficits		
					Length	*Branching*	
C9ORF72	Ciura *et al.* 2013	***C9ORF72 loss-of-function:*** MO-mediated KD of *C9ORF72*	- No TDP-43 pathology - *C9ORF72* transcript levels↘				
	Lee *et al.* 2013	***C9ORF72 RNA toxicity:*** Transient overexpression of *C9ORF72* repeat DNA	- ≥72 repeats: RNA foci				Apoptosis (from 38 repeats)
	Ohki *et al.* 2017	***C9ORF72 DPR toxicity:*** Transgenic lines containing 80 *C9ORF72* repeats (1) alone or (2) co-expressed with polyGA	- No TDP-43 pathology				(1) Mild edema phenotype (2) Severe pericardial edema
	Swaminathan *et al.* 2018	***C9ORF72 DPR toxicity:*** (1) Transient overexpression of DPRs with repeat length 40, 200 and 1000 (2) Stable overexpression of GR with repeat length 100		(1) >> in long Arg-rich DPRs (2)			(1) Edema, more severe in long Arg-rich DPRs
	Swinnen *et al.* 2018	***C9ORF72 RNA and DPR toxicity:*** Transient overexpression of (1) *C9ORF72* repeat RNA and (2) DPR-encoding RNA	(1) RNA foci, no detectable DPRs		(1,2) induced by repeat RNA and Arg-rich DPRs		
	Shaw *et al.* 2018	***C9ORF72 RNA and DPR toxicity:*** Transgenic line containing 89 *C9ORF72* repeats	- RNA foci and DPRs (RAN translation) - MN loss and muscle atrophy				Cognitive impairment and survival ↘
SOD1	Lemmens *et al.* 2007	Transient overexpression of *SOD1* RNA (***SOD1^{G93A}***, ***SOD1^{G37R}***, ***SOD1^{A4V}***)			Specific for motor neurons		
	Sakowski *et al.* 2012	(1) Transient and (2) stable overexpression of ***SOD1^{G93A}***	- Abnormal NMJ - MN loss	(2)	(1)		
	(1) Ramesh *et al.* 2010 (2) McGown *et al.* 2013	Transgenic lines containing ***SOD1^{G93R}*** (1) alone or (2) fused with heat shock response reporter hsp70-DsRed	(1) Abnormal NMJ and mitochondria (2) NM synaptic volume ↘ and MN stress (1,2) MN loss and muscle atrophy				Survival ↘ and paralysis
	Da Costa *et al.* 2014	TILLING-induced (*) point mutation ***SOD1^{T70I}***	- SOD1 enzymatic acitivity ↘ - Oxidative stress ↗ - Abnormal NMJ				
TARDBP	Kabashi *et al.* 2009	(1) Transient overexpression of *TARDBP* RNA (***TARDBP^{A315T}***, ***TARDBP^{G348C}***, ***TARDBP^{A382T}***) (2) MO-mediated **KD of *tardbp***					Curly tail
	Hewamadduma *et al.* 2013	TILLING-induced (*) null mutation ***tardpb$^{fh301/fh301}$*** and MO-mediated **KD of *tardbpl***	- Alternative *tardbp* splicing				Curly tail, weight ↘ and survival ↘
	Schmid *et al.* 2013	ZFN-induced (**) **KO of *tardbp* and *tardbpl***	- Muscle atrophy				Blood circulation and survival ↘
	Bose *et al.* 2019	CRISPR/Cas9-mediated **KO of *tardbp* and *tardbpl***	- Abnormal NMJ				Morphological abnormalities and survival ↘
	Asakawa *et al.* 2020	Reversible optogenetic-induced **TDP-43 mislocalization**	- Abnormal NMJ				
FUS	Dormann *et al.* 2010	Transient overexpression of ***FUS^{P525L}*** RNA fused with GST-GFP reporter	- FUS cytoplasmic mislocalization				
	Bosco *et al.* 2010	Transient overexpression of *FUS* RNA (***FUS^{H517Q}***, ***FUS^{R521G}***, ***FUS^{R495X}***, ***FUS^{G515X}***)	- FUS cytoplasmic mislocalization (R495X, G515X) upon heat shock (H517Q) - FUS in SG upon heat shock (R521G, R495X, G515X)				
	Kabashi *et al.* 2011	(1) Transient overexpression of ***FUS^{R521H}*** (2) MO-mediated **KD of *fus***					
	Armstrong and Drapeau *et al.* 2013	(1) Transient overexpression of ***FUS^{R521H}*** (2) MO-mediated **KD of *fus***	- Abnormal NMJ				
	Bourefis *et al.* 2020	ENU-induced (***) null mutation in *Fus* (***Fus^{C155T}***)	- Abnormal NMJ				Survival ↘
Others	Bercier *et al.* 2019 (*Dynactin1*)	ENU-induced (***) null mutation in *dynactin1a* (***dctn1a^{C2395T}***)	- Synapses ↘ - Abnormal NMJ				
	Kustermann *et al.* 2018 (*VCP*)	(1) MO-mediated **KD of *vcp*** (2) CRISPR/Cas9-mediated **KO of *vcp***	- Autophagy impairment - Myopathy				Heart failure

ALS. First, the whole-genome duplication that occurred in the zebrafish lineage especially complicates the modeling of gene silencing, as one human gene could have multiple orthologs in zebrafish (e.g. tardbp and tardbpl). Second, even though the zebrafish is the only vertebrate reviewed here, and thereby, has the highest complexity, its distinctive physiology and environmental conditions compared to humans still need to be taken into consideration. Third, *Drosophila* may lack orthologs for some important genes to be studied, i.e *C9ORF72*. Nevertheless, this problem can be overcome by integration and expression of the human genes. In support of this, key biological pathways were discovered in fly models, and later validated in iPSC-motor neurons and mice, suggesting that flies are an important and reliable model [110,214]. Finally, *C. elegans* nematodes have a simple body plan and lack multiple organs compared to humans, which also impedes the direct translation of findings to ALS patients.

Altogether, studies using small animal models do not replace rodent studies. Nevertheless, they can diminish the number of rodents needed. More specifically, small animal models can act as multi-systemic platforms providing promising candidates and directions that can be subsequently tested in other model systems and could lead to the discovery of new therapeutic targets for ALS.

4. Conclusion

Summarizing the last decades of ALS research highlights the large amount of available animal models, with *C. elegans, Drosophila melanogaster*, zebrafish (*Danio rerio*) and rodents being the best-established ones. The majority of these models recapitulate some aspects of the pathology seen in patients, including cytoplasmic accumulation of disease-associated proteins and neuronal loss. Nevertheless, the translation of findings observed in these models to the clinic is not straightforward. In particular, many therapies effective in rodent-based studies have subsequently failed in clinical trials, which might be partially due to the heterogeneity typical for ALS. However, great advances have been made, and in particular small animal models constitute a powerful additional tool for the dissection of pathogenic mechanisms involved in ALS, as well as for the investigation of disease modifiers. The further implementation of these small animal models will certainly help to increase our understanding of the mechanisms associated with disease-associated genes and they could establish a platform to discover new treatment options for ALS patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Research of the authors is supported by VIB, KU Leuven (C1 and "Opening the Future" Fund), the "Fund for Scientific Research Flanders" (FWO-Vlaanderen, G0C1620N), the Thierry Latran Foundation, the "Association Belge contre les Maladies neuro-Musculaires – aide à la recherché ASBL" (ABMM), the Muscular Dystrophy Association (MDA), the ALS Liga België (A Cure for ALS), Target ALS and the ALS Association (ALSA). Elke Braems and Paraskevi Tziortzouda are PhD fellows of FWO-Vlaanderen (1145619N, 11C7621N). We thank Thomas Moens for reading our manuscript and providing useful comments.

Author contributions

Elke Braems and Paraskevi Tziortzouda equally contributed to the original draft preparation and writing of the manuscript. Ludo Van Den Bosch reviewed and edited the manuscript before submission and supervised the writing process.

References

[1] O. Dols-Icardo, A. García-Redondo, R. Rojas-García, D. Borrego-Hernández, I. Illán-Gala, J.L. Muñoz-Blanco, A. Rábano, L. Cervera-Carles, A. Juárez-Rufián, N. Spataro, N. De Luna, L. Galán, E. Cortes-Vicente, J. Fortea, R. Blesa, O. Grau-Rivera, A. Lleó, J. Esteban-Pérez, E. Gelpi, J. Clarimón, Analysis of known amyotrophic lateral sclerosis and frontotemporal dementia genes reveals a substantial genetic burden in patients manifesting both diseases not carrying the C9orf72 expansion mutation, J. Neurol. Neurosurg. Psychiatry 89 (2018) 162–168, https://doi.org/10.1136/jnnp-2017-316820.

[2] P. Van Damme, W. Robberecht, L. Van Den Bosch, Modelling amyotrophic lateral sclerosis: Progress and possibilities, DMM Dis. Model. Mech. 10 (2017) 537–549, https://doi.org/10.1242/dmm.029058.

[3] C.A. Johnston, B.R. Stanton, M.R. Turner, R. Gray, A.H.M. Blunt, D. Butt, M. A. Ampong, C.E. Shaw, P.N. Leigh, A. Al-Chalabi, Amyotrophic lateral sclerosis in an urban setting: A population based study of inner city London, J. Neurol. 253 (2006) 1642–1643, https://doi.org/10.1007/s00415-006-0195-y.

[4] A.E. Volk, J.H. Weishaupt, P.M. Andersen, A.C. Ludolph, C. Kubisch, Current knowledge and recent insights into the genetic basis of amyotrophic lateral sclerosis, Medizinische Genet. 30 (2018) 252–258, https://doi.org/10.1007/s11825-018-0185-3.

[5] B. Swinnen, W. Robberecht, The phenotypic variability of amyotrophic lateral sclerosis, Nat. Rev. Neurol. 10 (2014) 661–670, https://doi.org/10.1038/nrneurol.2014.184.

[6] M.R. Turner, A. Al-Chalabi, A. Chio, O. Hardiman, M.C. Kiernan, J.D. Rohrer, J. Rowe, W. Seeley, K. Talbot, Genetic screening in sporadic ALS and FTD, J. Neurol. Neurosurg. Psychiatry. 88 (2017) 1042–1044, https://doi.org/10.1136/jnnp-2017-315995.

[7] A. Al-Chalabi, L.H. van den Berg, J. Veldink, Gene discovery in amyotrophic lateral sclerosis: Implications for clinical management, Nat. Rev. Neurol. 13 (2016) 96–104, https://doi.org/10.1038/nrneurol.2016.182.

[8] M. DeJesus-Hernandez, I.R. Mackenzie, B.F. Boeve, A.L. Boxer, M. Baker, N. J. Rutherford, A.M. Nicholson, N.C.A. Finch, H. Flynn, J. Adamson, N. Kouri, A. Wojtas, P. Sengdy, G.Y.R. Hsiung, A. Karydas, W.W. Seeley, K.A. Josephs, G. Coppola, D.H. Geschwind, Z.K. Wszolek, H. Feldman, D.S. Knopman, R. C. Petersen, B.L. Miller, D.W. Dickson, K.B. Boylan, N.R. Graff-Radford, R. Rademakers, Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS, Neuron. 72 (2011) 245–256, https://doi.org/10.1016/j.neuron.2011.09.011.

[9] A.E. Renton, E. Majounie, A. Waite, J. Simón-sánchez, S. Rollinson, J.R. Gibbs, J. C. Schymick, H. Laaksovirta, J.C. Van Swieten, A. Kaganovich, S.W. Scholz, J. Duckworth, J. Ding, D. Trabzuni, R.J. Guerreiro, R.W. Orrell, J. Neal, A. Murray, N. Halliwell, J. Callister, G. Toulson, A. Richardson, A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD, Neuron. 72 (2011) 257–268, https://doi.org/10.1016/j.neuron.2011.09.010.A.

[10] M. Neumann, D.M. Sampathu, L.K. Kwong, A.C. Truax, M.C. Micsenyi, T.T. Chou, J. Bruce, T. Schuck, M. Grossman, C.M. Clark, L.F. McCluskey, B.L. Miller, E. Masliah, I.R. Mackenzie, H. Feldman, W. Feiden, H.A. Kretzschmar, J. Q. Trojanowski, V.M.Y. Lee, Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis, Science 314 (2006) 130–133, https://doi.org/10.1126/science.1134108.

[11] G. Sala, A. Arosio, E. Conti, S. Beretta, C. Lunetta, N. Riva, C. Ferrarese, L. Tremolizzo, Riluzole selective antioxidant effects in cell models expressing amyotrophic lateral sclerosis endophenotypes, Clin. Psychopharmacol. Neurosci. 17 (2019) 438–442, https://doi.org/10.9758/cpn.2019.17.3.438.

[12] M.E. Gurney, H. Pu, A.Y. Chiu, M.C. Dal Canto, C.Y. Polchow, D.D. Alexander, J. Caliendo, A. Hentati, Y.W. Kwon, H.X. Deng, W. Chen, P. Zhai, R.L. Sufit, T. Siddique, Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation, Science 264 (1994) 1772–1775, https://doi.org/10.1126/science.8209258.

[13] M.E. Ripps, G.W. Huntley, P.R. Hof, J.H. Morrison, J.W. Gordon, Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. USA 92 (1995) 689–693, https://doi.org/10.1073/pnas.92.3.689.

[14] P.C. Wong, C.A. Pardo, D.R. Borchelt, M.K. Lee, N.G. Copeland, N.A. Jenkins, S. S. Sisodia, D.W. Cleveland, D.L. Price, An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria, Neuron. 14 (1995) 1105–1116, https://doi.org/10.1016/0896-6273(95)90259-7.

[15] L.I. Bruijn, M.W. Becher, M.K. Lee, K.L. Anderson, N.A. Jenkins, N.G. Copeland, S. S. Sisodia, J.D. Rothstein, D.R. Borchelt, D.L. Price, D.W. Cleveland, ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions, Neuron. 18 (1997) 327–338, https://doi.org/10.1016/S0896-6273(00)80272-X.

[16] C. Lutz, Mouse models of ALS: Past, present and future, Brain Res. 1693 (2018) 1–10, https://doi.org/10.1016/j.brainres.2018.03.024.

[17] J. Stephenson, S. Amor, Modelling amyotrophic lateral sclerosis in mice, Drug Discov. Today Dis. Model. 25–26 (2017) 35–44, https://doi.org/10.1016/j.ddmod.2018.10.001.

[18] Y.F. Xu, Y.J. Zhang, W.L. Lin, X. Cao, C. Stetler, D.W. Dickson, J. Lewis, L. Petrucelli, Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice, Mol. Neurodegener. 6 (2011) 73, https://doi.org/10.1186/1750-1326-6-73.

[19] N.R. Stallings, K. Puttaparthi, C.M. Luther, D.K. Burns, J.L. Elliott, Progressive motor weakness in transgenic mice expressing human TDP-43, Neurobiol. Dis. 40 (2010) 404–414, https://doi.org/10.1016/j.nbd.2010.06.017.

[20] J.C. Mitchell, P. McGoldrick, C. Vance, T. Hortobagyi, J. Sreedharan, B. Rogelj, E. L. Tudor, B.N. Smith, C. Klasen, C.C.J. Miller, J.D. Cooper, L. Greensmith, C. E. Shaw, Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion, Acta Neuropathol. 125 (2013) 273–288, https://doi.org/10.1007/s00401-012-1043-z.

[21] L.S. Wu, W.C. Cheng, C.K.J. Shen, Targeted depletion of TDP-43 expression in the spinal cord motor neurons leads to the development of amyotrophic lateral sclerosis-like phenotypes in mice, J. Biol. Chem. 287 (2012) 27335–27344, https://doi.org/10.1074/jbc.M112.359000.

[22] Y. Iguchi, M. Katsuno, J.I. Niwa, S. Takagi, S. Ishigaki, K. Ikenaka, K. Kawai, H. Watanabe, K. Yamanaka, R. Takahashi, H. Misawa, S. Sasaki, F. Tanaka, G. Sobue, Loss of TDP-43 causes age-dependent progressive motor neuron degeneration, Brain. 136 (2013) 1371–1382, https://doi.org/10.1093/brain/awt029.

[23] C. Yang, H. Wang, T. Qiao, B. Yang, L. Aliaga, L. Qiu, W. Tan, J. Salameh, D. M. McKenna-Yasek, T. Smith, L. Peng, M.J. Moore, R.H. Brown, H. Cai, Z. Xu, Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis, Proc. Natl. Acad. Sci. USA 111 (2014), https://doi.org/10.1073/pnas.1322641111.

[24] Y. Kino, C. Washizu, M. Kurosawa, M. Yamada, H. Miyazaki, T. Akagi, T. Hashikawa, H. Doi, T. Takumi, G.G. Hicks, N. Hattori, T. Shimogori, N. Nukina, FUS/TLS deficiency causes behavioral and pathological abnormalities distinct from amyotrophic lateral sclerosis, Acta Neuropathol. Commun. 3 (2015) 24, https://doi.org/10.1186/s40478-015-0202-6.

[25] G.G. Hicks, N. Singh, A. Nashabi, S. Mai, G. Bozek, L. Klewes, D. Arapovic, E. K. White, M.J. Koury, E.M. Oltz, L. Van Kaer, H.E. Ruley, Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death, Nat. Genet. 24 (2000) 175–179, https://doi.org/10.1038/72842.

[26] M. van Blitterswijk, M. DeJesus-Hernandez, R. Rademakers, How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: Can we learn from other noncoding repeat expansion disorders? Curr. Opin. Neurol. 25 (2012) 689–700, https://doi.org/10.1097/WCO.0b013e32835a3efb.

[27] J.G. O'Rourke, L. Bogdanik, A.K.M.G. Muhammad, T.F. Gendron, K.J. Kim, A. Austin, J. Cady, E.Y. Liu, J. Zarrow, S. Grant, R. Ho, S. Bell, S. Carmona, M. Simpkinson, D. Lall, K. Wu, L. Daughrity, D.W. Dickson, M.B. Harms, L. Petrucelli, E.B. Lee, C.M. Lutz, R.H. Baloh, C9orf72 BAC Transgenic Mice Display Typical Pathologic Features of ALS/FTD, Neuron. 88 (2015) 892–901, https://doi.org/10.1016/j.neuron.2015.10.027.

[28] O.M. Peters, G.T. Cabrera, H. Tran, T.F. Gendron, J.E. McKeon, J. Metterville, A. Weiss, N. Wightman, J. Salameh, J. Kim, H. Sun, K.B. Boylan, D. Dickson, Z. Kennedy, Z. Lin, Y.-J. Zhang, L. Daughrity, C. Jung, F.-B. Gao, P.C. Sapp, H. R. Horvitz, D.A. Bosco, S.P. Brown, P. de Jong, L. Petrucelli, C. Mueller, R. H. Brown, Human C9ORF72 Hexanucleotide Expansion Reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC Transgenic Mice, Neuron. 88 (2015) 902–909, https://doi.org/10.1016/j.neuron.2015.11.018.

[29] Y. Liu, A. Pattamatta, T. Zu, T. Reid, O. Bardhi, D.R. Borchelt, A.T. Yachnis, L.P. W. Ranum, C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD, Neuron. 90 (2016) 521–534, https://doi.org/10.1016/j.neuron.2016.04.005.

[30] J. Chew, T.F. Gendron, M. Prudencio, H. Sasaguri, Y.J. Zhang, M. Castanedes-Casey, C.W. Lee, K. Jansen-West, A. Kurti, M.E. Murray, K.F. Bieniek, P.O. Bauer, E.C. Whitelaw, L. Rousseau, J.N. Stankowski, C. Stetler, L.M. Daughrity, E. A. Perkerson, P. Desaro, A. Johnston, K. Overstreet, D. Edbauer, R. Rademakers, K.B. Boylan, D.W. Dickson, J.D. Fryer, L. Petrucelli, C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits, Science 348 (2015) 1151–1154, https://doi.org/10.1126/science.aaa9344.

[31] D.A. Mordes, B.M. Morrison, X.H. Ament, C. Cantrell, J. Mok, P. Eggan, C. Xue, J. Y. Wang, K. Eggan, J.D. Rothstein, Absence of survival and motor deficits in 500 Repeat C9ORF72 BAC Mice, Neuron. 108 (2020) 775–783.e4, https://doi.org/10.1016/j.neuron.2020.08.009.

[32] M. Therrien, J.A. Parker, Worming forward: Amyotrophic lateral sclerosis toxicity mechanisms and genetic interactions in Caenorhabditis elegans, Front. Genet. 5 (2014) 85, https://doi.org/10.3389/fgene.2014.00085.

[33] J. Li, W. Le, Modeling neurodegenerative diseases in Caenorhabditis elegans, Exp. Neurol. 250 (2013) 94–103, https://doi.org/10.1016/j.expneurol.2013.09.024.

[34] J. Apfeld, S. Alper, What Can We Learn About Human Disease from the Nematode C. elegans? Methods Mol. Biol. 1706 (2018) 53–75, https://doi.org/10.1007/978-1-4939-7471-9_4.

[35] M. Markaki, N. Tavernarakis, Modeling human diseases in Caenorhabditis elegans, Biotechnol. J. 5 (2010) 1261–1276, https://doi.org/10.1002/biot.201000183.

[36] X. Chen, X. Feng, S. Guang, Targeted genome engineering in Caenorhabditis elegans, Cell Biosci. 6 (2016) 60, https://doi.org/10.1186/s13578-016-0125-3.

[37] T. Boulin, O. Hobert, From genes to function: The C. elegans genetic toolbox, Wiley Interdiscip, Rev. Dev. Biol. 1 (2012) 114–137, https://doi.org/10.1002/wdev.1.

[38] M. Therrien, J.A. Parker, Deciphering genetic interactions between ALS genes using C. elegans, Worm. 3 (2014), e29047, https://doi.org/10.4161/worm.29047.

[39] S.D. Buckingham, D.B. Sattelle, Fast, automated measurement of nematode swimming (thrashing) without morphometry, BMC Neurosci. 10 (2009) 84, https://doi.org/10.1186/1471-2202-10-84.

[40] E.M. Jorgensen, GABA. WormBook, 2005, pp. 1–13, https://doi.org/10.1895/wormbook.1.14.1.

[41] Y. Zheng, P.J. Brockie, J.E. Mellem, D.M. Madsen, A.V. Maricq, Neuronal Control of Locomotion in C. elegans Is Modified by a Dominant Mutation in the GLR-1 Ionotropic Glutamate Receptor, Neuron. 24 (1999) 347–361, https://doi.org/10.1016/S0896-6273(00)80849-1.

[42] E. Braems, B. Swinnen, L. Van Den Bosch, C9orf72 loss-of-function: A trivial, stand-alone or additive mechanism in C9 ALS/FTD? Acta Neuropathol. 140 (2020) 625–643, https://doi.org/10.1007/s00401-020-02214-x.

[43] V. Kumar, G.M. Hasan, M.I. Hassan, Unraveling the Role of RNA Mediated Toxicity of C9orf72 Repeats in C9-FTD/ALS, Front. Neurosci. 11 (2017) 711, https://doi.org/10.3389/fnins.2017.00711.

[44] B. Swinnen, W. Robberecht, L. Van Den Bosch, RNA toxicity in non-coding repeat expansion disorders, EMBO J. 39 (2020), e101112, https://doi.org/10.15252/embj.2018101112.

[45] Z.T. McEachin, J. Parameswaran, N. Raj, G.J. Bassell, J. Jiang, RNA-mediated toxicity in C9orf72 ALS and FTD, Neurobiol. Dis. 145 (2020), 105055, https://doi.org/10.1016/j.nbd.2020.105055.

[46] B.D. Freibaum, J.P. Taylor, The Role of Dipeptide Repeats in C9ORF72-Related ALS-FTD, Front. Mol. Neurosci. 10 (2017) 35, https://doi.org/10.3389/fnmol.2017.00035.

[47] M. Therrien, G.A. Rouleau, P.A. Dion, J.A. Parker, Deletion of C9ORF72 Results in motor neuron degeneration and stress sensitivity in C. elegans, PLoS One. 8 (2013), e83450, https://doi.org/10.1371/journal.pone.0083450.

[48] A. Corrionero, H.R. Horvitz, A C9orf72 ALS/FTD ortholog acts in endolysosomal degradation and lysosomal homeostasis, Curr. Biol. 28 (2018) 1522–1535, https://doi.org/10.1016/j.cub.2018.03.063.

[49] M.A. Farg, V. Sundaramoorthy, J.M. Sultana, S. Yang, R.A.K. Atkinson, V. Levina, M.A. Halloran, P.A. Gleeson, I.P. Blair, K.Y. Soo, A.E. King, J.D. Atkin, C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking, Hum. Mol. Genet. 23 (2014) 3579–3595, https://doi.org/10.1093/hmg/ddu068.

[50] J. Amick, A. Roczniak-Ferguson, S.M. Ferguson, C9orf72 binds SMCR8, localizes to lysosomes, and regulates mTORC1 signaling, Mol. Biol. Cell. 27 (2016) 3040–3051, https://doi.org/10.1091/mbc.e16-01-0003.

[51] Y.J. Ji, J. Ugolino, T. Zhang, J. Lu, D. Kim, J. Wang, C9orf72/ALFA-1 controls TFEB/HLH-30-dependent metabolism through dynamic regulation of Rag GTPases, PLOS Genet. 16 (2020), e1008738, https://doi.org/10.1371/journal.pgen.1008738.

[52] X. Wang, L. Hao, T. Saur, K. Joyal, Y. Zhao, D. Zhai, J. Li, M. Pribadi, G. Coppola, B.M. Cohen, E.A. Buttner, Forward Genetic Screen in Caenorhabditis elegans Suggests F57A10.2 and acp-4 As Suppressors of C9ORF72 Related Phenotypes, Front. Mol. Neurosci. 9 (2016) 113, https://doi.org/10.3389/fnmol.2016.00113.

[53] P. Rudich, C. Snoznik, S.C. Watkins, J. Monaghan, U.B. Pandey, S.T. Lamitina, Nuclear localized C9orf72-associated arginine-containing dipeptides exhibit age-dependent toxicity in C. elegans, Hum. Mol. Genet. 26 (2017) 4916–4928, https://doi.org/10.1093/hmg/ddx372.

[54] M. Sanhueza, A. Chai, C. Smith, B.A. McCray, T.I. Simpson, J.P. Taylor, G. Pennetta, Network Analyses Reveal Novel Aspects of ALS Pathogenesis, PLOS Genet. 11 (2015), e1005107, https://doi.org/10.1371/journal.pgen.1005107.

[55] T.P. Levine, R.D. Daniels, A.T. Gatta, L.H. Wong, M.J. Hayes, The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs, Bioinformatics. 29 (2013) 499–503, https://doi.org/10.1093/bioinformatics/bts725.

[56] B.L. Tang, C9orf72's Interaction with Rab GTPases—Modulation of Membrane Traffic and Autophagy, Front. Cell. Neurosci. 10 (2016) 228, https://doi.org/10.3389/fncel.2016.00228.

[57] C.P. Webster, E.F. Smith, A.J. Grierson, K.J. De Vos, C9orf72 plays a central role in Rab GTPase-dependent regulation of autophagy, Small GTPases. 9 (2018) 399–408, https://doi.org/10.1080/21541248.2016.1240495.

[58] H.X. Deng, A. Hentati, J.A. Tainer, Z. Iqbal, A. Cayabyab, W.Y. Hung, E. D. Getzoff, P. Hu, B. Herzfeldt, R.P. Roos, C. Warner, G. Deng, E. Soriano, C. Smyth, H.E. Parge, A. Ahmed, A.D. Roses, R.A. Hallewell, M.A. Pericak-Vance, T. Siddique, Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase, Science 261 (1993) 1047–1051, https://doi.org/10.1126/science.8351519.

[59] D.R. Rosen, T. Siddique, D. Patterson, D.A. Figlewicz, P. Sapp, A. Hentati, D. Donaldson, J. Goto, J.P. O'Regan, H.-X. Deng, Z. Rahmani, A. Krizus, D. McKenna-Yasek, A. Cayabyab, S.M. Gaston, R. Berger, R.E. Tanzi, J. J. Halperin, B. Herzfeldt, R. Van den Bergh, W.-Y. Hung, T. Bird, G. Deng, D. W. Mulder, C. Smyth, N.G. Laing, E. Soriano, M.A. Pericak-Vance, J. Haines, G. A. Rouleau, J.S. Gusella, H.R. Horvitz, R.H. Brown, Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature. 362 (1993) 59–62, https://doi.org/10.1038/362059a0.

[60] J. Li, T. Li, X. Zhang, Y. Tang, J. Yang, W. Le, Human superoxide dismutase 1 overexpression in motor neurons of Caenorhabditis elegans causes axon guidance defect and neurodegeneration, Neurobiol. Aging. 35 (2014) 837–846, https://doi.org/10.1016/j.neurobiolaging.2013.09.003.

[61] S.N. Baskoylu, J. Yersak, P. O'Hern, S. Grosser, J. Simon, S. Kim, K. Schuch, M. Dimitriadi, K.S. Yanagi, J. Lins, A.C. Hart, Single copy/knock-in models of ALS SOD1 in C. elegans suggest loss and gain of function have different contributions to cholinergic and glutamatergic neurodegeneration, PLOS Genet. 14 (2018), e1007682, https://doi.org/10.1371/journal.pgen.1007682.

[62] J. Wang, G.W. Farr, D.H. Hall, F. Li, K. Furtak, L. Dreier, A.L. Horwich, An ALS-Linked Mutant SOD1 Produces a Locomotor Defect Associated with Aggregation and Synaptic Dysfunction When Expressed in Neurons of Caenorhabditis elegans, PLoS Genet. 5 (2009), e1000350, https://doi.org/10.1371/journal.pgen.1000350.

[63] T. Gidalevitz, T. Krupinski, S. Garcia, R.I. Morimoto, Destabilizing protein polymorphisms in the genetic background direct phenotypic expression of mutant SOD1 Toxicity, PLoS Genet. 5 (2009), e1000399, https://doi.org/10.1371/journal.pgen.1000399.

[64] M. Boccitto, T. Lamitina, R.G. Kalb, Daf-2 Signaling Modifies Mutant SOD1 Toxicity in C. elegans, PLoS One. 7 (2012), e33494, https://doi.org/10.1371/journal.pone.0033494.

[65] M.S. Gami, C.A. Wolkow, Studies of Caenorhabditis elegans DAF-2/insulin signaling reveal targets for pharmacological manipulation of lifespan, Aging Cell. 5 (2006) 31–37, https://doi.org/10.1111/j.1474-9726.2006.00188.x.

[66] A.M. Jablonski, T. Lamitina, N.F. Liachko, M. Sabatella, J. Lu, L. Zhang, L. W. Ostrow, P. Gupta, C.-Y. Wu, S. Doshi, J. Mojsilovic-Petrovic, H. Lans, J. Wang, B. Kraemer, R.G. Kalb, Loss of RAD-23 protects against models of motor neuron disease by enhancing mutant protein clearance, J. Neurosci. 35 (2015) 14286–14306, https://doi.org/10.1523/JNEUROSCI.0642-15.2015.

[67] M. Cornaglia, G. Krishnamani, L. Mouchiroud, V. Sorrentino, T. Lehnert, J. Auwerx, M.A.M. Gijs, Automated longitudinal monitoring of in vivo protein aggregation in neurodegenerative disease C. elegans models, Mol. Neurodegener. 11 (2016) 17, https://doi.org/10.1186/s13024-016-0083-6.

[68] A.M. Blokhuis, E.J.N. Groen, M. Koppers, L.H. van den Berg, R.J. Pasterkamp, Protein aggregation in amyotrophic lateral sclerosis, Acta Neuropathol. 125 (2013) 777–794, https://doi.org/10.1007/s00401-013-1125-6.

[69] T. Zhang, H.-Y. Hwang, H. Hao, C. Talbot, J. Wang, Caenorhabditis elegans RNA-processing Protein TDP-1 Regulates Protein Homeostasis and Life Span, J. Biol. Chem. 287 (2012) 8371–8382, https://doi.org/10.1074/jbc.M111.311977.

[70] N.F. Liachko, C.R. Guthrie, B.C. Kraemer, Phosphorylation Promotes Neurotoxicity in a Caenorhabditis elegans Model of TDP-43 Proteinopathy, J. Neurosci. 30 (2010) 16208–16219, https://doi.org/10.1523/JNEUROSCI.2911-10.2010.

[71] D. Aggad, J. Veriepe, A. Tauffenberger, J.A. Parker, TDP-43 toxicity proceeds via calcium dysregulation and necrosis in aging caenorhabditis elegans motor neurons, J. Neurosci. 34 (2014) 12093–12103, https://doi.org/10.1523/JNEUROSCI.2495-13.2014.

[72] A. Vaccaro, A. Tauffenberger, P.E.A. Ash, Y. Carlomagno, L. Petrucelli, J. A. Parker, TDP-1/TDP-43 Regulates Stress Signaling and Age-Dependent Proteotoxicity in Caenorhabditis elegans, PLoS Genet. 8 (2012), e1002806, https://doi.org/10.1371/journal.pgen.1002806.

[73] T. Zhang, P.C. Mullane, G. Periz, J. Wang, TDP-43 neurotoxicity and protein aggregation modulated by heat shock factor and insulin/IGF-1 signaling, Hum. Mol. Genet. 20 (2011) 1952–1965, https://doi.org/10.1093/hmg/ddr076.

[74] A. Vaccaro, A. Tauffenberger, D. Aggad, G. Rouleau, P. Drapeau, J.A. Parker, Mutant TDP-43 and FUS Cause Age-Dependent Paralysis and Neurodegeneration in C. elegans, PLoS One. 7 (2012), e31321, https://doi.org/10.1371/journal.pone.0031321.

[75] S.A. Patten, D. Aggad, J. Martinez, E. Tremblay, J. Petrillo, G.A. Armstrong, A. La Fontaine, C. Maios, M. Liao, S. Ciura, X.-Y. Wen, V. Rafuse, J. Ichida, L. Zinman, J.-P. Julien, E. Kabashi, R. Robitaille, L. Korngut, J.A. Parker, P. Drapeau, Neuroleptics as therapeutic compounds stabilizing neuromuscular transmission in amyotrophic lateral sclerosis, JCI Insight. 2 (2017), e97152, https://doi.org/10.1172/jci.insight.97152.

[76] S.Q. Wong, M.G. Pontifex, M.M. Phelan, C. Pidathala, B.C. Kraemer, J.W. Barclay, N.G. Berry, P.M. O'Neill, R.D. Burgoyne, A. Morgan, α-Methyl-α-phenylsuccinimide ameliorates neurodegeneration in a C. elegans model of TDP-43 proteinopathy, Neurobiol. Dis. 118 (2018) 40–54, https://doi.org/10.1016/j.nbd.2018.06.013.

[77] L. Ma, Y. Zhao, Y. Chen, B. Cheng, A. Peng, K. Huang, Caenorhabditis elegans as a model system for target identification and drug screening against neurodegenerative diseases, Eur. J. Pharmacol. 819 (2018) 169–180, https://doi.org/10.1016/j.ejphar.2017.11.051.

[78] S. Markert, M. Skoruppa, B. Yu, B. Mulcahy, M. Zhen, S. Gao, M. Sendtner, C. Stigloher, An ALS-associated mutation in human FUS reduces neurotransmission from C. elegans motor neurons to muscles, BioRxiv. (2019), 860536, https://doi.org/10.1101/860536.

[79] T. Murakami, S.-P. Yang, L. Xie, T. Kawano, D. Fu, A. Mukai, C. Bohm, F. Chen, J. Robertson, H. Suzuki, G.G. Tartaglia, M. Vendruscolo, G.S. Kaminski Schierle, F.T.S. Chan, A. Moloney, D. Crowther, C.F. Kaminski, M. Zhen, St George-Hyslop P., ALS mutations in FUS cause neuronal dysfunction and death in Caenorhabditis elegans by a dominant gain-of-function mechanism, Hum. Mol. Genet. 21 (2012) 1–9, https://doi.org/10.1093/hmg/ddr417.

[80] T. Murakami, S. Qamar, J.Q. Lin, G.S.K. Schierle, E. Rees, A. Miyashita, A. R. Costa, R.B. Dodd, F.T.S. Chan, C.H. Michel, D. Kronenberg-Versteeg, Y. Li, S.-P. Yang, Y. Wakutani, W. Meadows, R.R. Ferry, L. Dong, G.G. Tartaglia, G. Favrin, W.-L. Lin, D.W. Dickson, M. Zhen, D. Ron, G. Schmitt-Ulms, P.E. Fraser, N. A. Shneider, C. Holt, M. Vendruscolo, C.F. Kaminski, P. St George-Hyslop, ALS/FTD Mutation-Induced Phase Transition of FUS Liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP Granule Function, Neuron. 88 (2015) 678–690, https://doi.org/10.1016/j.neuron.2015.10.030.

[81] T. Zhang, Y.-C. Wu, P. Mullane, Y.J. Ji, H. Liu, L. He, A. Arora, H.-Y. Hwang, A. F. Alessi, A.G. Niaki, G. Periz, L. Guo, H. Wang, E. Elkayam, L. Joshua-Tor, S. Myong, J.K. Kim, J. Shorter, S.-E. Ong, A.K.L. Leung, J. Wang, FUS Regulates Activity of MicroRNA-Mediated Gene Silencing, Mol. Cell. 69 (2018) 787–801, https://doi.org/10.1016/j.molcel.2018.02.001.

[82] A. Vaccaro, S.A. Patten, S. Ciura, C. Maios, M. Therrien, P. Drapeau, E. Kabashi, J. A. Parker, Methylene Blue Protects against TDP-43 and FUS Neuronal Toxicity in C. elegans and D. rerio, PLoS One 7 (2012), e42117, https://doi.org/10.1371/journal.pone.0042117.

[83] M. Yamashita, T. Nonaka, T. Arai, F. Kametani, V.L. Buchman, N. Ninkina, S. O. Bachurin, H. Akiyama, M. Goedert, M. Hasegawa, Methylene blue and dimebon inhibit aggregation of TDP-43 in cellular models, FEBS Lett. 583 (2009) 2419–2424, https://doi.org/10.1016/j.febslet.2009.06.042.

[84] J.O. Johnson S.M. Glynn J.R. Gibbs M.A. Nalls M. Sabatelli G. Restagno V.E. Drory A. Chiò E. Rogaeva B.J. Traynor Mutations in the CHCHD10 gene are a common cause of familial amyotrophic lateral sclerosis Brain. 137 2014 e311 e311 10.1093/brain/awu265.

[85] G.H.P. Tazelaar, W. van Rheenen, S.L. Pulit, R.A.A. van der Spek, A.M. Dekker, M. Moisse, R.L. McLaughlin, W. Sproviero, K.P. Kenna, M. Kooyman, P.T.C. van Doormaal, K.E. van Eijk, B.M. Middelkoop, R.D. Schellevis, W.J. Brands, A. Al-Chalabi, K.E. Morrison, P.J. Shaw, C.E. Shaw, S.E. Newhouse, M.A. van Es, A. N. Basak, F. Akçimen, C. Kocoglu, C. Tunca, M. Povedano, J.S. Mora, J.D. Glass, P. Van Damme, W. Robberecht, O. Hardiman, J.E. Landers, L.H. van den Berg, J. H. Veldink, CHCHD10 variants in amyotrophic lateral sclerosis: Where is the evidence? Ann. Neurol. 84 (2018) 110–116, https://doi.org/10.1002/ana.25273.

[86] L. Fumagalli, F.L. Young, S. Boeynaems, M. De Decker, A. Mehta, A. Swijsen, R. Fazal, W. Guo, M. Moisse, J. Beckers, L. Dedeene, T.S. Bhuvaneish, T. Vandoorne, V. Madan, M. van Blitterswijk, D. Raitcheva, A. McCampbell, K. Poesen, A.D. Gitler, P. Koch, P. Vanden Berghe, D.R. Thal, C. Verfaillie, S. Chandran, L. Van Den Bosch, S. Bullock, P. Van Damme, C9orf72-derived arginine-containing dipeptide repeats associate with axonal transport machinery and impede microtubule-based motility, Sci. Adv. 7 (2021), https://doi.org/10.1126/sciadv.abg3013.

[87] S. Bannwarth, S. Ait-El-Mkadem, A. Chaussenot, E.C. Genin, S. Lacas-Gervais, K. Fragaki, L. Berg-Alonso, Y. Kageyama, V. Serre, D.G. Moore, A. Verschueren, C. Rouzier, I. Le Ber, G. Augé, C. Cochaud, F. Lespinasse, K. N'Guyen, A. de Septenville, A. Brice, P. Yu-Wai-Man, H. Sesaki, J. Pouget, V. Paquis-Flucklinger, A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement, Brain. 137 (2014) 2329–2345, https://doi.org/10.1093/brain/awu138.

[88] K. Ikenaka, K. Kawai, M. Katsuno, Z. Huang, Y.-M. Jiang, Y. Iguchi, K. Kobayashi, T. Kimata, M. Waza, F. Tanaka, I. Mori, G. Sobue, dnc-1/dynactin 1 Knockdown Disrupts Transport of Autophagosomes and Induces Motor Neuron Degeneration, PLoS One. 8 (2013), e54511, https://doi.org/10.1371/journal.pone.0054511.

[89] V. Bercier, J.M. Hubbard, K. Fidelin, K. Duroure, T.O. Auer, C. Revenu, C. Wyart, F. Del Bene, Dynactin1 depletion leads to neuromuscular synapse instability and functional abnormalities, Mol. Neurodegener. 14 (2019) 27, https://doi.org/10.1186/s13024-019-0327-3.

[90] J.A.A. Woo, T. Liu, C. Trotter, C.C. Fang, E. De Narvaez, P. Lepochat, D. Maslar, A. Bukhari, X. Zhao, A. Deonarine, S.D. Westerheide, D.E. Kang, Loss of function CHCHD10 mutations in cytoplasmic TDP-43 accumulation and synaptic integrity, Nat. Commun. 8 (2017) 15558, https://doi.org/10.1038/ncomms15558.

[91] J. Vériène, L. Fossouo, J.A. Parker, Neurodegeneration in C. elegans models of ALS requires TIR-1/Sarm1 immune pathway activation in neurons, Nat. Commun. 6 (2015) 7319, https://doi.org/10.1038/ncomms8319.

[92] F.P. Diekstra, P.W.J. van Vught, W. van Rheenen, M. Koppers, R.J. Pasterkamp, M.A. van Es, H.J. Schelhaas, M. de Visser, W. Robberecht, P. Van Damme, P. M. Andersen, L.H. van den Berg, J.H. Veldink, UNC13A is a modifier of survival in amyotrophic lateral sclerosis, Neurobiol. Aging. 33 (2012) e3–e8, https://doi.org/10.1016/j.neurobiolaging.2011.10.029.

[93] A.E. Sturtevant, E.B. Lewis. History of genetics, Cold Spring Harbor Laboratory Press, 2001.

[94] M.D.W. Piper, D. Skorupa, L. Partridge, Diet, metabolism and lifespan in Drosophila, Exp. Gerontol. 40 (2005) 857–862, https://doi.org/10.1016/j.exger.2005.06.013.

[95] F. Hirth, Drosophila melanogaster in the study of human neurodegeneration, CNS Neurol. Disord. Drug Targets. 9 (2010) 504–523, https://doi.org/10.2174/187152710791556104.

[96] L.T. Reiter, L. Potocki, S. Chien, M. Gribskov, E. Bier, A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster, Genome Res. 11 (2001) 1114–1125, https://doi.org/10.1101/gr.169101.

[97] G.M. Rubin, M.D. Yandell, J.R. Wortman, G.L. Gabor Miklos, C.R. Nelson, I. K. Hariharan, M.E. Fortini, P.W. Li, R. Apweiler, W. Fleischmann, J.M. Cherry, S. Henikoff, M.P. Skupski, S. Misra, M. Ashburner, E. Birney, M.S. Boguski, T. Brody, P. Brokstein, S.E. Celniker, S.A. Chervitz, D. Coates, A. Cravchik, A. Gabrielian, R.F. Galle, W.M. Gelbart, R.A. George, L.S.B. Goldstein, F. Gong, P. Guan, N.L. Harris, B.A. Hay, R.A. Hoskins, J. Li, Z. Li, R.O. Hynes, S.J.M. Jones, P.M. Kuehl, B. Lemaitre, J.T. Littleton, D.K. Morrison, C. Mungall, P.H. O'Farrell, O.K. Pickeral, C. Shue, L.B. Vosshall, J. Zhang, Q. Zhao, X.H. Zheng, F. Zhong, W. Zhong, R. Gibbs, J.C. Venter, M.D. Adams, S. Lewis, Comparative genomics of the eukaryotes, Science 287 (2000) 2204–2215, https://doi.org/10.1126/science.287.5461.2204.

[98] B. Ugur, K. Chen, H.J. Bellen, Drosophila tools and assays for the study of human diseases, Dis. Model. Mech. 9 (2016) 235–244, https://doi.org/10.1242/dmm.023762.

[99] A. Berson, L.D. Goodman, A.N. Sartoris, C.G. Otte, J.A. Aykit, V.M.-Y. Lee, J. Q. Trojanowski, N.M. Bonini, Drosophila Ref1/ALYREF regulates transcription and toxicity associated with ALS/FTD disease etiologies, Acta Neuropathol. Commun. 7 (2019) 65, https://doi.org/10.1186/s40478-019-0710-x.

[100] D.A. Solomon, A. Stepto, W.H. Au, Y. Adachi, D.C. Diaper, R. Hall, A. Rekhi, A. Boudi, P. Tziortzouda, Y.-B. Lee, B. Smith, J.C. Bridi, G. Spinelli, J. Dearlove, D. M. Humphrey, J.-M. Gallo, C. Troakes, M. Fanto, M. Soller, B. Rogelj, R. B. Parsons, C.E. Shaw, T. Hortobágyi, F. Hirth, A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin-α mediates C9orf72-related

neurodegeneration, Brain 141 (2018) 2908–2924, https://doi.org/10.1093/brain/awy241.
[101] H.M. McKinney, L.M. Sherer, J.L. Williams, S.J. Certel, R.S. Stowers, Characterization of Drosophila octopamine receptor neuronal expression using MiMIC-converted Gal4 lines, J. Comp. Neurol. 528 (2020) 2174–2194, https://doi.org/10.1002/cne.24883.
[102] Y. Yuva-Aydemir, S. Almeida, G. Krishnan, T.F. Gendron, F.-B. Gao, Transcription elongation factor AFF2/FMR2 regulates expression of expanded GGGGCC repeat-containing C9ORF72 allele in ALS/FTD, Nat. Commun. 10 (2019) 5466, https://doi.org/10.1038/s41467-019-13477-8.
[103] W. Xu, J. Xu, C9orf72 dipeptide repeats cause selective neurodegeneration and cell-autonomous excitotoxicity in drosophila glutamatergic neurons, J. Neurosci. 38 (2018) 7741–7752, https://doi.org/10.1523/JNEUROSCI.0908-18.2018.
[104] R.J.H. West, J.L. Sharpe, A. Voelzmann, A.L. Munro, I. Hahn, R.A. Baines, S. Pickering-Brown, Co-expression of C9orf72 related dipeptide-repeats over 1000 repeat units reveals age- and combination-specific phenotypic profiles in Drosophila, Acta Neuropathol. Commun. 8 (2020) 158, https://doi.org/10.1186/s40478-020-01028-y.
[105] Z. Xu, M. Poidevin, X. Li, Y. Li, L. Shu, D.L. Nelson, H. Li, C.M. Hales, M. Gearing, T.S. Wingo, P. Jin, Expanded GGGGCC repeat RNA associated with amyotrophic lateral sclerosis and frontotemporal dementia causes neurodegeneration, Proc. Natl. Acad. Sci. 110 (2013) 7778–7783, https://doi.org/10.1073/pnas.1219643110.
[106] S. Mizielinska, S. Gronke, T. Niccoli, C.E. Ridler, E.L. Clayton, A. Devoy, T. Moens, F.E. Norona, I.O.C. Woollacott, J. Pietrzyk, K. Cleverley, A.J. Nicoll, S. Pickering-Brown, J. Dols, M. Cabecinha, O. Hendrich, P. Fratta, E.M.C. Fisher, L. Partridge, A.M. Isaacs, C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins, Science 345 (2014) 1192–1194, https://doi.org/10.1126/science.1256800.
[107] B.D. Freibaum, Y. Lu, R. Lopez-Gonzalez, N.C. Kim, S. Almeida, K.-H. Lee, N. Badders, M. Valentine, B.L. Miller, P.C. Wong, L. Petrucelli, H.J. Kim, F.-B. Gao, J.P. Taylor, GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport, Nature. 525 (2015) 129–133, https://doi.org/10.1038/nature14974.
[108] K. Zhang, C.J. Donnelly, A.R. Haeusler, J.C. Grima, J.B. Machamer, P. Steinwald, E.L. Daley, S.J. Miller, K.M. Cunningham, S. Vidensky, S. Gupta, M.A. Thomas, I. Hong, S.-L. Chiu, R.L. Huganir, L.W. Ostrow, M.J. Matunis, J. Wang, R. Sattler, T.E. Lloyd, J.D. Rothstein, The C9orf72 repeat expansion disrupts nucleocytoplasmic transport, Nature. 525 (2015) 56–61, https://doi.org/10.1038/nature14973.
[109] K.Y. Shi, E. Mori, Z.F. Nizami, Y. Lin, M. Kato, S. Xiang, L.C. Wu, M. Ding, Y. Yu, J. G. Gall, S.L. McKnight, Toxic PR n poly-dipeptides encoded by the C9orf72 repeat expansion block nuclear import and export, Proc. Natl. Acad. Sci. 114 (2017) e1111–e1117, https://doi.org/10.1073/pnas.1620293114.
[110] S. Boeynaems, E. Bogaert, E. Michiels, I. Gijselinck, A. Sieben, A. Jovičić, G. De Baets, W. Scheveneels, J. Steyaert, I. Cuijt, K.J. Verstrepen, P. Callaerts, F. Rousseau, J. Schymkowitz, M. Cruts, C. Van Broeckhoven, P. Van Damme, A. D. Gitler, W. Robberecht, L. Van Den Bosch, Drosophila screen connects nuclear transport genes to DPR pathology in c9ALS/FTD, Sci. Rep. 6 (2016) 20877, https://doi.org/10.1038/srep20877.
[111] C.-C. Chou, Y. Zhang, M.E. Umoh, S.W. Vaughan, I. Lorenzini, F. Liu, M. Sayegh, P.G. Donlin-Asp, Y.H. Chen, D.M. Duong, N.T. Seyfried, M.A. Powers, T. Kukar, C. M. Hales, M. Gearing, N.J. Cairns, K.B. Boylan, D.W. Dickson, R. Rademakers, Y.-J. Zhang, L. Petrucelli, R. Sattler, D.C. Zarnescu, J.D. Glass, W. Rossoll, TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD, Nat. Neurosci. 21 (2018) 228–239, https://doi.org/10.1038/s41593-017-0047-3.
[112] X. Wen, P. An, H. Li, Z. Zhou, Y. Sun, J. Wang, L. Ma, B. Lu, Tau Accumulation via Reduced Autophagy Mediates GGGGCC Repeat Expansion-Induced Neurodegeneration in Drosophila Model of ALS, Neurosci. Bull. (2020), https://doi.org/10.1007/s12264-020-00518-2.
[113] G.H.P. Tazelaar, S. Boeynaems, M. De Decker, J.J.F.A. van Vugt, L. Kool, H. S. Goedee, R.L. McLaughlin, W. Sproviero, A. Iacoangeli, M. Moisse, M. Jacquemyn, D. Daelemans, A.M. Dekker, R.A. van der Spek, H.-J. Westeneng, K.P. Kenna, A. Assialioui, N. Da Silva, P.M.A.S. Consortium, M. Povedano, J.S. M. Pardina, O. Hardiman, F. Salachas, S. Millecamps, P. Vourc'h, P. Corcia, P. Couratier, K.E. Morrison, P.J. Shaw, C.E. Shaw, R.J. Pasterkamp, J.E. Landers, L. Van Den Bosch, W. Robberecht, A. Al-Chalabi, L.H. van den Berg, P. Van Damme, J.H. Veldink, M.A. van Es, ATXN1 repeat expansions confer risk for amyotrophic lateral sclerosis and contribute to TDP-43 mislocalization, Brain Commun. 2 (2020), https://doi.org/10.1093/braincomms/fcaa064.
[114] J.H. Park, C.G. Chung, J. Seo, B.-H. Lee, Y.-S. Lee, J.H. Kweon, S.B. Lee, C9orf72-associated arginine-rich dipeptide repeat proteins reduce the number of golgi outposts and dendritic branches in drosophila neurons, Mol. Cells. 43 (2020) 821–830, https://doi.org/10.14348/molcells.2020.0130.
[115] T.G. Moens, T. Niccoli, K.M. Wilson, M.L. Atilano, N. Birsa, L.M. Gittings, B. V. Holbling, M.C. Dyson, A. Thoeng, J. Neeves, I. Glaria, L. Yu, J. Bussmann, E. Storkebaum, M. Pardo, J.S. Choudhary, P. Fratta, L. Partridge, A.M. Isaacs, C9orf72 arginine-rich dipeptide proteins interact with ribosomal proteins in vivo to induce a toxic translational arrest that is rescued by eIF1A, Acta Neuropathol. 137 (2019) 487–500, https://doi.org/10.1007/s00401-018-1946-4.
[116] J.P. Phillips, S.D. Campbell, D. Michaud, M. Charbonneau, A.J. Hilliker, Null mutation of copper/zinc superoxide dismutase in Drosophila confers hypersensitivity to paraquat and reduced longevity, Proc. Natl. Acad. Sci. 86 (1989) 2761–2765, https://doi.org/10.1073/pnas.86.8.2761.
[117] T.L. Parkes, A.J. Elia, D. Dickinson, A.J. Hilliker, J.P. Phillips, G.L. Boulianne, Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons, Nat. Genet. 19 (1998) 171–174, https://doi.org/10.1038/534.
[118] I. Martin, M.A. Jones, M. Grotewiel, Manipulation of Sod1 expression ubiquitously, but not in the nervous system or muscle, impacts age-related parameters in Drosophila, FEBS Lett. 583 (2009) 2308–2314, https://doi.org/10.1016/j.febslet.2009.06.023.
[119] M.R. Watson, R.D. Lagow, K. Xu, B. Zhang, N.M. Bonini, A drosophila model for amyotrophic lateral sclerosis reveals motor neuron damage by human SOD1, J. Biol. Chem. 283 (2008) 24972–24981, https://doi.org/10.1074/jbc.M804817200.
[120] A. Şahin, A. Held, K. Bredvik, P. Major, T.-M. Achilli, A.G. Kerson, K. Wharton, G. Stilwell, R. Reenan, Human SOD1 ALS Mutations in a drosophila knock-in model cause severe phenotypes and reveal dosage-sensitive gain- and loss-of-function components, Genetics. 205 (2017) 707–723, https://doi.org/10.1534/genetics.116.190850.
[121] L. Cragnaz, R. Klima, L. De Conti, G. Romano, F. Feiguin, E. Buratti, M. Baralle, F. E. Baralle, An age-related reduction of brain TBPH/TDP-43 levels precedes the onset of locomotion defects in a Drosophila ALS model, Neuroscience. 311 (2015) 415–421, https://doi.org/10.1016/j.neuroscience.2015.10.037.
[122] F.C. Fiesel, A. Voigt, S.S. Weber, C. Van den Haute, A. Waldenmaier, K. Görner, M. Walter, M.L. Anderson, J.V. Kern, T.M. Rasse, T. Schmidt, W. Springer, R. Kirchner, M. Bonin, M. Neumann, V. Baekelandt, M. Alunni-Fabbroni, J. B. Schulz, P.J. Kahle, Knockdown of transactive response DNA-binding protein (TDP-43) downregulates histone deacetylase 6, EMBO J. 29 (2010) 209–221, https://doi.org/10.1038/emboj.2009.324.
[123] S. Sanna, S. Esposito, A. Masala, P. Sini, G. Nieddu, M. Galioto, M. Fais, C. Iaccarino, G. Cestra, C. Crosio, HDAC1 inhibition ameliorates TDP-43-induced cell death in vitro and in vivo, Cell Death Dis. 11 (2020) 1–14, https://doi.org/10.1038/s41419-020-2580-3.
[124] F. Feiguin, V.K. Godena, G. Romano, A. D'Ambrogio, R. Klima, F.E. Baralle, Depletion of TDP-43 affects Drosophila motoneurons terminal synapsis and locomotive behavior, FEBS Lett. 583 (2009) 1586–1592, https://doi.org/10.1016/j.febslet.2009.04.019.
[125] Y. Lu, J. Ferris, F.-B. Gao, Frontotemporal dementia and amyotrophic lateral sclerosis-associated disease protein TDP-43 promotes dendritic branching, Mol. Brain. 2 (2009) 30, https://doi.org/10.1186/1756-6606-2-30.
[126] D.C. Diaper, Y. Adachi, B. Sutcliffe, D.M. Humphrey, C.J.H. Elliott, A. Stepto, Z. N. Ludlow, L. Vanden Broeck, P. Callaerts, B. Dermaut, A. Al-Chalabi, C.E. Shaw, I.M. Robinson, F. Hirth, Loss and gain of Drosophila TDP-43 impair synaptic efficacy and motor control leading to age-related neurodegeneration by loss-of-function phenotypes, Hum. Mol. Genet. 22 (2013) 1539–1557, https://doi.org/10.1093/hmg/ddt005.
[127] N. Strah, G. Romano, C. Introna, R. Klima, M. Marzullo, L. Ciapponi, A. Megighian, M. Nizzardo, F. Feiguin, TDP-43 promotes the formation of neuromuscular synapses through the regulation of Disc-large expression in Drosophila skeletal muscles, BMC Biol. 18 (2020) 34, https://doi.org/10.1186/s12915-020-00767-7.
[128] Y. Li, P. Ray, E.J. Rao, C. Shi, W. Guo, X. Chen, E.A. Woodruff, K. Fushimi, J. Y. Wu, A Drosophila model for TDP-43 proteinopathy, Proc. Natl. Acad. Sci. 107 (2010) 3169–3174, https://doi.org/10.1073/pnas.0913602107.
[129] M. Pons, L. Miguel, C. Miel, T. Avequin, F. Juge, T. Frebourg, D. Campion, M. Lecourtois, Splicing factors act as genetic modulators of TDP-43 production in a new autoregulatory TDP-43 Drosophila model, Hum. Mol. Genet. 26 (2017) 3396–3408, https://doi.org/10.1093/hmg/ddx229.
[130] G. Riboldi, M. Nizzardo, C. Simone, M. Falcone, N. Bresolin, G.P. Comi, S. Corti, ALS genetic modifiers that increase survival of SOD1 mice and are suitable for therapeutic development, Prog. Neurobiol. 95 (2011) 133–148, https://doi.org/10.1016/j.pneurobio.2011.07.009.
[131] A. Jovičić, J. Mertens, S. Boeynaems, E. Bogaert, N. Chai, S.B. Yamada, J.W.P. Iii, S. Sun, J.R. Herdy, N.J. Kramer, F.H. Gage, L. Van Den Bosch, A.D. Gitler, Modifiers of C9orf72 DPR toxicity implicate nucleocytoplasmic transport impairments in c9FTD/ALS, Nat Neurosci. 18 (2015) 1226–1229, https://doi.org/10.1038/nn.4085.Modifiers.
[132] C. Appocher, F. Mohagheghi, S. Cappelli, C. Stuani, M. Romano, F. Feiguin, E. Buratti, Major hnRNP proteins act as general TDP-43 functional modifiers both in Drosophila and human neuronal cells, Nucleic Acids Res. 45 (2017) 8026–8045, https://doi.org/10.1093/nar/gkx477.
[133] A.C. Elden, H.-J. Kim, M.P. Hart, A.S. Chen-Plotkin, B.S. Johnson, X. Fang, M. Armakola, F. Geser, R. Greene, M.M. Lu, A. Padmanabhan, D. Clay-Falcone, L. McCluskey, L. Elman, D. Juhr, P.J. Gruber, U. Rüb, G. Auburger, J. Q. Trojanowski, V.M.Y. Lee, V.M. Van Deerlin, N.M. Bonini, A.D. Gitler, Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS, Nature. 466 (2010) 1069–1075, https://doi.org/10.1038/nature09320.
[134] P. Van Damme, J.H. Veldink, M. van Blitterswijk, A. Corveleyn, P.W.J. van Vught, V. Thijs, B. Dubois, G. Matthijs, L.H. van den Berg, W. Robberecht, Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2, Neurology. 76 (2011) 2066–2072, https://doi.org/10.1212/WNL.0b013e31821f445b.
[135] W. Sproviero, A. Shatunov, D. Stahl, M. Shoai, W. van Rheenen, A.R. Jones, S. Al-Sarraj, P.M. Andersen, N.M. Bonini, F.L. Conforti, P. Van Damme, H. Daoud, M. Del Mar Amador, I. Fogh, M. Forzan, B. Gaastra, C. Gellera, A.D. Gitler, J. Hardy, P. Fratta, V. La Bella, I. Le Ber, T. Van Langenhove, S. Lattante, Y.-C. Lee, A. Malaspina, V. Meininger, S. Millecamps, R. Orrell, R. Rademakers, W. Robberecht, G. Rouleau, O.A. Ross, F. Salachas, K. Sidle, B.N. Smith, B.-W. Soong, G. Sorarù, G. Stevanin, E. Kabashi, C. Troakes, C. van Broeckhoven, J.

H. Veldink, L.H. van den Berg, C.E. Shaw, J.F. Powell, A. Al-Chalabi, ATXN2 trinucleotide repeat length correlates with risk of ALS, Neurobiol. Aging. 51 (2017) e1–e9, https://doi.org/10.1016/j.neurobiolaging.2016.11.010.
[136] Z. Melamed, J. López-Erauskin, M.W. Baughn, O. Zhang, K. Drenner, Y. Sun, F. Freyermuth, M.A. McMahon, M.S. Beccari, J.W. Artates, T. Ohkubo, M. Rodriguez, N. Lin, D. Wu, C.F. Bennett, F. Rigo, S. Da Cruz, J. Ravits, C. Lagier-Tourenne, D.W. Cleveland, Premature polyadenylation-mediated loss of stathmin-2 is a hallmark of TDP-43-dependent neurodegeneration, Nat. Neurosci. 22 (2019) 180–190, https://doi.org/10.1038/s41593-018-0293-z.
[137] W. Guo, Y. Chen, X. Zhou, A. Kar, P. Ray, X. Chen, E.J. Rao, M. Yang, H. Ye, L. Zhu, J. Liu, M. Xu, Y. Yang, C. Wang, D. Zhang, E.H. Bigio, M. Mesulam, Y. Shen, Q. Xu, K. Fushimi, J.Y. Wu, An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity, Nat. Struct. Mol. Biol. 18 (2011) 822–831, https://doi.org/10.1038/nsmb.2053.
[138] C.G. Otte, T.R. Fortuna, J.R. Mann, A.M. Gleixner, N. Ramesh, N.J. Pyles, U. B. Pandey, C.J. Donnelly, Optogenetic TDP-43 nucleation induces persistent insoluble species and progressive motor dysfunction in vivo, Neurobiol. Dis. 146 (2020), https://doi.org/10.1016/j.nbd.2020.105078.
[139] J.C. Chang, D.B. Morton, Drosophila lines with mutant and wild type human TDP-43 replacing the endogenous gene reveals phosphorylation and ubiquitination in mutant lines in the absence of viability or lifespan defects, PLoS One. 12 (2017), https://doi.org/10.1371/journal.pone.0180828.
[140] C. Lagier-Tourenne, M. Polymenidou, K.R. Hutt, A.Q. Vu, M. Baughn, S.C. Huelga, K.M. Clutario, S.-C. Ling, T.Y. Liang, C. Mazur, E. Wancewicz, A.S. Kim, A. Watt, S. Freier, G.G. Hicks, J.P. Donohue, L. Shiue, C.F. Bennett, J. Ravits, D. W. Cleveland, G.W. Yeo, Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs, Nat. Neurosci. 15 (2012) 1488–1497, https://doi.org/10.1038/nn.3230.
[141] J.B. Machamer, S.E. Collins, T.E. Lloyd, The ALS gene FUS regulates synaptic transmission at the Drosophila neuromuscular junction, Hum. Mol. Genet. 23 (2014) 3810–3822, https://doi.org/10.1093/hmg/ddu094.
[142] J.B. Machamer, B.M. Woolums, G.G. Fuller, T.E. Lloyd, FUS causes synaptic hyperexcitability in Drosophila dendritic arborization neurons, Brain Res. 1693 (2018) 55–66, https://doi.org/10.1016/j.brainres.2018.03.037.
[143] M. Shahidullah, S.J. Le Marchand, H. Fei, J. Zhang, U.B. Pandey, M.B. Dalva, P. Pasinelli, I.B. Levitan, Defects in Synapse Structure and Function Precede Motor Neuron Degeneration in Drosophila Models of FUS-Related ALS, J. Neurosci. 33 (2013) 19590–19598, https://doi.org/10.1523/JNEUROSCI.3396-13.2013.
[144] J. Steyaert, W. Scheveneels, J. Vanneste, P. Van Damme, W. Robberecht, P. Callaerts, E. Bogaert, L. Van Den Bosch, FUS-induced neurotoxicity in Drosophila is prevented by downregulating nucleocytoplasmic transport proteins, Hum. Mol. Genet. 27 (2018) 4103–4116, https://doi.org/10.1093/hmg/ddy303.
[145] I. Casci, K. Krishnamurthy, S. Kour, V. Tripathy, N. Ramesh, E.N. Anderson, L. Marrone, R.A. Grant, S. Oliver, L. Gochenaur, K. Patel, J. Sterneckert, A. M. Gleixner, C.J. Donnelly, M.D. Ruepp, A.M. Sini, E. Zuccaro, M. Pennuto, P. Pasinelli, U. Bhan Pandey, Muscleblind acts as a modifier of FUS toxicity by modulating stress granule dynamics and SMN localization, Nat. Commun. 10 (2019) 1–20, https://doi.org/10.1038/s41467-019-13383-z.
[146] E. Bogaert, S. Boeynaems, M. Kato, L. Guo, T.R. Caulfield, J. Steyaert, W. Scheveneels, N. Wilmans, W. Haeck, N. Hersmus, J. Schymkowitz, F. Rousseau, J. Shorter, P. Callaerts, W. Robberecht, P. Van Damme, L. Van Den Bosch, Molecular Dissection of FUS Points at Synergistic Effect of Low-Complexity Domains in Toxicity, Cell Rep. 24 (2018) 529–537, https://doi.org/10.1016/j.celrep.2018.06.070.
[147] M.W. Kankel, A. Sen, L. Lu, M. Theodorou, D.N. Dimlich, A. McCampbell, C. E. Henderson, N.A. Shneider, S. Artavanis-Tsakonas, Amyotrophic lateral sclerosis modifiers in drosophila reveal the phospholipase D Pathway as a Potential Therapeutic Target, Genetics. 215 (2020) 747–766, https://doi.org/10.1534/genetics.119.302985.
[148] L. Marrone, H.C.A. Drexler, J. Wang, P. Tripathi, T. Distler, P. Heisterkamp, E. N. Anderson, S. Kour, A. Moraiti, S. Maharana, R. Bhatnagar, T.G. Belgard, V. Tripathy, N. Kalmbach, Z. Hosseinzadeh, V. Crippa, M. Abo-Rady, F. Wegner, A. Poletti, D. Troost, E. Aronica, V. Busskamp, J. Weis, U.B. Pandey, A.A. Hyman, S. Alberti, A. Goswami, J. Sterneckert, FUS pathology in ALS is linked to alterations in multiple ALS-associated proteins and rescued by drugs stimulating autophagy, Acta Neuropathol. 138 (2019) 67–84, https://doi.org/10.1007/s00401-019-01998-x.
[149] A.Z. Ozsoy, S. Cayli, C. Sahin, S. Ocakli, T.O. Sanci, D.B. Ilhan, Altered expression of p97/Valosin containing protein and impaired autophagy in preeclamptic human placenta, Placenta. 67 (2018) 45–53, https://doi.org/10.1016/j.placenta.2018.05.013.
[150] A.E. Johnson, H. Shu, A.G. Hauswirth, A. Tong, G.W. Davis, VCP-dependent muscle degeneration is linked to defects in a dynamic tubular lysosomal network in vivo, Elife. 4 (2015), https://doi.org/10.7554/eLife.07366.
[151] G.P. Ritson, S.K. Custer, B.D. Freibaum, J.B. Guinto, D. Geffel, J. Moore, W. Tang, M.J. Winton, M. Neumann, J.Q. Trojanowski, V.M.Y. Lee, M.S. Forman, J. P. Taylor, TDP-43 mediates degeneration in a novel Drosophila model of disease caused by mutations in VCP/p97, J. Neurosci. 30 (2010) 7729–7739, https://doi.org/10.1523/JNEUROSCI.5894-09.2010.
[152] Z. Mushtaq, S.D. Choudhury, S.K. Gangwar, G. Orso, V. Kumar, Human Senataxin Modulates Structural Plasticity of the Neuromuscular Junction in Drosophila through a Neuronally Conserved TGFβ Signalling Pathway, Neurodegener. Dis. 16 (2016) 324–336, https://doi.org/10.1159/000445435.
[153] A. Ratnaparkhi, G.M. Lawless, F.E. Schweizer, P. Golshani, G.R. Jackson, A Drosophila Model of ALS: Human ALS-Associated Mutation in VAP33A Suggests a Dominant Negative Mechanism, PLoS One. 3 (2008), e2334, https://doi.org/10.1371/journal.pone.0002334.
[154] K. Chaplot, L. Pimpale, B. Ramalingam, S. Deivasigamani, S.S. Kamat, G. S. Ratnaparkhi, SOD1 activity threshold and TOR signalling modulate VAP(P58S) aggregation via reactive oxygen species-induced proteasomal degradation in a Drosophila model of amyotrophic lateral sclerosis, Dis. Model. Mech. 12 (2019) dmm033803, https://doi.org/10.1242/dmm.033803.
[155] H. Tsuda, S.M. Han, Y. Yang, C. Tong, Y.Q. Lin, K. Mohan, C. Haueter, A. Zoghbi, Y. Harati, J. Kwan, M.A. Miller, H.J. Bellen, The Amyotrophic Lateral Sclerosis 8 Protein VAPB Is Cleaved Secreted, and Acts as a Ligand for Eph Receptors, Cell. 133 (2008) 963–977, https://doi.org/10.1016/j.cell.2008.04.039.
[156] E.E. Patton, D.M. Tobin, Spotlight on zebrafish: The next wave of translational research, Dis. Model. Mech. 12 (2019) dmm039370, https://doi.org/10.1242/dmm.039370.
[157] J.B. Phillips, M. Westerfield, Zebrafish models in translational research: tipping the scales toward advancements in human health, Dis. Model. Mech. 7 (2014) 739–743, https://doi.org/10.1242/dmm.015545.
[158] A.M. Stewart, O. Braubach, J. Spitsbergen, R. Gerlai, A.V. Kalueff, Zebrafish models for translational neuroscience research: from tank to bedside, Trends Neurosci. 37 (2014) 264–278, https://doi.org/10.1016/j.tins.2014.02.011.
[159] C. Wyatt, E.M. Bartoszek, E. Yaksi, Methods for studying the zebrafish brain: past, present and future, Eur. J. Neurosci. 42 (2015) 1746–1763, https://doi.org/10.1111/ejn.12932.
[160] R. Koster, W.A. Sassen, A molecular toolbox for genetic manipulation of zebrafish, Adv. Genomics Genet. (2015) 151–163, https://doi.org/10.2147/AGG.S57585.
[161] N.G. Holtzman, M.K. Iovine, J.O. Liang, J. Morris, Learning to Fish with Genetics: A Primer on the Vertebrate Model Danio rerio, Genetics. 203 (2016) 1069–1089, https://doi.org/10.1534/genetics.116.190843.
[162] B. Schmid, C. Haass, Genomic editing opens new avenues for zebrafish as a model for neurodegeneration, J. Neurochem. 127 (2013) 461–470, https://doi.org/10.1111/jnc.12460.
[163] S. Albadri, F. Del Bene, C. Revenu, Genome editing using CRISPR/Cas9-based knock-in approaches in zebrafish, Methods. 121–122 (2017) 77–85, https://doi.org/10.1016/j.ymeth.2017.03.005.
[164] M.L. Suster, G. Abe, A. Schouw, K. Kawakami, Transposon-mediated BAC transgenesis in zebrafish, Nat. Protoc. 6 (2011) 1998–2021, https://doi.org/10.1038/nprot.2011.416.
[165] A. Hruscha, B. Schmid, Generation of Zebrafi sh Models by CRISPR/Cas9 Genome Editing, Methods Mol. Biol. 1254 (2015) 341–350, https://doi.org/10.1007/978-1-4939-2152-2_24.
[166] A. McGown, M.J. Stopford, High-throughput drug screens for amyotrophic lateral sclerosis drug discovery, Expert Opin. Drug Discov. 13 (2018) 1015–1025, https://doi.org/10.1080/17460441.2018.1533953.
[167] S. Ciura, S. Lattante, I. Le Ber, M. Latouche, H. Tostivint, A. Brice, E. Kabashi, Loss of function of C9orf72 causes motor deficits in a zebrafish model of Amyotrophic Lateral Sclerosis, Ann. Neurol. 74 (2013) 180–187, https://doi.org/10.1002/ana.23946.
[168] A. Swaminathan, M. Bouffard, M. Liao, S. Ryan, J.B. Callister, S.M. Pickering-Brown, G.A.B. Armstrong, P. Drapeau, Expression of C9orf72-related dipeptides impairs motor function in a vertebrate model, Hum. Mol. Genet. 27 (2018) 1754–1762, https://doi.org/10.1093/hmg/ddy083.
[169] M.P. Shaw, A. Higginbottom, A. McGown, L.M. Castelli, E. James, G. M. Hautbergue, P.J. Shaw, T.M. Ramesh, Stable transgenic C9orf72 zebrafish model key aspects of the ALS/FTD phenotype and reveal novel pathological features, Acta Neuropathol. Commun. 6 (2018) 125, https://doi.org/10.1186/s40478-018-0629-7.
[170] B. Swinnen, A. Bento-Abreu, T.F. Gendron, S. Boeynaems, E. Bogaert, R. Nuyts, M. Timmers, W. Scheveneels, N. Hersmus, J. Wang, S. Mizielinska, A.M. Isaacs, L. Petrucelli, R. Lemmens, P. Van Damme, L. Van Den Bosch, W. Robberecht, A zebrafish model for C9orf72 ALS reveals RNA toxicity as a pathogenic mechanism, Acta Neuropathol. 135 (2018) 427–443, https://doi.org/10.1007/s00401-017-1796-5.
[171] Y.-B. Lee, H.-J. Chen, J.N. Peres, J. Gomez-Deza, J. Attig, M. Štalekar, C. Troakes, A.L. Nishimura, E.L. Scotter, C. Vance, Y. Adachi, V. Sardone, J.W. Miller, B. N. Smith, J.-M. Gallo, J. Ule, F. Hirth, B. Rogelj, C. Houart, C.E. Shaw, Hexanucleotide Repeats in ALS/FTD Form Length-Dependent RNA Foci, Sequester RNA Binding Proteins, and Are Neurotoxic, Cell Rep. 5 (2013) 1178–1186, https://doi.org/10.1016/j.celrep.2013.10.049.
[172] Y. Ohki, A. Wenninger-Weinzierl, A. Hruscha, K. Asakawa, K. Kawakami, C. Haass, D. Edbauer, B. Schmid, Glycine-alanine dipeptide repeat protein contributes to toxicity in a zebrafish model of C9orf72 associated neurodegeneration, Mol. Neurodegener. 12 (2017) 6, https://doi.org/10.1186/s13024-016-0146-8.
[173] S. Ciura, C. Sellier, M.-L. Campanari, N. Charlet-Berguerand, E. Kabashi, The most prevalent genetic cause of ALS-FTD, C9orf72 synergizes the toxicity of ATXN2 intermediate polyglutamine repeats through the autophagy pathway, Autophagy. 12 (2016) 1406–1408, https://doi.org/10.1080/15548627.2016.1189070.
[174] A. Bento-Abreu, G. Jager, B. Swinnen, L. Rué, S. Hendrickx, A. Jones, K.A. Staats, I. Taes, C. Eykens, A. Nonneman, R. Nuyts, M. Timmers, L. Silva, A. Chariot, L. Nguyen, J. Ravits, R. Lemmens, D. Cabooter, L. Van Den Bosch, P. Van Damme, A. Al-Chalabi, A. Bystrom, W. Robberecht, Elongator subunit 3 (ELP3) modifies ALS through tRNA modification, Hum. Mol. Genet. 27 (2018) 1276–1289, https://doi.org/10.1093/hmg/ddy043.
[175] A. Corman, B. Jung, M. Häggblad, L. Bräutigam, V. Lafarga, L. Lidemalm, D. Hühn, J. Carreras-Puigvert, O. Fernandez-Capetillo, A Chemical Screen Identifies Compounds Limiting the Toxicity of C9ORF72 Dipeptide Repeats, Cell

Chem. Biol. 26 (2019) 235–243, https://doi.org/10.1016/j.chembiol.2018.10.020.
[176] R. Lemmens, A. Van Hoecke, N. Hersmus, V. Geelen, I. D'Hollander, V. Thijs, L. Van Den Bosch, P. Carmeliet, W. Robberecht, Overexpression of mutant superoxide dismutase 1 causes a motor axonopathy in the zebrafish, Hum. Mol. Genet. 16 (2007) 2359–2365, https://doi.org/10.1093/hmg/ddm193.
[177] S.A. Sakowski, J. Lunn, A.S. Busta, S. Oh, G. Zamora-Berridi, M. Palmer, A. A. Rosenberg, S.G. Philip, J.J. Dowling, E.L. Feldman, Neuromuscular effects of G93A-SOD1 expression in zebrafish, Mol. Neurodegener. 7 (2012) 44, https://doi.org/10.1186/1750-1326-7-44.
[178] T. Ramesh, A.N. Lyon, R.H. Pineda, C. Wang, P.M.L. Janssen, B.D. Canan, A.H. M. Burghes, C.E. Beattie, A genetic model of amyotrophic lateral sclerosis in zebrafish displays phenotypic hallmarks of motoneuron disease, Dis. Model. Mech. 3 (2010) 652–662, https://doi.org/10.1242/dmm.005538.
[179] M.M.J. Da Costa, C.E. Allen, A. Higginbottom, T. Ramesh, P.J. Shaw, C. J. McDermott, A new zebrafish model produced by TILLING of SOD1-related amyotrophic lateral sclerosis replicates key features of the disease and represents a tool for in vivo therapeutic screening, Dis. Model. Mech. 7 (2014) 73–81, https://doi.org/10.1242/dmm.012013.
[180] A. McGown, J.R. McDearmid, N. Panagiotaki, H. Tong, S. Al Mashhadi, N. Redhead, A.N. Lyon, C.E. Beattie, P.J. Shaw, T.M. Ramesh, Early interneuron dysfunction in ALS: Insights from a mutant sod1 zebrafish model, Ann. Neurol. 73 (2013) 246–258, https://doi.org/10.1002/ana.23780.
[181] A. Van Hoecke, L. Schoonaert, R. Lemmens, M. Timmers, K.A. Staats, A.S. Laird, E. Peeters, T. Philips, A. Goris, B. Dubois, P.M. Andersen, A. Al-Chalabi, V. Thijs, A.M. Turnley, P.W. van Vught, J.H. Veldink, O. Hardiman, L. Van Den Bosch, P. Gonzalez-Perez, P. Van Damme, R.H. Brown, L.H. van den Berg, W. Robberecht, EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans, Nat. Med. 18 (2012) 1418–1422, https://doi.org/10.1038/nm.2901.
[182] P. Bose, E. Tremblay, C. Maois, V. Narasimhan, G.A.B. Armstrong, M. Liao, J. A. Parker, R. Robitaille, X.Y. Wen, C. Barden, P. Drapeau, The Novel Small Molecule TRVA242 Stabilizes Neuromuscular Junction Defects in Multiple Animal Models of Amyotrophic Lateral Sclerosis, Neurotherapeutics. 16 (2019) 1149–1166, https://doi.org/10.1007/s13311-019-00765-w.
[183] L. Benedetti, A. Ghilardi, E. Rottoli, M. De Maglie, L. Prosperi, C. Perego, M. Baruscotti, A. Bucchi, L. Del Giacco, M. Francolini, INaP selective inhibition reverts precocious inter- and motorneurons hyperexcitability in the Sod1-G93R zebrafish ALS model, Sci. Rep. 6 (2016) 24515, https://doi.org/10.1038/srep24515.
[184] B.C. Kraemer, T. Schuck, J.M. Wheeler, L.C. Robinson, J.Q. Trojanowski, V.M. Y. Lee, G.D. Schellenberg, Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis, Acta Neuropathol. 119 (2010) 409–419, https://doi.org/10.1007/s00401-010-0659-0.
[185] Z.-S. Xu, Does a loss of TDP-43 function cause neurodegeneration? Mol. Neurodegener. 7 (2012) 27, https://doi.org/10.1186/1750-1326-7-27.
[186] P. Fratta, P. Sivakumar, J. Humphrey, K. Lo, T. Ricketts, H. Oliveira, J.M. Brito-Armas, B. Kalmar, A. Ule, Y. Yu, N. Birsa, C. Bodo, T. Collins, A.E. Conicella, A. Mejia Maza, A. Marrero-Gagliardi, M. Stewart, J. Mianne, S. Corrochano, W. Emmett, G. Codner, M. Groves, R. Fukumura, Y. Gondo, M. Lythgoe, E. Pauws, E. Peskett, P. Stanier, L. Teboul, M. Hallegger, A. Calvo, A. Chiò, A.M. Isaacs, N. L. Fawzi, E. Wang, D.E. Housman, F. Baralle, L. Greensmith, E. Buratti, V. Plagnol, E.M. Fisher, A. Acevedo-Arozena, Mice with endogenous TDP-43 mutations exhibit gain of splicing function and characteristics of amyotrophic lateral sclerosis, EMBO J. 37 (2018), e98684, https://doi.org/10.15252/embj.201798684.
[187] M.A. White, E. Kim, A. Duffy, R. Adalbert, B.U. Phillips, O.M. Peters, J. Stephenson, S. Yang, F. Massenzio, Z. Lin, S. Andrews, A. Segonds-Pichon, J. Metterville, L.M. Saksida, R. Mead, R.R. Ribchester, Y. Barhomi, T. Serre, M. P. Coleman, J.R. Fallon, T.J. Bussey, R.H. Brown, J. Sreedharan, TDP-43 gains function due to perturbed autoregulation in a Tardbp knock-in mouse model of ALS-FTD, Nat. Neurosci. 21 (2018) 552–563, https://doi.org/10.1038/s41593-018-0113-5.
[188] C.A.A. Hewamadduma, A.J. Grierson, T.P. Ma, L. Pan, C.B. Moens, P.W. Ingham, T. Ramesh, P.J. Shaw, Tardbpl splicing rescues motor neuron and axonal development in a mutant tardbp zebrafish, Hum. Mol. Genet. 22 (2013) 2376–2386, https://doi.org/10.1093/hmg/ddt082.
[189] E. Kabashi, L. Lin, M.L. Tradewell, P.A. Dion, V. Bercier, P. Bourgouin, D. Rochefort, S. Bel Hadj, H.D. Durham, C. Vande Velde, G.A. Rouleau, P. Drapeau, Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo, Hum. Mol. Genet. 19 (2010) 671–683, https://doi.org/10.1093/hmg/ddp534.
[190] B. Schmid, A. Hruscha, S. Hogl, J. Banzhaf-Strathmann, K. Strecker, J. van der Zee, M. Teucke, S. Eimer, J. Hegermann, M. Kittelmann, E. Kremmer, M. Cruts, B. Solchenberger, L. Hasenkamp, F. van Bebber, C. Van Broeckhoven, D. Edbauer, S.F. Lichtenthaler, C. Haass, Loss of ALS-associated TDP-43 in zebrafish causes muscle degeneration, vascular dysfunction, and reduced motor neuron axon outgrowth, Proc. Natl. Acad. Sci. 110 (2013) 4986–4991, https://doi.org/10.1073/pnas.1218311110.
[191] P. Bose, G.A.B. Armstrong, P. Drapeau, Neuromuscular junction abnormalities in a zebrafish loss-of-function model of TDP-43, J. Neurophysiol. 121 (2019) 285–297, https://doi.org/10.1152/jn.00265.2018.
[192] D. Lou Demy, M.L. Campanari, R. Munoz-Ruiz, H.D. Durham, B.J. Gentil, E. Kabashi, Functional Characterization of Neurofilament Light Splicing and Misbalance in Zebrafish, Cells. 9 (2020) 1238, https://doi.org/10.3390/cells9051238.
[193] A. Lissouba, M. Liao, E. Kabashi, P. Drapeau, Transcriptomic Analysis of Zebrafish TDP-43 Transgenic Lines, Front. Mol. Neurosci. 11 (2018) 463, https://doi.org/10.3389/fnmol.2018.00463.
[194] K. Kapeli, G.A. Pratt, A.Q. Vu, K.R. Hutt, F.J. Martinez, B. Sundararaman, R. Batra, P. Freese, N.J. Lambert, S.C. Huelga, S.J. Chun, T.Y. Liang, J. Chang, J. P. Donohue, L. Shiue, J. Zhang, H. Zhu, F. Cambi, E. Kasarskis, S. Hoon, M. Ares, C.B. Burge, J. Ravits, F. Rigo, G.W. Yeo, Distinct and shared functions of ALS-associated proteins TDP-43, FUS and TAF15 revealed by multisystem analyses, Nat. Commun. 7 (2016) 12143, https://doi.org/10.1038/ncomms12143.
[195] C. Lagier-Tourenne, M. Polymenidou, D.W. Cleveland, TDP-43 and FUS/TLS: Emerging roles in RNA processing and neurodegeneration, Hum. Mol. Genet. 19 (2010) 46–64, https://doi.org/10.1093/hmg/ddq137.
[196] E. Kabashi, V. Bercier, A. Lissouba, M. Liao, E. Brustein, G.A. Rouleau, P. Drapeau, FUS and TARDBP but Not SOD1 Interact in Genetic Models of Amyotrophic Lateral Sclerosis, PLoS Genet. 7 (2011), e1002214, https://doi.org/10.1371/journal.pgen.1002214.
[197] G.A.B. Armstrong, P. Drapeau, Loss and gain of FUS function impair neuromuscular synaptic transmission in a genetic model of ALS, Hum. Mol. Genet. 22 (2013) 4282–4292, https://doi.org/10.1093/hmg/ddt278.
[198] D. Dormann, R. Rodde, D. Edbauer, E. Bentmann, I. Fischer, A. Hruscha, M. E. Than, I.R.A. Mackenzie, A. Capell, B. Schmid, M. Neumann, C. Haass, ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import, EMBO J. 29 (2010) 2841–2857, https://doi.org/10.1038/emboj.2010.143.
[199] D.A. Bosco, N. Lemay, H.K. Ko, H. Zhou, C. Burke, T.J. Kwiatkowski, P. Sapp, D. McKenna-Yasek, R.H. Brown, L.J. Hayward, Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules, Hum. Mol. Genet. 19 (2010) 4160–4175, https://doi.org/10.1093/hmg/ddq335.
[200] N.A. Lanson, A. Maltare, H. King, R. Smith, J.H. Kim, J.P. Taylor, T.E. Lloyd, U. B. Pandey, A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43, Hum. Mol. Genet. 20 (2011) 2510–2523, https://doi.org/10.1093/hmg/ddr150.
[201] S.-C. Ling, C.P. Albuquerque, J.S. Han, C. Lagier-Tourenne, S. Tokunaga, H. Zhou, D.W. Cleveland, ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS, Proc. Natl. Acad. Sci. 107 (2010) 13318–13323, https://doi.org/10.1073/pnas.1008227107.
[202] M. Kustermann, L. Manta, C. Paone, J. Kustermann, L. Lausser, C. Wiesner, L. Eichinger, C.S. Clemen, R. Schröder, H.A. Kestler, M. Sandri, W. Rottbauer, S. Just, Loss of the novel Vcp (valosin containing protein) interactor Washc4 interferes with autophagy-mediated proteostasis in striated muscle and leads to myopathy in vivo, Autophagy. 14 (2018) 1911–1927, https://doi.org/10.1080/15548627.2018.1491491.
[203] M. Atkins, L. Gasmi, V. Bercier, C. Revenu, F. Del Bene, J. Hazan, C. Fassier, FIGNL1 associates with KIF1Bβ and BICD1 to restrict dynein transport velocity during axon navigation, J. Cell Biol. 218 (2019) 3290–3306, https://doi.org/10.1083/jcb.201805128.
[204] T.O. Auer, T. Xiao, V. Bercier, C. Gebhardt, K. Duroure, J.P. Concordet, C. Wyart, M. Suster, K. Kawakami, J. Wittbrodt, H. Baier, F. Del Bene, Deletion of a kinesin I motor unmasks a mechanism of homeostatic branching control by neurotrophin-3, Elife. 4 (2015), e05061, https://doi.org/10.7554/eLife.05061.
[205] M. van Blitterswijk, M.A. van Es, E.A.M. Hennekam, D. Dooijes, W. van Rheenen, J. Medic, P.R. Bourque, H.J. Schelhaas, A.J. van der Kooi, M. de Visser, P.I.W. de Bakker, J.H. Veldink, L.H. van den Berg, Evidence for an oligogenic basis of amyotrophic lateral sclerosis, Hum. Mol. Genet. 21 (2012) 3776–3784, https://doi.org/10.1093/hmg/dds199.
[206] D. Petrov, C. Mansfield, A. Moussy, O. Hermine, ALS Clinical Trials Review: 20 Years of Failure. Are we any closer to registering a new treatment? Front. Aging Neurosci. 9 (2017) 68, https://doi.org/10.3389/fnagi.2017.00068.
[207] U.B. Pandey, C.D. Nichols, Human disease models in drosophila melanogaster and the role of the fly in therapeutic drug discovery, Pharmacol. Rev. 63 (2011) 411–436, https://doi.org/10.1124/pr.110.003293.
[208] A. Vagnoni, S.L. Bullock, A simple method for imaging axonal transport in aging neurons using the adult Drosophila wing, Nat. Protoc. 11 (2016) 1711–1723, https://doi.org/10.1038/nprot.2016.112.
[209] C. Germeys, T. Vandoorne, V. Bercier, L. Van Den Bosch, Existing and emerging metabolomic tools for ALS research, Genes (Basel). 10 (2019) 1011, https://doi.org/10.3390/genes10121011.
[210] K. Asakawa, H. Handa, K. Kawakami, Optogenetic modulation of TDP-43 oligomerization accelerates ALS-related pathologies in the spinal motor neurons, Nat. Commun. 11 (2020) 1–16, https://doi.org/10.1038/s41467-020-14815-x.
[211] A.J. Svahn, E.K. Don, A.P. Badrock, N.J. Cole, M.B. Graeber, J.J. Yerbury, R. Chung, M. Morsch, Nucleo-cytoplasmic transport of TDP-43 studied in real time: Impaired microglia function leads to axonal spreading of TDP-43 in degenerating motor neurons, Acta Neuropathol. 136 (2018) 445–459, https://doi.org/10.1007/s00401-018-1875-2.
[212] V. Bercier, M. Rosello, F. Del Bene, C. Revenu, Zebrafish as a model for the study of live in vivo processive transport in neurons, Front. Cell Dev. Biol. 7 (2019) 17, https://doi.org/10.3389/fcell.2019.00017.
[213] V. Petel Légaré, Z.A. Harji, C.J. Rampal, X. Allard-Chamard, E.C. Rodríguez, G.A. B. Armstrong, Augmentation of spinal cord glutamatergic synaptic currents in zebrafish primary motoneurons expressing mutant human TARDBP (TDP-43), Sci. Rep. 9 (2019) 9122, https://doi.org/10.1038/s41598-019-45530-3.
[214] J. Vanneste, T. Vercruysse, S. Boeynaems, A. Sicart, P. Van Damme, D. Daelemans, L. Van Den Bosch, C9orf72-generated poly-GR and poly-PR do not directly interfere with nucleocytoplasmic transport, Sci. Rep. 9 (2019) 15728, https://doi.org/10.1038/s41598-019-52035-6.