



HAL
open science

Novel FUS and CHCHD10 models to investigate pathogenic mechanisms in Amyotrophic Lateral Sclerosis

Annis-Rayan Bourefis

► **To cite this version:**

Annis-Rayan Bourefis. Novel FUS and CHCHD10 models to investigate pathogenic mechanisms in Amyotrophic Lateral Sclerosis. *Neurons and Cognition [q-bio.NC]*. Sorbonne Université, 2019. English. NNT : 2019SORUS177 . tel-03065826

HAL Id: tel-03065826

<https://theses.hal.science/tel-03065826>

Submitted on 15 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

PhD THESIS – SORBONNE UNIVERSITE

DOCTORAL SCHOOL “CERVEAU, COGNITION, COMPORTEMENT”



**Novel FUS and CHCHD10 models to investigate pathogenic mechanisms in
Amyotrophic Lateral Sclerosis**

Annis-Rayan Bourefis

Under the supervision of Doctor Edor Kabashi

U1163 “Translational Research for Neurological Diseases”, Institut Imagine, Paris

Defended on 13th December 2019

Composition of the jury:

Dr Valérie Buée-Scherrer – rapporteur

Dr Luc Dupuis – rapporteur

Dr Sophie Saunier – examiner

Dr Daniel Zytnicki – examiner

Dr Edor Kabashi – Thesis supervisor

Dr Maria-Letizia Campanari – Thesis co-supervisor

Remerciements

Je tiens à remercier particulièrement le Dr. Edor Kabashi pour m'avoir accueilli dans son équipe durant ma thèse. Les nombreux conseils que tu m'as fournis m'ont permis d'aborder le projet dans de bonnes conditions et la confiance que tu m'as accordé m'a permis de m'émanciper scientifiquement.

Je remercie toute l'équipe pour avoir fourni un environnement de travail unique, débordant de bienveillance, d'amour et de sympathie. Merci à Sorana, Anca, Corinne pour votre soutien constant. Merci à Hortense et Doris, mes grandes sœurs de labo sur qui je prenais exemple constamment. Merci à Solène, Grégoire, Mélissa, Claire et Chloé pour l'ambiance drôle et chaleureuse que vous apportez tous les jours au labo. Vous côtoyer au quotidien est un vrai bonheur.

A Letizia. Il me faudrait tout un autre manuscrit pour exprimer ma gratitude envers toi. Tu as été plus qu'une superviseure, tu as été un mentor pour moi. Travailler à tes côtés m'a fait grandir scientifiquement, mais aussi humainement, et j'espère avoir été à la hauteur de tes exigences.

A Raphaël. Cette aventure, on l'a fait ensemble et je n'aurais pas pu rêver d'un meilleur acolyte que toi. Je te remercie de m'avoir accompagné durant cette thèse. Ton soutien infailible m'a été vital.

A Sonia, la personne la plus fantastique et la plus funky qui soit. Tu le sais, sans toi, je ne serai pas allé bien loin. Merci d'avoir été constamment à mes côtés.

Je remercie aussi de tout mon cœur tous les membres de ma famille qui m'ont soutenu durant ces dernières années, et mes parents qui ont toujours été derrière moi. J'espère vous avoir rendu fiers.

Je remercie aussi tous mes amis qui m'ont suivi durant ces dernières années. Merci Pierre le philosophe, Antoine, mon étoile, ainsi qu'Oscar et Malik, mes plus vieux amis.

Enfin, je tiens à remercier chaleureusement tous les membres du jury d'avoir accepté d'examiner mon travail. Merci aux Drs Sophie Saunier et Daniel Zytnicki pour votre disponibilité. Un grand merci aux Drs Valérie Buée-Scherrer et Luc Dupuis pour m'avoir suivi durant ma thèse lors des différents comités de suivi, ainsi que pour avoir pris le temps de lire et évaluer mon manuscrit de thèse.

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disorder caused by progressive degeneration of upper and lower motor neurons (MNs), with a very rapid clinical course. It leads to muscle weakness and atrophy progressing to paralysis, with respiratory failure being the major cause of death within years following clinical diagnosis. ALS also shares neuropathological and genetic features with Frontotemporal Dementia (FTD), as the prevalence of cognitive and behavioral symptoms in ALS patients is high. Thus, these two disorders constitute the ends of a same spectrum of disease.

A major gene mutated in ALS patients is the RNA-binding protein FUS (FUSed in sarcoma; also known as TLS, translocated in liposarcoma). FUS is implicated in multiple aspects of RNA metabolism including RNA splicing, trafficking and translation. This factor shuttles between the nucleus and the cytoplasm where it controls more than 5,500 identified RNA targets. Another recently identified gene implicated in ALS is coiled-coil-helix-coiled-coil-helix domain 10 (CHCHD10), which plays a role in mitochondria stability.

Both these genes have been investigated through different model systems, from small invertebrate models to patient biopsies. However, the major phenotypic features obtained in these models are complex and often controversial. The objective of this work is to provide new insights on the implication of these genes in ALS through the use of new models.

To investigate the pathogenic mechanisms induced by FUS and CHCHD10, we generated and characterized two novel stable non-sense mutant zebrafish models for the orthologues of these genes and highlighted several ALS phenotypic features.

For *fus*, we demonstrated that loss of its function reduces the lifespan and leads to locomotor disabilities. We associated these phenotypic features with the observation of aberrant axonal projections from MNs and disorganized neuromuscular junction (NMJ) morphology. Furthermore, we described a significant dysregulation of several Acetylcholine receptor subunits associated with a decrease of *hdac4*, highlighting possible denervation and reinnervation processes in our model. We also demonstrated altered muscle morphology as well as disrupted mitochondrial network. At the molecular level, we demonstrated transcriptomic and protein expression changes for the genes encoding for different isoforms of the protein tau in the model, as well as changes in the expression of kinases acting on tau phosphorylation.

For *chchd10*, we observed non-persistent locomotion defects associated with fragmented mitochondrial network and collapse of the mitochondrial membrane potential, suggesting the enhancement of apoptotic conditions. However, these observations were not significant, suggesting a lack of loss of function effect from CHCHD10, possibly tempered by compensatory pathways.

These findings indicate that loss of *fus* expression is responsible for the occurrence of distal pathological signs at the NMJ, thus supporting a “dying-back” neuronopathy, in which early disease hallmarks start at the level of the NMJ and progress towards MN cell bodies.

Résumé

La sclérose latérale amyotrophique (SLA) est une maladie neurodégénérative dévastatrice causée par la dégénérescence progressive des motoneurons (MNs) supérieurs et inférieurs. Cela mène à une faiblesse et une atrophie musculaire qui progresse jusqu'à la paralysie. Des défaillances respiratoires sont la cause majeure de décès qui survient quelques années après diagnostic clinique. Les patients SLA présentant une importante prévalence de symptômes cognitifs et comportementaux, il a été montré qu'elle partage aussi des traits génétiques et neuropathologiques avec la démence fronto-temporale (DFT). Ainsi, ses maladies sont considérées comme faisant partie d'un même spectre pathologique.

Un des gènes majeurs identifiés chez des patients SLA est le gène FUS (FUSed in sarcoma), codant pour une protéine de liaison aux ARNs. FUS est impliqué dans de nombreux aspects du métabolisme de l'ARN, comme son épissage, son transport et sa traduction. La protéine navigue entre le noyau et le cytoplasme où il contrôle plus de 5500 ARNs. Un autre gène, nouvellement identifié comme étant impliqué dans la SLA, est le gène coiled-coil-helix-coiled-coil-helix domain 10 (CHCHD10), qui joue un rôle majeur dans le maintien et la stabilité des mitochondries.

Ces deux gènes ont été étudiés à travers différents modèles, de petits modèles invertébrés aux biopsies de patients. Cependant, les différents traits phénotypiques observés dans ses modèles sont complexes et parfois controversés. L'objectif de cette thèse est de fournir de nouvelles informations sur l'implication de ces deux gènes dans la SLA à travers l'utilisation de nouveaux modèles.

Pour étudier les mécanismes pathologiques induits par FUS et CHCHD10, nous avons généré et caractérisé deux nouveaux modèles de poisson-zèbres présentant une mutation non-sens des orthologues de ces gènes, et nous avons mis en évidence différents traits phénotypiques propres à la SLA.

Pour fus, nous avons démontré que la perte de sa fonction réduit la durée de vie du modèle et mène à des déficits locomoteurs. Nous avons associé ses phénotypes à l'observation de projections axonales des MNs aberrantes et une désorganisation de la jonction neuromusculaire (JNM). De plus, nous avons décrit une dérégulation significative des sous-unités des récepteurs à l'acétylcholine associé à une diminution de l'expression de hdac4, soulignant de possibles phénomènes de dénervation et de réinnervation dans ce modèle. Nous avons aussi montré une structure musculaire altérée ainsi qu'un réseau mitochondrial perturbé. Au niveau moléculaire, nous avons montré des changements d'expression au niveau protéique et transcriptomique pour des gènes codant pour différents isoformes de la protéine tau, ainsi que des changements d'expression de kinases phosphorylant tau.

Pour chchd10, nous avons observés des déficits locomoteurs non persistants associé à un réseau mitochondrial fragmenté et une diminution du potentiel de membrane mitochondrial, suggérant une favorisation de conditions apoptotiques. Cependant, ces observations n'étaient pas significatives, supposant une absence d'effet pathologique de la perte de fonction de CHCHD10, possiblement atténué par des processus compensatoires.

Ces résultats montrent que la perte de fonction de fus est responsable de l'apparition de signes pathologiques distaux au niveau de la JNM, indiquant une neuronopathie en « dying-back », dans laquelle les traits pathologiques de la SLA commencent au niveau de la JNM et progressent vers les corps cellulaires des MNs.

Table of contents

Abstract/Résumé	7
-----------------------	---

CHAPTER 1 - INTRODUCTION

1. Amyotrophic Lateral Sclerosis.....	16
1.1. Definition and Clinical Features:	16
1.2. Incidence of ALS (clinical epidemiology)	17
1.3. Diagnostic challenge [revised from (Campanari, Bourefis & Kabashi, 2019)]	19
1.4. Diagnostic Methods	20
1.5. Treatment challenges	24
1.6. Environmental causes of ALS	25
2. Genetic causes and pathology of ALS.....	26
2.1. Genetics	26
2.2. Pathophysiology/cellular mechanisms	31
2.3. Zebrafish models to study neurodegeneration.....	47
3. Neuromuscular junction involvement in ALS and skeletal muscle implication	49
3.1. The NMJ.....	49
3.2. The zebrafish NMJ	50
3.3. NMJ degeneration in ALS	52
3.4. The role of skeletal muscle in ALS-related NMJ degradation	54
4. The gene FUS and its implication in ALS	58
4.1. FUS structure and properties.....	58
4.2. ALS-FUS pathology	66
4.3. Models of FUS in ALS pathology.....	70
4.4. FUS pathogenicity	73
5. Beyond FUS: RNA targets and their implication in ALS/FTD.....	79
6. Working hypothesis and objectives	86

CHAPTER 2 - RESULTS

1. Functional characterization of the novel FUS mutant zebrafish model.....	90
1.1. Generation and characterization of the model	90
1.2. <i>fus</i> inactivation causes locomotion defects in zebrafish	92
1.3. <i>fus</i> depletion causes behavioral impairment.....	95
1.4. <i>fus</i> loss-of-function causes defects at the zebrafish NMJs	97
1.5. TDP-43 overexpression does not restore <i>fus</i>^{-/-} phenotype.....	100
1.6. <i>Tau</i> isoforms imbalance in <i>fus</i>^{-/-} zebrafish.....	101

1.7.	Kinase expression changes in <i>fus</i>^{-/-} zebrafish	105
1.8.	Discussion	107
1.9.	Material and methods	115
1.10.	Supplementary Material	119
2.	FUS and CHCHD10 implication in muscle and mitochondria pathology	126
2.1.	Objectives	126
2.2.	<i>fus</i> KO causes slow muscle disorganization	127
2.3.	<i>fus</i> KO impacts the mitochondrial network in the zebrafish embryos muscles	130
2.4.	Expression of mitochondrial genes in <i>fus</i> mutants	133
3.	Functional characterization of the novel CHCHD10 mutant zebrafish model	135
3.1.	Generation and characterization of the model	135
3.2.	Locomotion assessment in <i>chchd10</i> zebrafish models	137
3.3.	<i>Chchd10</i> mutation does not impact NMJ formation in zebrafish	138
3.4.	Muscle morphology of <i>chchd10</i> zebrafish mutants	140
3.5.	Mitochondrial network and membrane potential permeability in <i>chchd10</i> mutants .	141
3.6.	Mitochondrial gene expression changes in <i>chchd10</i> mutants	144
3.7.	Expression of <i>tardbp</i> and <i>fus</i> in <i>chchd10</i> mutant zebrafish	145
3.8.	Expression of <i>chchd10</i> in the <i>fus</i> mutant zebrafish model	147
3.9.	Discussion	148
 CHAPTER 3 - CONCLUSION AND PERSPECTIVES		
	Conclusion and Perspectives	164
	Bibliography	172
	Abbreviations	220

CHAPTER 1 – INTRODUCTION

1. Amyotrophic Lateral Sclerosis

1.1. Definition and Clinical Features:

Described by Jean Martin Charcot in 1869, Amyotrophic Lateral Sclerosis (ALS) is a rare heterogeneous neurodegenerative disease that is characterized by a progressive degeneration of the upper motor neurons (UMNs) in the motor cortex and the lower motor neurons (LMNs) in the brainstem and the spinal cord (**Figure 1**). This leads to focal weakness, spasticity and atrophy of most of the muscles, progressing to paralysis. Death occurs predominantly through respiratory failure within 3 to 5 years of diagnosis (Chiò et al., 2009).

As a heterogeneous disease, the presentation of ALS can vary significantly between patients. Indeed, some patients present with spinal-onset disease, involving muscle weakness of the limbs, while others can present with bulbar-onset disease, characterized by a difficulty to speak (dysarthria) and to swallow (dysphagia) (Hardiman, Al-Chalabi, Chio, Corr, Logroscino, Robberecht, Shaw, Simmons, & Van Den Berg, 2017). This creates a difficulty to diagnose ALS accurately that can be rather challenging for neurologists (reviewed in the section 1.3 *Diagnostic challenge*).

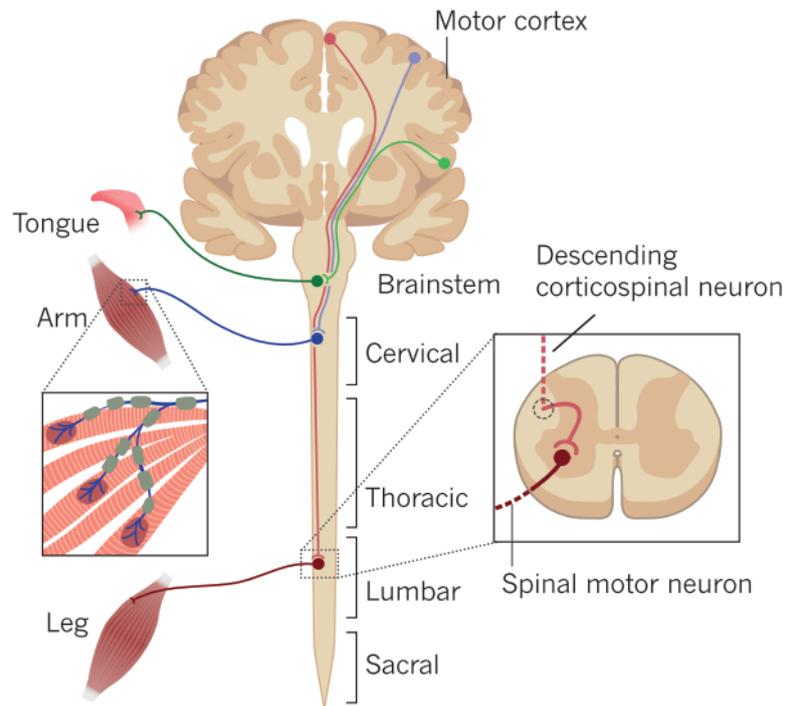


Figure 1: Clinical affections caused by ALS. Upper motor neurons from the motor cortex and lower motor neurons projecting from the brain stem and the spinal cord to the skeletal muscles are affected in ALS. (Derived from Taylor et al., 2016)

ALS also shares neuropathological and genetic features with another disorder, Frontotemporal Dementia (FTD) (Morita et al., 2006; Caroline Vance et al., 2006), as the prevalence of cognitive and behavioral symptoms in ALS patients is high. This has contributed to redefine ALS as a neurodegenerative rather than a neuromuscular disorder (Hardiman, Al-Chalabi, Chio, Corr, Logroscino, Robberecht, Shaw, Simmons, & Van Den Berg, 2017).

1.2. Incidence of ALS (clinical epidemiology)

Thanks to high-quality European patient registers that were combined to form the European ALS Epidemiology Consortium (EURALS), epidemiological studies of ALS based on population were efficiently conducted and provided data to assess the incidence of ALS in Europe (Logroscino et al., 2010; Rooney et al., 2017). Meta-analysis of numerous international

studies with homogenous rates in populations from Europe, North America and New Zealand (Marin et al., 2017) has established the incidence of ALS at around 2 per 100,000 individuals each year.

The heterogeneity of ALS is also highlighted by sex differences in the onset. Indeed, it has been reported that in Europe, most men have spinal-onset disease whereas women have mostly bulbar-onset disease (Logroscino et al., 2010). This difference of onset can also be observed depending on the region. There are more individuals with bulbar-onset disease in Europe than in Asia, and a difference between Northern and Southern Europe has been noted, with more patients presenting a spinal-onset disease in southern Europe (Logroscino et al., 2010).

The onset of the disease occurs prevalently during adulthood, with a peak age of 58-63 years for sporadic ALS (meaning the causes of the disease remain elusive [covered in section 2.1 *Genetics*]) and 47-52 years for familial cases (meaning the disease is due to inherited genetic mutations [covered in section 2.1 *Genetics*]) (Logroscino et al., 2010), but several studies reported the existence of a young onset ALS defined by a peak age under 40 years and representing 10% of cases (Corcia et al., 2017; Sabatelli et al., 2008). In this particular type of ALS, males are predominant and spinal onset occurs in 80% of cases. Also, survival is longer than in classical ALS with a median around 70 months (Corcia et al., 2017; Sabatelli et al., 2008). Some ALS cases involve patients with motor onset before the age of 25 years and are referred as juvenile ALS. It is usually characterized by spinal onset, a predominant long duration of evolution of upper and lower motor neurons (MN) degeneration leading to facial muscle spasticity with spastic dysarthria and gait (Corcia et al., 2017; Hübers et al., 2015; Orban, Devon, Hayden, & Leavitt, 2007).

1.3. Diagnostic challenge [revised from (Campanari, Bourefis & Kabashi, 2019)]

The difficulty to diagnose ALS resides mainly in the existence of several mimic syndromes, unrelated to ALS but which present similar clinical features (Ghasemi, 2016; B. J. Traynor et al., 2000). Motor neuron diseases (MNDs) are classified in four main groups in which ALS represents the most common form (**Table 1**). While these diseases affect individuals in different ways, they also share several symptoms due to MN loss of function. These disorders present progressive weakening of skeletal muscles, which eventually affects the ability to speak, swallow and breathe. ALS diagnosis is even more difficult if we add to the list other neurological conditions unrelated to MNDs which can mimic its early symptoms. Moreover, increasing evidence points to a possible direct implication of muscle in the early stage of the disease, adding myopathies to the list of ALS-mimic pathologies (reviewed in **Table 1**). Standard diagnostic criteria for ALS have been established in 1991 [El Escorial criteria [EEC] (Brooks, Miller, Swash, & Munsat, 2000)] and were revised in 1997 [AirlieHouse criteria [AHC] or El Escorial Revisited (Miller, Munsat, Swash, & Brooks, 1999)]. Even if the essential requirements for ALS diagnosis were defined by these criteria, many neurologists and neuromuscular clinicians were missing the diagnosis, proving the low clinical accuracy of these diagnostic parameters (Bryan J. Traynor et al., 2000). In 2008, electrodiagnostic studies, known as the Awaji criteria (Mamede De Carvalho & Swash, 2009), were included in the clinical procedure to allow earlier and more accurate assessment of ALS diagnosis. However, the application of those sets of defined features are still insufficient to rule out other similar and related diseases (Davenport, Swingler, Chancellor, & Warlow, 1996; Haverkamp, Appel, & Appel, 1995).

Motor Neuron Diseases (MND):

Amyotrophic Lateral Sclerosis (ALS)

Primary Lateral Sclerosis (PLS)
Progressive Muscular Atrophy (PMA)
Progressive Bulbar Palsy (PBP)

Other neurological conditions that can mimic ALS:

Mitochondrial Disorder (MID)
Pseudobulbar Palsy
Spinal muscular atrophy (SMA)
Primary lateral sclerosis (some subtypes not related to ALS)
Progressive spinal muscular atrophy (some subtypes not related to ALS)
Spinobulbar muscular atrophy (SBMA or Kennedy's disease)
Autoimmune syndromes Monoclonal
Myopathies

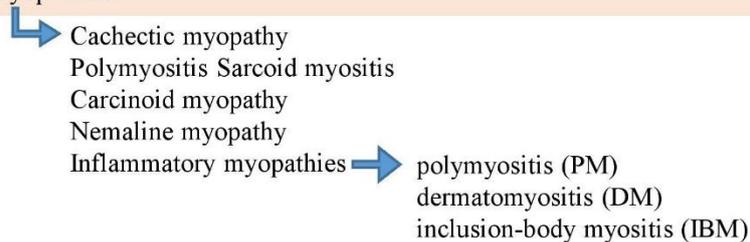


Table 1: General overview of neuromuscular diseases and ALS-mimic pathologies. (Campanari, Bourefis & Kabashi, 2019)

1.4. Diagnostic Methods

ALS diagnosis goes mainly through clinical evaluation. However, in order to obtain an accurate diagnosis, laboratory testing based on advanced techniques of electrodiagnosis, neuroimaging, immunobiochemistry, and neurogenetics, is required. Tests to rule out other neuromuscular conditions may include:

Electromyogram (EMG): The needle EMG is the most important study in determining diagnostic certainty of ALS (Daube, 1985). During this test, a needle electrode is inserted through the skin into various muscles, starting with the most severely involved limb (**Figure 2**). The examination then progresses through four anatomical regions: bulbar, cervical, thoracic, and lumbar. At least three anatomical regions have to be positive to this test to define ALS. The fasciculation potential (FP) has been included in Awaji criteria as a hallmark of ALS muscular

denervation. In general, a decreased number of motor unit recruitment, with long duration of the motor unit potential, and abnormal spontaneous activity, are measured at the EMG in ALS patients [reviewed in (Joyce & Carter, 2013)].

Nerve Conduction Study (NCS): This test measures how fast an electrical impulse moves through the nerve (**Figure 2**). During the test, one electrode placed on the skin stimulates the nerve of interest with a very mild electrical impulse. Variations in time spent to reach a second electrode can help in identifying a nerve damage. While EMG measures the electrical activity in the muscles, the nerve conduction study is specific for nerves and helps to localize the disorder among nerve, neuromuscular junction, and muscle. NCS is a powerful tool to discriminate ALS from axonal demyelination or conduction block impairments (Duleep & Shefner, 2013). NCS parameters are generally normal in ALS, albeit the presence of prolonged distal motor latency and slowed conduction velocity could be consistent with the diagnosis of ALS (Argyriou, Polychronopoulos, Talelli, & Chroni, 2006; M de Carvalho & Swash, 2000). These changes suggest loss of large myelinated fibers, but also motor axons regeneration phenomena (M de Carvalho & Swash, 2000).

Magnetic Resonance Imaging (MRI): This technique is able to produce detailed images of the brain and spinal cord, the latter with the advantage of simultaneously investigating the upper and lower MNs. During several years, its application was related to the exclusion of other disorders, as tumors or hernias that can display certain of the ALS-mimic symptoms (Filippi et al., 2010). The evolution and improvement of this multimodal tool has recently become essential for the diagnosis of ALS. MRI scans can show cerebral degeneration and gray/white matter atrophy [reviewed in (Martin R Turner & Modo, 2010)], and also detect abnormalities in ALS muscle, likely due to denervation atrophy process (Staff, Amrami, & Howe, 2015).

Blood and Urine Tests: Testing hematological factors is helpful to exclude diseases that are capable of mimicking ALS symptoms. Recently, a population- based study, proposed serum

albumin, creatinine levels, and lymphocyte count as markers for ALS, indicating muscle waste and inflammation respectively (Chiò et al., 2014). Other markers potentially related to a better ALS outcome have been proposed: LDL/HDL levels, which are elevated in ALS plasma and represent a general unexplained hypermetabolism (Dorst et al., 2011; L. Dupuis et al., 2008); serum uric acid levels, which are decreased among ALS patients, further demonstrating the possible role of oxidative stress in the induction and propagation of the disease (Keizman et al., 2009); serum ferritin levels which are elevated in ALS patients and could reflect perturbation in iron metabolism (Qureshi, Brown, Rogers, & Cudkowicz, 2008); concentrations of certain amino acids, which are decreased in ALS (Ilzecka, Stelmasiak, Solski, Wawrzycki, & Szpetnar, 2003); levels of serum proinflammatory cytokines, such as IL-6, which are increased in ALS (Ono, Hu, Shimizu, Imai, & Nakagawa, 2001). Finally, high level of circulating Acetylcholinesterase (AChE) and metalloproteinases (MMP) have been reported in ALS plasma (Festoff & Fernandez, 1981; Niebroj-Dobosz, Janik, Sokołowska, & Kwiecinski, 2010) and although the exact source of these two classes of enzymes remains uncertain, it could in part reflect a disruption of extracellularly bound AChE at the NMJ and early change in the nerve-muscle integrity.

Spinal Tap (Lumbar Puncture): Using this particular test, a small amount of cerebrospinal fluid (CSF) is taken from the lower back of the patient for laboratory tests. Thanks to its proximity to the central nervous system, the CSF is considered one preferred tissue to search for ALS biomarkers [reviewed in (Barschke, Oeckl, Steinacker, Ludolph, & Otto, 2017)]. Several markers for ALS have been identified in CSF such as Tau, TDP43, Nefl, and MMP levels [reviewed in (Shaw & Williams, 2000)]. In particular, MMPs with their ability to digest collagen, proteoglycan, and laminin (Vincenti & Brinckerhoff, 2007), may reflect ongoing destruction of the matrix which wraps synapses (Lim et al., 1996) and pathological changes at the brain-blood barrier (Niebroj-Dobosz et al., 2010).

Muscle Biopsy: With this technique, a small portion of muscle is removed by needle biopsy and sent to a laboratory for histopathological analysis. Rarely performed because of its painful and invasive nature, this tool is useful when ALS diagnosis is not clear. Generally, ALS muscles present signs of active denervation/reinnervation and an increased number of atrophic fibers (Jensen, Jørgensen, Bech, Frandsen, & Schrøder, 2016).

Genetic Testing: People with familial ALS (fALS) background can get an efficient diagnosis through genetic testing (Chiò et al., 2014). This technique may help ALS patients to understand the basis of their condition, and improve the genotype-specific treatments (Roggenbuck, Quick, & Kolb, 2017). Unluckily, there is a lack of consensus among clinicians above the definition of fALS, since new genes related to ALS are continuously found (Vajda et al., 2017). Nowadays, genetic testing is not wildly used because of its high cost and the belief that ALS genetics is not well-enough understood to provide a better treatment plan, as reported in 2017 in a study which involved 167 clinicians from 21 different countries around the world (Vajda et al., 2017).

Accurate diagnosis is crucial to provide adequate counseling and information about the prognosis and disease course, and to avoid inappropriate therapy. Moreover, a good diagnosis could provide a more equal stratification of cases and be important in the choice of additional medical support, as for example nutritional intervention strategies or physical therapy. Currently, there is no common consensus in the use of laboratory analysis for ALS diagnosis. Basically, clinicians decide for the application of certain techniques based on their experience, expertise and hospital practice. Progress in molecular genetics and identification of specific biomarkers is ongoing, which will translate to a refined diagnostic certitude. Therefore, there is the emerging need to establish a widely accepted protocol for laboratory tests to discriminate the majority of cases that present clinical features resembling ALS.

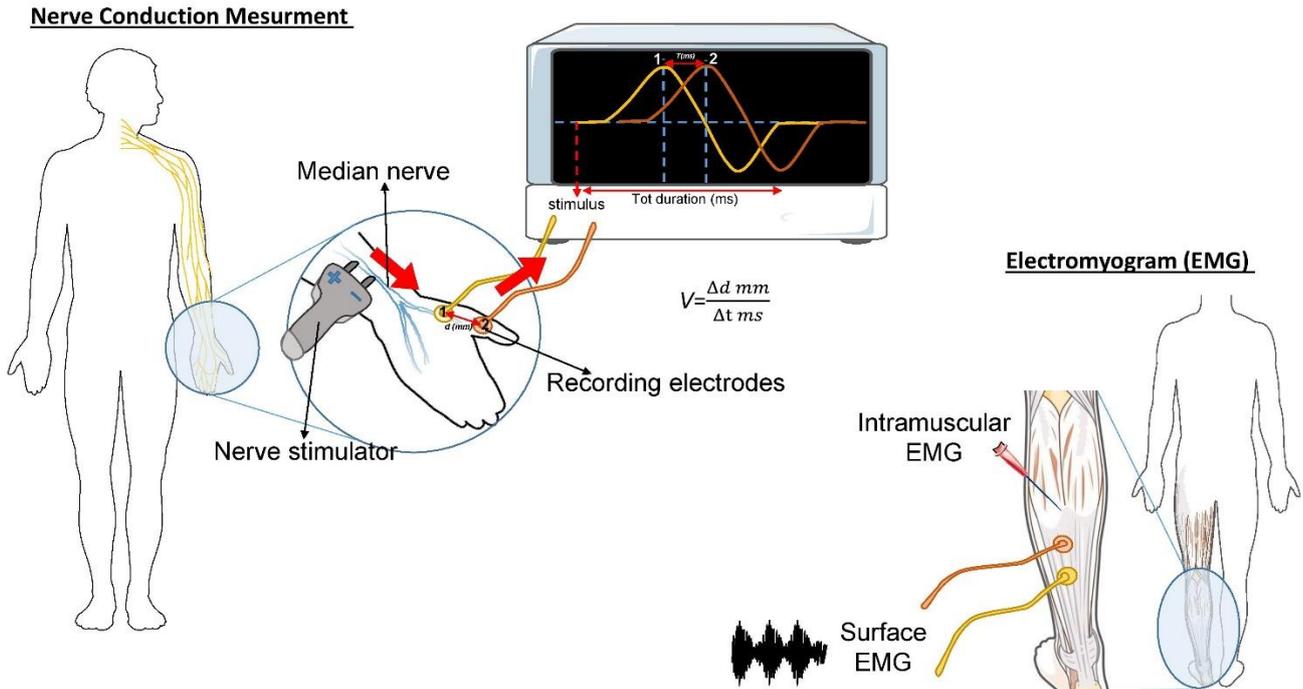


Figure 2: Schematic representation of the nerve conduction and muscle contraction studies. Nerve Conduction Velocity (NCV - left) measures the velocity and the quality of conduction of the electrical signal in a nerve. During the test, the nerve is electrically stimulated and two recording electrodes, placed on the skin at a specific distance from each other (in the order of millimeters), receive the impulse. The time (in milliseconds) spent by the impulse to move from a point to another represent the velocity. In ALS, the impulse conduction is slower than control cases and is worsened by the increase of axonal degeneration. The electromyogram (EMG-right) measures the electrical activity of the muscles at rest and during contraction. There are two kinds of EMG: surface EMG and intramuscular EMG. In the first, the muscle activity is recorded by one or more electrodes patched on the skin and it assess the contractile response of superficial muscles. However, the result can be influenced by the depth of the subcutaneous tissue and the discharges of adjacent muscles. With the intramuscular EMG, specific deep muscle activity is recorded by using one needle electrode inserted into the muscle. EMG and NCV tests are often done together to give more complete information. (Campanari, Bourefis & Kabashi, 2019).

1.5. Treatment challenges

In 150 years of recorded history on ALS, there has been no treatment that significantly slows down or reverses symptoms in the majority of patients despite more than 200 ALS clinical trials having been conducted worldwide (Jaiswal, 2019). Riluzole was the first drug approved in the

USA by the Food and Drug Administration (FDA) for clinical use in the 1990s (Lacomblez, Bensimon, Meininger, Leigh, & Guillet, 1996). It clinically acts to increase the survival of patients of approximately 3 months (18,19 from Jaiswal), and many pharmaceutical experiments showed disease progression delays in transgenic ALS animal models, but overall it failed to show significant efficacy in clinical trials or are still in Phase I-III trials (Jaiswal, 2019). Very recently, in 2017, ederavone, a new drug developed by Mitsubishi Tanabe Pharma, was approved by the FDA after showing efficiency in significantly reducing ALS progression during early disease stages (Abe et al., 2017). However it was not approved by the European Medicines Agency and the idea of providing ederavone to all patients with ALS regardless of clinical presentation is under debate (Hardiman & van den Berg, 2017).

1.6. Environmental causes of ALS

Environmental factors were the focus on several epidemiological studies. From these analyses, various factors have been put to light to extend the current understanding of the implication of the environment, such as exercise, smoking, pesticides, exposure to heavy metals, cyanotoxins found in food, electric shocks or geographical clustering [reviewed in (Al-Chalabi & Hardiman, 2013)]. However, for most of the studies, the evidence for the implication of these factors is not clear-cut. Actually, many reasons make the identification of environmental risk factors difficult. Indeed, contrary to the genetic profile that does not change from birth, the exposition to the environment changes over time and can only be measured by prospective observation or recall. The only solution would be very detailed longitudinal observations over a lifetime in large numbers of people with similar genetic backgrounds (Al-Chalabi & Hardiman, 2013). Another point to consider is that environmental exposures most likely act on specific genetic background, highlighting a gene-environment interaction. Thus, without knowing the genetic background of the participants, interpreting the study results can be challenging. Hence, the

majority of studies that constituted most of the knowledge around ALS are focused on genetics, and will be covered in the next section.

2. Genetic causes and pathology of ALS

2.1. Genetics

Although the majority of ALS cases occur sporadically (sALS), there is a Mendelian inheritance in about 10% of the cases (familial ALS, fALS), mainly in an autosomal, dominant fashion (Strong, Hudson, & Alvord, 1991). The two are clinically indistinguishable and a variety of genetic defects in more than 20 genetic loci have been linked with the ALS phenotype (R. H. Brown & Al-Chalabi, 2017), with new genes constantly being identified in subsets of ALS patients (Brenner et al., 2016; Freischmidt et al., 2015; I. R. Mackenzie et al., 2017) (**Figure 3**). In all these studies, evidence points out oligogenic (phenotype determined by more than one gene) and pleiotropic (multiple phenotypes from a single gene) implications, and among these familial cases, four major genes, which mutations are known to cause ALS, are the following: chromosome 9 open reading frame 72 (C9orf72), superoxide dismutase 1 (SOD1), transactive response DNA-binding protein (TARDBP) and fused in sarcoma (FUS) (Kwiatkowski et al., 2009; Renton et al., 2011; Rosen et al., 1993; Sreedharan et al., 2008). Other genes have been identified to be associated to ALS, including ATXN2, TBK1 or TUBA4A, and provide additional information on the complex genetic spectrum of ALS pathology, but they will not be covered in this overview. Further information can be found in recent extensive reviews (R. H. Brown & Al-Chalabi, 2017; Ragagnin, Shadfar, Vidal, Jamali, & Atkin, 2019).

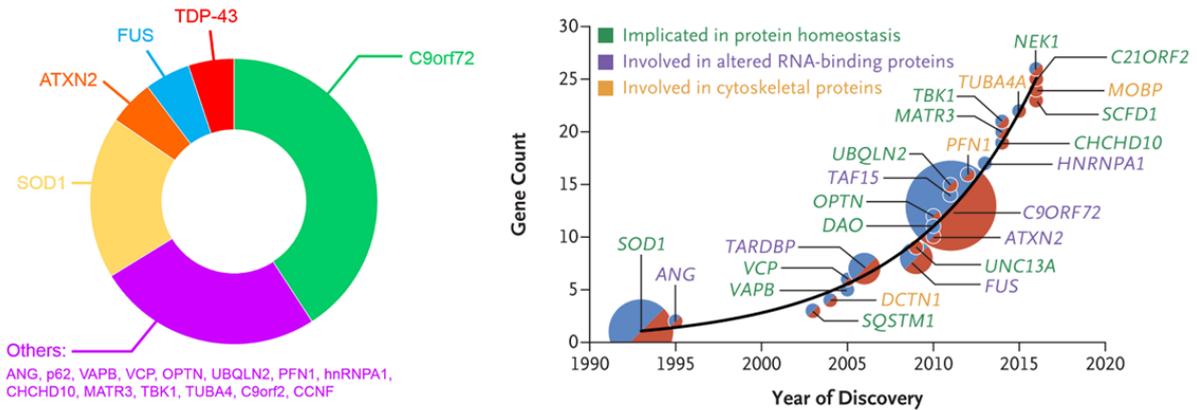


Figure 3: Frequency of mutated genes in fALS patients and ALS gene discovery since 1990 (derived from Ragagnin et al., 2019 and Brown et al., 2017)

Superoxide dismutase 1 (SOD1): In 1993, the identification of missense mutations in SOD1 represented the first significant clue that highlighted the underlying genetic cause of ALS (Rosen et al., 1993). More than 160 mutations have been identified in ALS patients with a frequency of mutations varying from 12 to 23% in familial cases and from 0 to 7% in sporadic cases (Kabashi, Valdmanis, Dion, & Rouleau, 2007; Kaur, McKeown, & Rashid, 2016; Saccon, Bunton-Stasyshyn, Fisher, & Fratta, 2013). These mutations are responsible of a toxic gain of function of SOD1, even though some reported the implication of a SOD1 loss of function in the appearance of ALS (Saccon et al., 2013). The gene encodes for a homodimeric Cu/Zn-binding enzyme that catalyzes the inactivation of superoxide into oxygen and hydrogen peroxide, providing antioxidant defense mechanism to the cells (Gurney, Liu, Althaus, Hall, & Decker, 1998). In pathological conditions, several mechanism alterations have been linked to SOD1 mutations, including excitotoxicity, mitochondrial metabolism alteration, axonal transport defect or apoptosis (Pasinelli & Brown, 2006).

The discovery of SOD1 mutations in ALS patients was followed by the development of several SOD1 transgenic rodent models. These models recapitulate main feature of ALS, notably motor neuron degeneration, reduced lifespan, SOD1/ubiquitin-positive aggregates, or aberrant

oxidative metabolism (reviewed in Orietta Pansarasa et al., 2018). They thus allowed to elucidate various mechanisms in the pathology of ALS and underpinned most of the current knowledge of the disease (reviewed in Orietta Pansarasa et al., 2018; Van Damme, Robberecht, & Van Den Bosch, 2017). However, therapeutic strategies based on these models failed to provide significant effects in ALS patients. The identification of other major genes implicated in ALS in the following years provided more insights toward therapeutic clues.

Chromosome 9 open reading frame 72 (C9orf72): the C9orf72 gene is located on the short arm of chromosome 9 and encodes for a protein located in the cytoplasm of neurons and in presynaptic terminals. Linkage analysis of North European patients with ALS and FTD suggested a susceptibility locus on the short arm of chromosome 9 (Morita et al., 2006; Van Es et al., 2009; Caroline Vance et al., 2006). In 2011, hexanucleotide repeat GGGGCC (G4C2) expansion (HRE) within the first intron of C9orf72 was identified as a pathogenic mutation in ALS and FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). In healthy conditions, there are less than 20-30 HRE, whereas in mutated patients, hundreds of repeats can occur. It has been later proven that HRE of the C9orf72 gene is a major genetic factor in ALS and FTD patients, as it is detected in approximately 40% of fALS and FTD, and 9% of sALS cases (Majounie et al., 2012), as well as other neurodegenerative disorders like Alzheimer's disease or Parkinson's disease (Beck et al., 2013; DeJesus-Hernandez et al., 2013; Harms et al., 2013; Theuns et al., 2014).

C9orf72 HRE-linked pathogenicity in ALS/FTD has been suggested to be explained by loss and gain of function processes through different hypotheses: the first supposed that inhibition of endosomal trafficking and the perturbation of endocytosis, leading to autophagy, caused by a reduction in the C9ORF72 protein level (Koppers et al., 2015). The second suggested a neurotoxicity of HRE by forming RNA foci causing a sequestration of RNA binding proteins

and disabling the RNA processing machinery (Y.-B. Lee et al., 2013). The third hypothesis was based on the repeat-associated non-AUG translation of G4C2 RNA to generate five different toxic dipeptide repeat proteins (DPR) called “polyGA, “polyGP, “polyGR”, “polyPA” and “polyPR” (Y.-B. Lee et al., 2017) that can have an influence on activation of programmed cell death and trans-activation response (TAR) DNA- binding protein 43 (TDP-43) cleavage (Y.-B. Lee et al., 2017).

The first vertebrate model of C9orf72 loss of function was developed by Ciura and colleagues in 2013 and allowed to assess the effects of a knockdown of the zebrafish homolog to human C9orf72. It resulted in reduced motor function that was rescued by co-expression with human C9orf72 mRNA (Sorana Ciura et al., 2013). Other models, ranging from *Drosophila* to mouse models (Morrice, Gregory-Evans, & Shaw, 2018; W. Xu & Xu, 2018), were developed and presented features similar to ALS, including paralysis, MN loss, impaired neuromuscular junction integrity, DPR protein aggregation, TDP-43 cytoplasmic aggregation and reduced lifespan (Y. Liu et al., 2016; Morrice et al., 2018).

Transactive response DNA-binding protein (TARDBP): In 2006, studies reported the identification of the gene coding for the protein TDP-43, a nuclear RNA/DNA binding protein, as a major actor in ALS and FTD pathogenesis, and the main component of ubiquitinated inclusions (Arai et al., 2006; M. Neumann et al., 2006). These inclusions have been found in approximately 97% of ALS cases (22,23). TDP-43 is involved in many essential roles for cell survival, including transcriptional repression, pre-mRNA maturation and alternative splicing, mRNA transportation, microRNA biogenesis, interaction with noncoding RNA, autoregulation, and translational regulation of key proteins [reviewed in (Guerrero et al., 2016)].

TDP-43 is mostly characterized by cytoplasmic mislocalization and aggregation in ALS, as well as FTD, and its aggregated forms are described by abnormal phosphorylation, truncation, and cytoplasmic mislocalization (Kabashi et al., 2008; Sreedharan et al., 2008). From this, it has been hypothesized that neurodegeneration might occur through gain of toxic TDP-43 or through loss of function of TDP-43 and a number of studies explored these hypotheses through different animal models. Indeed, overexpression of TDP-43 was shown to recapitulates TDP-43 proteinopathy and ALS phenotypes, supporting a role for gain of toxic function in disease (Ash et al., 2010; Y. Li et al., 2010; Wils et al., 2010). Knock-in mouse models for mutant TARDBP were also used and displayed neurodegenerative features, suggesting disease progression contribution by gain of toxicity function (Gao, Wang, Huntley, Perry, & Wang, 2018; Sreedharan et al., 2008).

To examine loss of function, TDP-43 knockout mice were first used and revealed embryonic lethality (Kraemer et al., 2010; Chantelle F. Sephton et al., 2010; L. S. Wu et al., 2010). Afterwards, other models demonstrated progressive motor phenotype and typical TDP-43 proteinopathy (Iguchi et al., 2013; Kabashi et al., 2009a; L. S. Wu, Cheng, & Shen, 2012). From these reports, it is estimated that both gain and loss of function of TDP-43 may be involved in ALS pathomechanisms [reviewed in (Scotter, Chen, & Shaw, 2015)].

Fused in sarcoma (FUS): Like TDP-43, FUS is an RNA/DNA binding protein, ubiquitously expressed in both the nucleus and cytoplasm in many cell types, but almost exclusively in the nucleus in neurons and glial cells (Åman et al., 1996b; Andersson et al., 2008a). The gene was first identified as a fusion oncogene on chromosome 16 in human liposarcoma (Crozat, Åman, Mandahl, & Ron, 1993). It was then associated with ALS pathology in 2009 (Kwiatkowski et al., 2009; Vance et al., 2009), with currently more than 50 missense mutations reported mostly located in the exon 15 which encodes for the NLS (nuclear localization signal) at the C-terminal

region of the protein (Lattante et al., 2013). These mutations are known to cause the redistribution of FUS into the cytoplasm and the accumulation of protein inclusions (Lagier-Tourenne et al., 2012a; Lattante et al., 2013; Rademakers et al., 2010). This suggests a loss of FUS normal function or gain of toxic function in the cytoplasm (Liuqing Yang, Gal, Chen, & Zhu, 2014).

As FUS is the main focus of this work, a more in-depth review of genetic features in patients carrying FUS mutations and features of FUS animal and cellular models will be presented in section 4 (The gene FUS).

2.2. Pathophysiology/cellular mechanisms

As stated before, from the identification of the genetic causes of ALS, a variety of animal models have been developed to model the mechanisms involved in disease pathobiology (**Figure 4**). They provided substantial clues that enriched our understanding of neurodegeneration, with various pathophysiological mechanisms having been identified that includes protein homeostasis, RNA metabolism, different transport processes, excitability, neuroinflammation and mitochondrial dysfunction. I will review here the major pathogenic mechanisms in ALS.

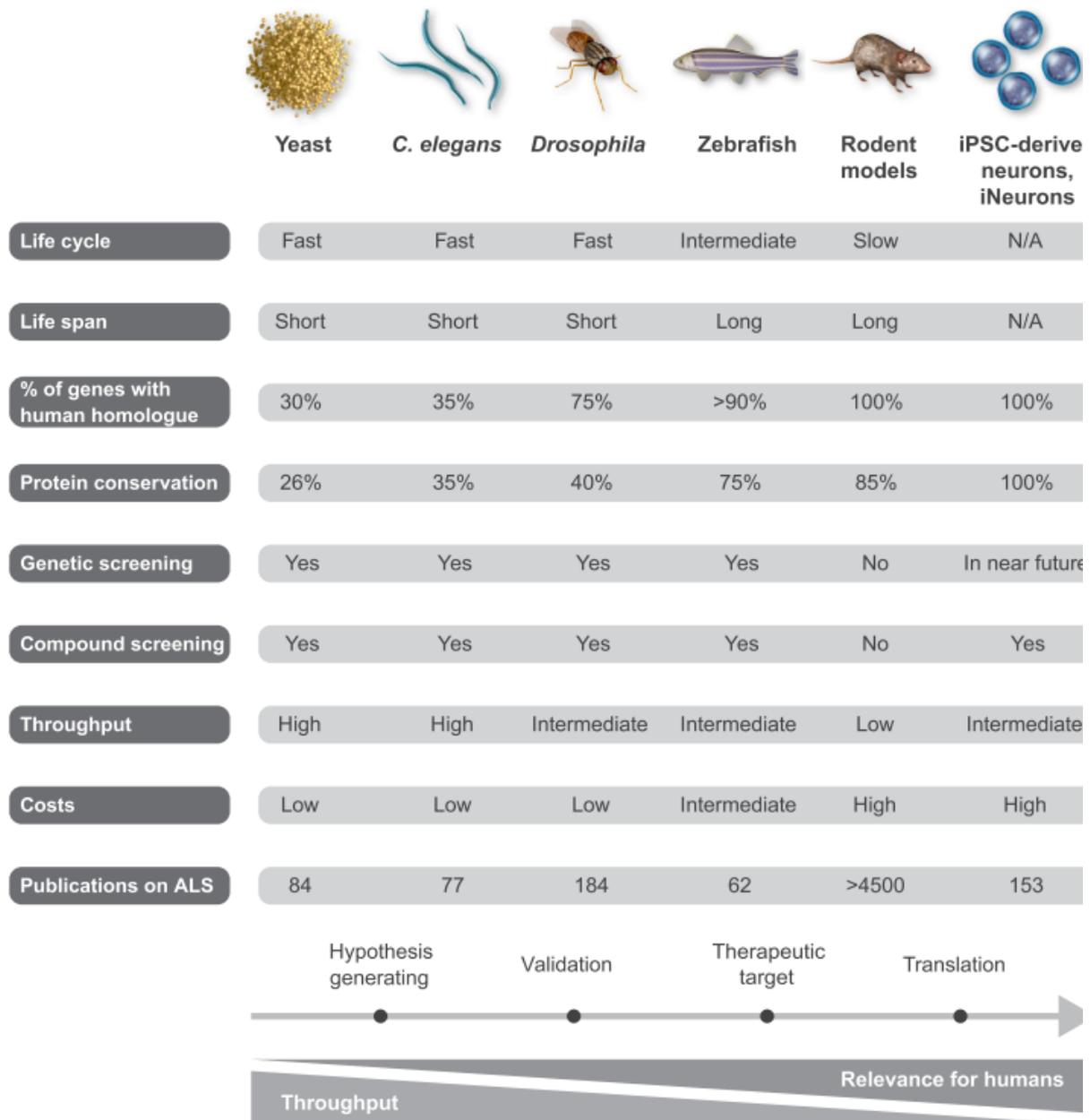


Figure 4: Model organisms used in ALS with their advantages and limitations (derived from Van Damme et al., 2017)

Altered protein homeostasis: The hallmark of ALS pathology is the formation of misfolded and mislocalized proteins, referred as toxic aggregates that are known to disrupt protein homeostasis in affected neurons (Bertram & Tanzi, 2005; Soto, 2003). Several ALS-associated genes have been linked to the detection of the misfolded and aggregated forms of their respective proteins in affected MNs of sALS and fALS patients (Bosco, Morfini, et al., 2010;

Forsberg et al., 2010; M. Neumann et al., 2006; Renton, Chiò, & Traynor, 2014; Sharma et al., 2016a). For instance, cytoplasmic proteinaceous aggregates of TDP-43 have been found in different cell types and tissues of ALS patients, including neurons and glial cells, the spinal cord, the primary motor cortex or the brain stem (Arai et al., 2006; Hasegawa et al., 2008; M. Neumann et al., 2006).

Apart from protein aggregation, genes involved in proteostasis control have been linked to ALS and increasing evidence demonstrated a role of the protein-folding and degradation machinery in the MN impairments found in ALS (Rozas, Bargsted, Martínez, Hetz, & Medinas, 2017; Ruegsegger & Saxena, 2016). This cell machinery uses two systems for protein degradation: the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathway. The UPS is a cell mechanism consisting of tagging short-lived and soluble proteins with ubiquitin molecules in order to be degraded by the proteasome. Autophagy, on the other hand, will degrade long-lived cytoplasmic proteins, soluble and insoluble misfolded proteins, and even entire organelles [reviewed in (Kurtishi, Rosen, Patil, Alves, & Møller, 2019; Medinas, Valenzuela, & Hetz, 2017; N. Ramesh & Pandey, 2017)].

UPS: Genes encoding for proteins involved in the UPS have been associated with fALS. One study reported reduced expression of components of the UPS in a neuronal cell mutant of SOD1 (Urushitani, Kurisu, Tsukita, & Takahashi, 2002). The same was found in mice expressing a SOD1 mutation (Kabashi, Agar, Taylor, Minotti, & Durham, 2004). Others showed that the alteration of the roles of valosin-containing protein (VCP) and ubiquilin-2 (UBQLN2) in substrate delivery to the proteasome were due to the presence of ALS-associated mutations (L. Chang & Monteiro, 2015; H. X. Deng et al., 2011; Johnson et al., 2010). In addition, it was demonstrated that TDP-43 was a substrate of the UPS (Scotter et al., 2014), and TDP-43 mislocalization associated with MN pathology was caused by knock-down of proteasome components (Tashiro et al., 2012).

Autophagy: Numerous studies reported the importance of autophagy in the contribution to the pathological formation of aggregates in ALS. C9orf72 has a major role in autophagy initiation and it was shown that its depletion in mouse cortical neurons caused the aggregation of p62, a key receptor protein in autophagy, which is a main pathological feature in ALS/FTD (Sellier et al., 2016; Sullivan et al., 2016). Other genes implicated in autophagy have been reported to be associated with ALS. Among them, mutations in UBQLN2, optineurin (OPTN) and sequestosome 1/p62 (SQSTM1/p62) play a role in ALS disease appearance (Goode et al., 2016; Hjerpe et al., 2016; Katsuragi, Ichimura, & Komatsu, 2015; Y. C. Wong & Holzbaur, 2014). A regulator of p62 and OPTN, serine/threonine-protein kinase TBK1 is also associated with ALS pathology (Matsumoto, Shimogori, Hattori, & Nukina, 2015; Weidberg & Elazar, 2011). VCP mutations causing a reduction of VCP activity led to a decrease of autophagosome maturation (Ju et al., 2009). Also, SOD1 and TDP-43 were reported to be substrates of autophagy, which can explain their aggregation in ALS in the case of a deficiency in autophagy (Bose, Huang, & Shen, 2011; Kabuta, Suzuki, & Wada, 2006; N. Ramesh & Pandey, 2017; Q. Xia et al., 2016; Yung, Sha, Li, & Chin, 2016).

RNA metabolism: With the identification of mutations in RNA-binding proteins such as TARDBP and FUS in ALS, mRNA processing has been the focus of many ALS-related studies [reviewed in (Conlon & Manley, 2017; Droppelmann, Campos-Melo, Ishtiaq, Volkening, & Strong, 2014)].

As stated before, RNA-binding proteins play a major role in the multiple processes of RNA metabolism from transcription to mRNA maturation and degradation (Dreyfuss, Kim, & Kataoka, 2002) (**Figure 5**). TDP-43 and FUS are mislocalized in the cytoplasm in pathological conditions, suggesting alteration of RNA processing of their targets (Amlie-Wolf et al., 2015; Y. Zhou, Liu, Öztürk, & Hicks, 2014). Indeed, loss of TDP-43 function could lead to disease-

specific splicing hallmarks (J. P. Ling, Pletnikova, Troncoso, & Wong, 2015; Polymenidou et al., 2011a; Xiao et al., 2011) and showed altered transport of distally targeted mRNAs (Ishiguro, Kimura, Watanabe, Watanabe, & Ishihama, 2016). Reports also highlighted transcriptome alteration in models of TARDBP-related ALS (Arnold et al., 2013), and misregulation of gene expression has also been observed (Chen-Plotkin, Lee, & Trojanowski, 2010; Ratti & Buratti, 2016; Walsh et al., 2015).

Apart from TDP-43, other genes involved in RNA metabolism have been associated to ALS, including ANG (Chan, Hieter, & Stirling, 2014; Skourti-Stathaki, Proudfoot, & Gromak, 2011), SETX (Pizzo et al., 2013; S. K. Saxena, Rybak, Davey, Youle, & Ackerman, 1992), ELP3, TAF15 or EWSR1, other DNA/RNA -binding proteins structurally similar to FUS (Couthouis et al., 2012; Kapeli et al., 2016; C. L. Simpson et al., 2008), that play different roles in transcription, elongation and alternative splicing (Han et al., 2008; B. Huang, Johansson, & Byström, 2005).

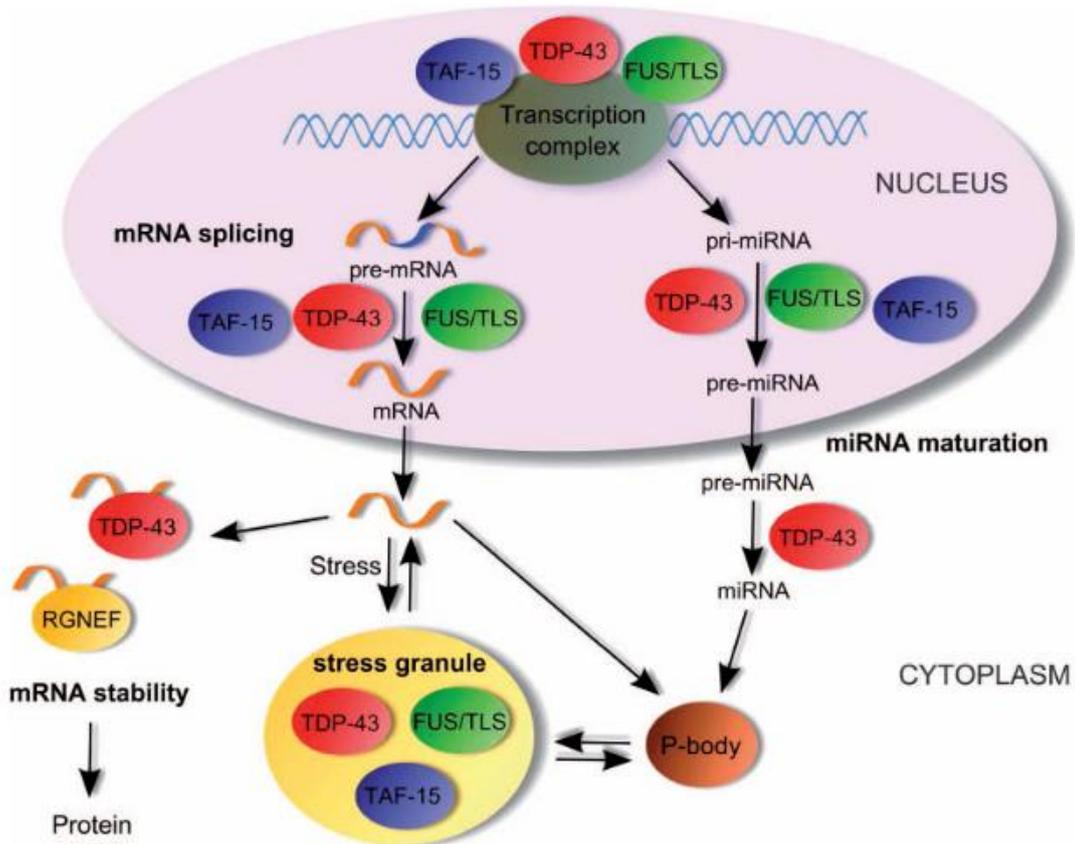


Figure 5: RNA metabolism pathways involving ALS-associated proteins (derived from Droppelman et al. 2014)

Another mechanism involved in the survival of cells that implicates RNA metabolism is the adaptation to stressful conditions through the formation of stress granules (SGs). SGs are a form of highly dynamic ribonucleoprotein granules that facilitates cell survival by translational arrest of non-essential transcripts and by sequestering pro-apoptotic proteins and regulators of cell growth [reviewed in (Droppelmann et al., 2014)]. TDP-43, like FUS, in response to stress, shuttle to the cytoplasm and colocalize with SGs (Ayala et al., 2008; H Zinszner, Sok, Immanuel, Yin, & Ron, 1997a). The link between TDP-43 and SGs is still unclear, with studies suggesting an induction of SG formation from TDP-43 overexpression (Freibaum, Chitta, High, & Taylor, 2010; Liu-Yesucevitz et al., 2010) but insufficient without additional stress like oxidative and osmotic stress (Dewey et al., 2011; Meyerowitz et al., 2011). Others showed a decrease in the number of cells containing SGs and the size of SGs after oxidative stress when

TDP-43 is knocked down (Aulas, Stabile, & Vande Velde, 2012). Another study from McDonald et al. (2011) proved that TDP-43 plays an active role in the cellular stress response by showing that TDP-43 and its binding partner, the heterogeneous nuclear ribonucleoprotein hnRNP A2, are components of SGs induced by oxidative stress and contribute to SG formation and maintenance (McDonald et al., 2011).

Other ALS-associated genes have been related to SGs, like TAF15 (Andersson et al., 2008a; Blechingberg, Luo, Bolund, Damgaard, & Nielsen, 2012; Marko, Vlassis, Guialis, & Leichter, 2012), TIA1 (Apicco et al., 2017; Mackenzie et al., 2017), or the polyglutamine (polyQ) protein Ataxin-2 (Ralser et al., 2005).

Aberrant excitability: Nerve excitability is a well-studied parameter that provides, through threshold-tracking techniques, *in vivo* assessment of changes in the properties of the axonal membrane, including the function of ion channels, pumps and transporters of myelinated axons (Bostock, Cikurel, & Burke, 1998; Kiernan & Kaji, 2013; Krarup & Moldovan, 2009; Matamala et al., 2018).

Excitability has been the focus of many studies on neuromuscular disorders (Krishnan, Lin, Park, & Kiernan, 2009). Indeed, impairments in Na⁺ and K⁺ conductances have been significantly linked to ALS (Bostock, Sharief, Reid, & Murray, 1995; Geevasinga et al., 2015; Kanai et al., 2006; Mogyoros, Kiernan, Burke, & Bostock, 1998; Tamura et al., 2006; Vucic & Kiernan, 2006b), causing muscle cramps and fasciculation in patients (that is, brief spontaneous contraction that affects a small number of muscle fibers, provoking a flicker of movement under the skin) (Weiss et al., 2016). Numerous studies referred to specific parameters to assess the excitability changes in ALS. Among them, the strength-duration time constant (STD) is a membrane time constant, assessing the relationship between stimulus strength and duration (S.

B. Park, Kiernan, & Vucic, 2017), and prolongation of this constant has been identified in sALS patients and in neurodegeneration (Kanai et al., 2003; Kuo, Siddique, Fu, & Heckman, 2005; Mogyoros et al., 1998; Shibuya et al., 2013; Vucic & Kiernan, 2006a, 2006b, 2010), as well as fALS forms linked to SOD1 and c9orf72 mutations (Geevasinga et al., 2015; Vucic & Kiernan, 2010; Vucic, Nicholson, & Kiernan, 2008). The changes in this STD constant, as briefly stated before, are underlined by upregulation of Na⁺ conductances and reduction in K⁺ channel conductances (Kanai et al., 2006; Kuo et al., 2005; Urbani & Belluzzi, 2000), with the one causing an increase in the tendency to membrane depolarization and the other causing a decrease of the hyperpolarizing drive, leading to membrane hyperexcitability (C Kiernan DSc et al., 2011).

These mechanisms have been studied thoroughly with the help of different ALS models. Reduced expression of Na⁺/K⁺ ATPase activity was reported in sALS and fALS patients, and expression of this ATPase seemed to be neuroprotective in transgenic mouse models (Ellis, Rabe, & Sweadner, 2003). Furthermore, a mouse model of SOD1 showed less resistant axons to activity-induced changes in intracellular ion concentrations and degeneration (Alvarez, Calin, Graffmo, Moldovan, & Krarup, 2013), highlighting the implication of Na⁺/K⁺ ATPase impairment in ALS. Another report identified a dysfunction of Na⁺/K⁺ ATPase activity in the spinal cords of SOD1^{G93A} mice and ALS patients that was associated with accelerated neuronal degeneration in the mouse model (Rueggsegger et al., 2016). Another information to note is that it has been suggested that an influx of Na⁺ ions causes an inversion of operation of the Na⁺/Ca²⁺ exchanger that would provoke an intra-axonal accumulation of Ca²⁺ ions, and this would activate Ca²⁺-dependent enzyme pathways ultimately leading to MN degeneration (Stys, 1998, 2005, 2007; Waxman, 2006). Such a mechanism has been explored and studies found that a reduction of Na⁺/K⁺ ATPase activity in transgenic ALS mouse models lead to an increase in intracellular Na⁺ ion concentration, resulting in Ca²⁺-mediated neurodegeneration (Stys, 2005).

Other studies brought to light significant clues associating hyperexcitability and abnormal Na⁺ conductances with ALS through transgenic mouse models (Vucic, Rothstein, & Kiernan, 2014). Indeed, SOD1^{G93A} mouse models revealed an upregulation of persistent Na⁺ currents (Kuo et al., 2005; Pieri, Carunchio, Curcio, Mercuri, & Zona, 2009; Quinlan, 2011) that could be inhibited by riluzole (Kuo et al., 2005). In addition, another report found that reduction of persistent Na⁺ conductances by specific (mexiletine) or nonspecific (spermidine and riluzole) agents inhibited hyperexcitability and prevented MN degeneration (Fritz et al., 2013), suggesting blocking Na⁺ channels as potential therapeutic lead in ALS.

Using induced pluripotent stem-cell (iPS) techniques, more evidence supporting the role of axonal ion-channel dysfunction in ALS has been put to light. More precisely, iPS-derived MNs from ALS patients carrying SOD1, C9orf72 and FUS mutations showed a decrease in K⁺ currents (Wainger et al., 2014). In the same study, they used retigabine to activate voltage-gated K⁺ channels and observed a reduction in hyperexcitability and improved survival (Wainger et al., 2014). Another study went through similar techniques to show that hyperexcitability is an early feature in ALS patients with C9orf72 and TARDBP mutations (Devlin et al., 2015).

However, another pool of studies suggested that hyperexcitability had a neuroprotective effect in ALS and failed to induce neurodegeneration in mouse models (Leroy, Lamotte d'Incamps, Imhoff-Manuel, & Zytnicki, 2014; S. Saxena et al., 2013). Thus, the importance of excitability in ALS still needs clarification, although several clinical trials used it as a potential biomarker for therapy, with some revealing stabilization of axonal ion channel function and reduced rate of lower motor neuron dysfunction in patients treated with flecainide (S. B. Park et al., 2015).

Axonal transport: Defects in axonal transport have also been linked to ALS pathogenesis and are one of the first hallmarks of the disease. It allows the distribution of cargo like proteins, mRNAs, vesicles or organelles between the cell body and different cellular sites, like synapses,

to ensure a good neuronal function and survival. For this, motor proteins, such as kinesins and dynein, play a key role in moving axonal cargo along microtubules, and numerous studies reported the implication of kinesins in ALS through the identification of kinesin-5A (KIF5A) mutations causing ALS (Brenner et al., 2018; Nicolas et al., 2018; K. Zhang et al., 2019). Another study using a loss-of-KIF5A-function mouse model showed neurodegeneration and paralysis (C. H. Xia et al., 2003). Dynein 1, responsible for retrograde axonal transport, is regulated by adapter complexes including dynactin, which contains p150^{Glued}, a protein encoded by the gene DCTN1, that was reported in sALS and fALS patients (Münch et al., 2007). Also, studies linked SOD1 mutation to dynein-mediated transport through findings in SOD1^{G93A} transgenic mice displaying defective dynein retrograde transport in their MNs (Bilsland et al., 2010; Kieran et al., 2005).

Other genes identified in ALS patients, such as neurofilament heavy chain (NFH) (Mizusawa et al., 1989), tubulin β -4A (TUBA4A) or peripherin (PRPH) (Gros-Louis et al., 2004), are implicated in axonal transport, and underlie, with all the discussed studies before, the importance of the role played by this machinery in ALS pathogenesis.

Intracellular trafficking: An increasing number of studies highlight an association between cell trafficking impairments and ALS pathogenesis. C9orf72 has been one of the most extensively studied gene in this matter, and reports showed its implication in surface expression, trafficking and recycling of cell surface receptors. Indeed, a study showed in induced motor neurons from C9orf72 patients elevated cell surface levels of NMDA and AMPA receptors, as well as a defective trafficking of Mannose-6-phosphate receptors (M6PRs), a transmembrane receptor playing a role in targeting lysosomal enzymes to lysosomes (Shi et al., 2018), suggesting defects in the intracellular trafficking pathway. Furthermore, it has been suggested that C9orf72 is linked to abnormal endosomal recycling with the finding of a decreased

expression of Vps26, a component of the retromer complex (a complex of proteins involved in recycling transmembrane receptors from endosomes to the Golgi network (Seaman, 2012)) (Aoki et al., 2017; Burd & Cullen, 2014). But most significantly, C9orf72 has been associated with Rab-GTPases, which are key controllers of the intracellular trafficking pathways including vesicle formation, movement and membrane fusion [reviewed in (Burk & Pasterkamp, 2019; Stenmark, 2009; Zhen & Stenmark, 2015)].

TDP-43 has also been reported to be implicated in trafficking dysfunction. Loss of TDP-43 has been shown to alter dendritic endosomes, impacting neuronal survival or axonal innervation (Schwenk et al., 2016). Other genes like ALS2 and UNC13A were associated with ALS and showed defects in endosomal and vesicle transport.

In these different transport pathways, disruption in the transport system between endoplasmic reticulum (ER) and Golgi has been linked to ALS-associated genes. Among these genes, C9orf72, OPTN, SOD1, TARDBP and FUS have been found to be implicated in ER-Golgi transport impairments (Atkin et al., 2014; Braakman et al., 2014; S. Liu & Storrie, 2012; Mitne-Neto et al., 2011; Nagabhushana et al., 2010; Soo et al., 2015). Studies found that Golgi-associated vesicular trafficking is inhibited in cells expressing these mutated proteins (Atkin et al., 2008; Soo et al., 2015; Sundaramoorthy et al., 2013; van Dis et al., 2014). Also, ER-Golgi transport is regulated by a family of proteins, the vesicle-associated membrane protein-associated protein or VAP, like VAPA and VAPB (Lev, Halevy, Peretti, & Dahan, 2008; Peretti, Dahan, Shimoni, Hirschberg, & Lev, 2008; Soussan et al., 1999), and it has been reported that mutations on VAPB cause ALS (Y.-B. Lee et al., 2017). Moreover, VAP protein levels were reduced in different ALS patients and models (Anagnostou et al., 2010; Mitne-Neto et al., 2011; Teuling et al., 2007).

Neuroinflammation: Studies demonstrated the association between neuroinflammation, characterized by microglial and astrocyte activation, T cells infiltration, as well as overproduction of inflammatory cytokines, and ALS in patient autopsies and rodent models (Brites & Vaz, 2014b; Corcia et al., 2012; Komine & Yamanaka, 2015).

Through positron emission tomography (PET), it has been observed that widespread microglial activation occurred in the brain of living ALS patients and SOD1^{G93A} mice (Corcia et al., 2012; Gargiulo et al., 2016; M R Turner et al., 2004). Furthermore, in mutant SOD1 transgenic mice, when the mutant SOD1 expression in microglia was reduced, and when microglia from the mutant was replaced by wild-type microglia, MN degeneration was delayed and animal survival was improved (Beers et al., 2006; Boillée et al., 2006). Another study demonstrated that loss of C9orf72 function in mice caused lysosomal trafficking impairments as well as different defects in microglia activity and neuroinflammation (O'Rourke et al., 2016).

Astrocytes are also major actors in ALS with many ALS-associated genes being expressed in this particular cell type (Bruijn et al., 1997; Forsberg, Andersen, Marklund, & Brännström, 2011; Nagai et al., 2007; H. Zhang et al., 2008). These cells usually clear excess glutamate from synaptic clefts through glutamate transporters. However, it has been reported that in ALS patients and mutant SOD1 mice, MN degeneration was exacerbated due to the loss of glutamate transporter EAAT2/GLT1 which led to impaired uptake of glutamate by astrocytes (Dunlop, Beal McIlvain, She, & Howland, 2003; Howland et al., 2002; Papadeas, Kraig, O'Banion, Lepore, & Maragakis, 2011; Pardo et al., 2006). Other studies reported toxicity from astrocytes expressing mutant SOD1 to MNs as well as MNs derived from embryonic stem cells carrying mutant SOD1 gene (Di Giorgio, Boulting, Bobrowicz, & Eggan, 2008; Nagai et al., 2007; Yamanaka et al., 2008). Moreover, inflammatory mediators such as prostaglandin E2, leukotriene B4, nitric oxide and NOX2 were secreted by astrocytes from ALS patient

postmortem tissues and SOD1^{G93A} mice and provoked toxic effects on MNs (Haidet-Phillips et al., 2011; Hensley et al., 2006; Marchetto et al., 2008).

Although less studied than other actors of neuroinflammation, macrophages and monocytes have also been linked to ALS and MN degeneration. Invasion of peripheral monocytes into the spinal cord of ALS patients and mice has been reported and demonstrated an effect on MN loss (Butovsky et al., 2012; Zondler et al., 2016). In addition, activated monocytes from ALS patients showed impaired phagocytosis and adhesion behavior, as well as altered proinflammatory cytokine secretion (Zondler et al., 2017, 2016).

Mitochondrial dysfunction: Mitochondria are part of the main actors of cell metabolism and evidence from different disease models significantly associates many ALS-linked genes to mitochondrial dysfunction, that can be manifested by production of reactive oxygen species (ROS), altered mitochondrial structure or impaired mitochondrial dynamics.

Indeed, one of the first clues to ALS pathology reported in ALS patients was the structural alteration of mitochondria in MNs, adopting a swollen and vacuolated appearance (Atsumi, 1981; Sasaki & Iwata, 2007). These reports were in accordance with studies on cell and animal models of ALS demonstrating changes in mitochondria morphology and fragmentation, like in SOD1^{G93A} transgenic mouse models where mitochondria were found to be less elongated and more spherical in MNs (Dal Canto & Gurney, 1994; De vos et al., 2007; C. M. J. Higgins, Jung, & Xu, 2003). Further studies demonstrated in these mutant mice a link between mitochondrial damage and changes in specific alternatively spliced mRNAs, including mRNAs coding for proteins involved in neuron-related pathways (Lenzken et al., 2011), highlighting the importance of SOD1 in mitochondrial homeostasis. The same was reported for TDP-43 with different models showing TDP-43-related impaired RNA metabolism associated with oxidative

stress and mitochondrial damage (Carrì, Valle, Bozzo, & Cozzolino, 2015; Cozzolino, Rossi, Mirra, & Carrì, 2015). Similar results were also observed with C9orf72 and another ALS-related gene, valosin-containing protein (VCP) (Dafinca et al., 2016; Nalbandian et al., 2013; Onesto et al., 2016; H. Z. Yin et al., 2012).

The discovery of mutations in the gene Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 10 (CHCHD10), encoding for a mitochondrial protein located between the inner and outer mitochondrial membranes, was a direct and strong evidence that mitochondrial changes in structure may be implicated in ALS pathology (Bannwarth et al., 2014a). It was shown in patients that CHCHD10 mutations provoke loss of mitochondrial cristae junctions and severe mitochondrial structure defects (Genin, Plutino, Bannwarth, Villa, Cisneros-Barroso, et al., 2016). The implication of this gene in ALS pathology will be further reviewed in section 5 (Beyond FUS).

Fragmentation and structure impairment of mitochondria has been reported to occur in early disease stages in several ALS models, suggesting that these alterations could be a source of degeneration in the future stages of disease (Magrané, Cortez, Gan, & Manfredi, 2014; Vande Velde et al., 2011; W. Wang et al., 2013a).

As stated before, mitochondria dysfunction has been linked to different ALS-associated proteins such as TDP-43, FUS, SOD1 and C9orf72. More precisely, it has been shown that these proteins could directly interact with mitochondria (Blokhuys et al., 2016; J. Deng et al., 2015; C. Higgins, Jung, Ding, & Xu, 2002; Lopez-Gonzalez et al., 2016; Mattiazzi et al., 2002; W. Wang et al., 2016) and thus taking part of the appearance of mitochondrial impairments linked to ALS.

Reduction of the electron transport chain complexes activity, responsible of ATP production, was found and associated with mutant SOD1 where it was located to the intermembrane space

of the mitochondria and aggregated (Ferri et al., 2006; Vijayvergiya, Beal, Buck, & Manfredi, 2005). TDP-43, in its wild type and mutant forms, has been found to accumulate in mitochondria and bind to the mRNAs of components of the complex I of the respiratory chain, impairing their transcription which causes the complex I to be dismantled (W. Wang et al., 2016).

By producing ATP through oxidative phosphorylation, mitochondria are responsible for the production of ROS, as mentioned before. When they are produced in excess, ROS can lead to cellular damage to DNA, proteins and lipids. This causes an impaired efficiency of cellular processes, induction of inflammatory pathways, excitotoxicity, protein aggregation and ER stress (Gandhi & Abramov, 2012; Shibata & Kobayashi, 2008). Many reports have highlighted the link between increased levels of ROS and ROS-associated damage and ALS (Barber & Shaw, 2010b), founding abnormal rates of ROS levels and ROS damage in sALS and fALS patients (Bogdanov et al., 2000; Mitsumoto et al., 2008; Onesto et al., 2016; Said Ahmed, Hung, Zu, Hockberger, & Siddique, 2000; E. P. Simpson, Henry, Henkel, Smith, & Appel, 2004; Smith, Henry, Mattson, & Appel, 1998) (**Figure 6**). SOD1^{G93A} cell and rodent models allowed to substantially demonstrate the presence of oxidative damage to DNA, RNA, proteins and lipids [reviewed in (Barber & Shaw, 2010a)] with mRNA oxidation occurring before motor neuron degeneration and potentially promoting it (Y. Chang et al., 2008). Reports on mutant TDP-43 and FUS also revealed their link to oxidative damage with augmented ROS levels (Cohen et al., 2015; Cohen, Hwang, Unger, Trojanowski, & Lee, 2012; J. Deng et al., 2015; K. Hong et al., 2012).

ALS-associated genes have also been reported to be linked to other mitochondrial dysfunction, including loss of calcium homeostasis (Carrì et al., 1997; Damiano et al., 2006; De vos et al., 2012; Mórotz et al., 2012; Siklós et al., 1996; Siklós et al., 1998; Stoica et al., 2014, 2016; Van Den Bosch, Vandenberghe, Klaassen, Van Houtte, & Robberecht, 2000) due to defects in ER-

mitochondria communication, pro-apoptotic signaling (Pasinelli et al., 2004; Pedrini et al., 2010; W. Tan et al., 2013), and impaired dynamics that comprises altered mitochondrial fusion and fission (Ferri et al., 2010; Luo et al., 2013; Y. F. Xu et al., 2011), as well as mitophagy failure (Lagier-Tourenne et al., 2012a; Polymenidou et al., 2011a; Song, Song, Kincaid, Bossy, & Bossy-Wetzel, 2013). A more detailed description of these mechanisms can be found in (Bozzo, Mirra, & Carrì, 2017).

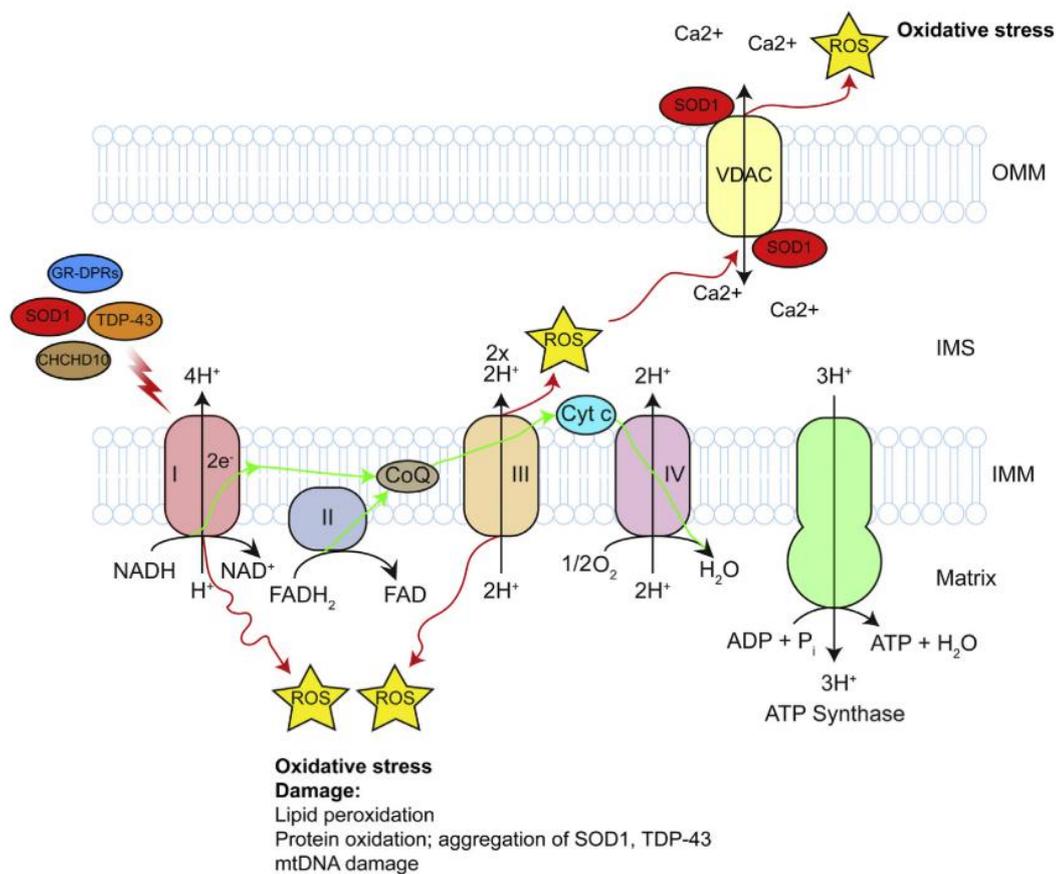


Figure 6: Oxidative stress and impaired mitochondrial respiration involving ALS-associated genes (from Smith et al., 2017). Mutations in *SOD1*, *TDP-43*, *CHCHD10* affect the activity of the electron transport chain complexes, which causes an over-production of ROS and altered TP production.

2.3. Zebrafish models to study neurodegeneration

As reported before, several animal models, especially rodents, have been used to try to understand the neurobiological basis of ALS and unravel new efficient treatment strategies (**Figure 4**) (Van Damme et al., 2017). Among these models, the zebrafish is at the heart of my project of ALS pathogenesis investigation and will be further reviewed here.

The zebrafish, or *Danio rerio*, is a vertebrate that is part of the Cyprinidae fish family, originally used to understand the genetic basis of vertebrate development (Streisinger, Walker, Dower, Knauber, & Singer, 1981). Over the years, the zebrafish has grown in popularity for modeling human diseases thanks to the high degree of conservation of molecules and processes between humans and zebrafish. One of the parameters that makes the zebrafish interesting is its fast development, with 2-days old larvae already presenting organ formation and function, and a short generation time of 3-5 months (Detrich, Westerfield, & Zon, 1999; Drapeau et al., 2002; Kimmel, Ballard, Kimmel, Ullmann, & Schilling, 1995; Schmid & Haass, 2013). Zebrafish also have large clutches of externally fertilized and transparent eggs, facilitating the observation and experimental manipulation of the embryos, including *in vivo* tracking and fluorescence imaging (Kabashi, Brusteijn, Champagne, & Drapeau, 2011; Kabashi, Champagne, Brusteijn, & Drapeau, 2010). Also, the zebrafish genome is fully sequenced (Howe et al., 2013) and there is more than 70% of homology between human and zebrafish proteins (Patten et al., 2014). In addition, the embryos are malleable to genetic manipulation by antisense morpholino oligonucleotide mediating transitory knockdown, and different genome-editing techniques, including TALEN and CRISPR-Cas9 systems (Hisano, Ota, & Kawahara, 2014; Hwang et al., 2013; Schmid & Haass, 2013).

Another important feature of the zebrafish model is that high-throughput screening of neuroactive compounds can be easily performed for drug identification thanks to the small size

of the embryos allowing them to fit in a single well of up to 384-well plates (Zon & Peterson, 2005) and their permeability to small molecules at these stages of development (Zon & Peterson, 2005).

Modeling ALS in the zebrafish

Several studies in ALS research have used the zebrafish to assess the effects of ALS-associated gene mutations. Through different genetic manipulation techniques, gain- and loss-of-function effects of major ALS genes were investigated and showed for example that overexpression of mutant SOD1 led to several hallmarks of the disease, including loss of MNs, muscle degeneration, loss of neuromuscular connectivity, locomotion defects and increased stress in the MNs (McGown et al., 2013; T. Ramesh et al., 2010; Sakowski et al., 2012; Van Hoecke et al., 2012). A zebrafish model of C9orf72 also displayed MN axonopathy and locomotion alterations through knockdown of the gene, which was rescued by expressing human C9orf72 (Sorana Ciura et al., 2013). Expression of mutant TARDBP, expressing TDP-43, by AMO knockdown showed similar results, including MN axonopathy (Kabashi et al., 2009b). Kabashi and colleagues also demonstrated MN toxicity through TDP-43 and FUS knockdown that could be rescued by injection of wild type TDP-43 and FUS, but not SOD1-induced toxicity (Kabashi, Bercier, et al., 2011). In addition, ephrin receptor EphA4 was identified as an actor in ALS pathogenesis in zebrafish as a modulator of SOD1, with lower EphA4 levels providing a reduction in the severity of SOD1-induced disease progression (Van Hoecke et al., 2012). Studies also performed drug tests on SOD1-ALS zebrafish models and observed reduced SOD1-induced neuronal stress response (McGown et al., 2013; McGown, Shaw, & Ramesh, 2016).

3. Neuromuscular junction involvement in ALS and skeletal muscle implication

Despite all the gathered evidence on MN dysfunction in ALS as reviewed above, it is still unclear where the MN dysfunction begins and the extrinsic factors that accelerate MN degeneration. This led to the consideration of ALS as either a *dying forward* process that proposes an anterograde degeneration of MNs by glutamate excitotoxicity or altered neuronal excitability (as already developed in section 2.2. Excitotoxicity) from the cortex, that would extend to corticospinal projections (Braak et al., 2013), or a *dying back* phenomenon in which MN degeneration starts distally at the nerve terminal or at the neuromuscular junction (NMJ) and progresses toward the cell body (Fischer et al., 2004b; Moloney, de Winter, & Verhaagen, 2014b). ALS being a complex disease, it is today considered that both processes occur and whether it is one hypothesis or the other, NMJ dismantlement causing muscle denervation is a major factor in ALS pathogenesis.

3.1. The NMJ

The NMJ is a tripartite synapse formed by the presynaptic MN nerve ending, the postsynaptic muscle and the synapse-associated glial cells (terminal Schwann cells, TSC). This allows the transmission of action potentials from MNs to muscles (Campanari, García-Ayllón, Ciura, Sáez-Valero, & Kabashi, 2016) and the release of the neurotransmitter acetylcholine (ACh) that bind to ACh receptors (AChRs) clustered on the muscle fibers initiating a muscle contraction through the depolarization of the muscle cells (L. Li, Xiong, & Mei, 2018). During development, before the elongation of motor axons to the muscle fibers, a process named muscle pre patterning occurs and consists in AChRs gathering in clusters at the central region to prepare for the future arrival of the motor nerve terminal (N. Kim & Burden, 2008; X. Yang, Li, Prescott, Burden, & Wang, 2000). Once elongated, the motor axons innervate the fibers and

form NMJs. The pattern of AChRs clustering is then refined with the dispersion of the clusters located in non-synaptic areas (L. Li et al., 2018). To do so, Agrin, a glycoprotein, is secreted by the nerve terminals to the synaptic cleft and promotes the transcription of AChRs subunit genes as well as other proteins involved in NMJ structure and function. It also promotes AChR transport to the postsynaptic membrane, their clustering and AChR stability (L. Li et al., 2018) (Figure 7).

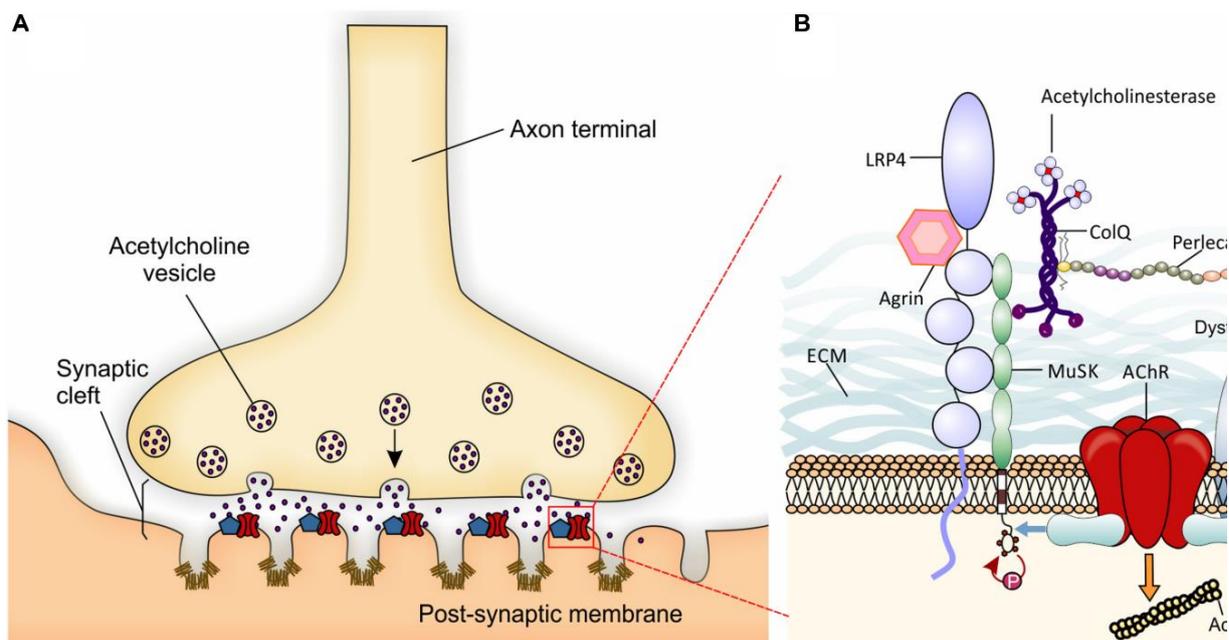


Figure 7: Pre- and post-synaptic elements of the neuromuscular junction and the post-synaptic complex involved in AChR clustering, anchoring and stability (from Campanari et al., 2016)

3.2. The zebrafish NMJ

The NMJ of the zebrafish is very similar to the mammal ones, including in its development and its function. Like in humans and mice, synaptic vesicles on the presynaptic side release ACh from MN endings after membrane excitation (Daikoku, Saito, & Ono, 2015; Kummer, Misgeld, & Sanes, 2006). On the post-synaptic side, AChRs, after receiving the released ACh, cause a cascade of events through current conduction leading to muscle fiber contraction. Five subunits compose an AChR: two $\alpha 1$, one $\beta 1$, one δ , and one γ or ϵ depending on the stage of

development. Indeed, the γ subunit is expressed early in development and, in mammals and fish, is replaced by the ϵ subunit later in the development (Daikoku et al., 2015; Mishina et al., n.d.; Walogorsky et al., 2012) (**Figure 8A**). Previous studies found that zebrafish slow muscle fibers present AChRs with two δ subunits instead of a γ or ϵ subunit (Mongeon et al., 2011; J. Y. Park et al., 2014) (**Figure 8A**).

In early development, zebrafish aneural AChR clusters are formed on the medial surface of adaxial muscle cells before the MN elongation (Flanagan-Steet, Fox, Meyer, & Sanes, 2005; Panzer et al., 2005a; J. Zhang, Lefebvre, Zhao, & Granato, 2004) (**Figure 8B**). As the motor axon grows, adaxial cells migrate to the lateral surface and are replaced by fast muscle fibers, which are innervated to form neural AChR clusters at the exact location of the previous aneural AChR clusters (Panzer et al., 2005a; H. Wu, Xiong, & Mei, 2010; J. Zhang et al., 2004) (**Figure 8B**).

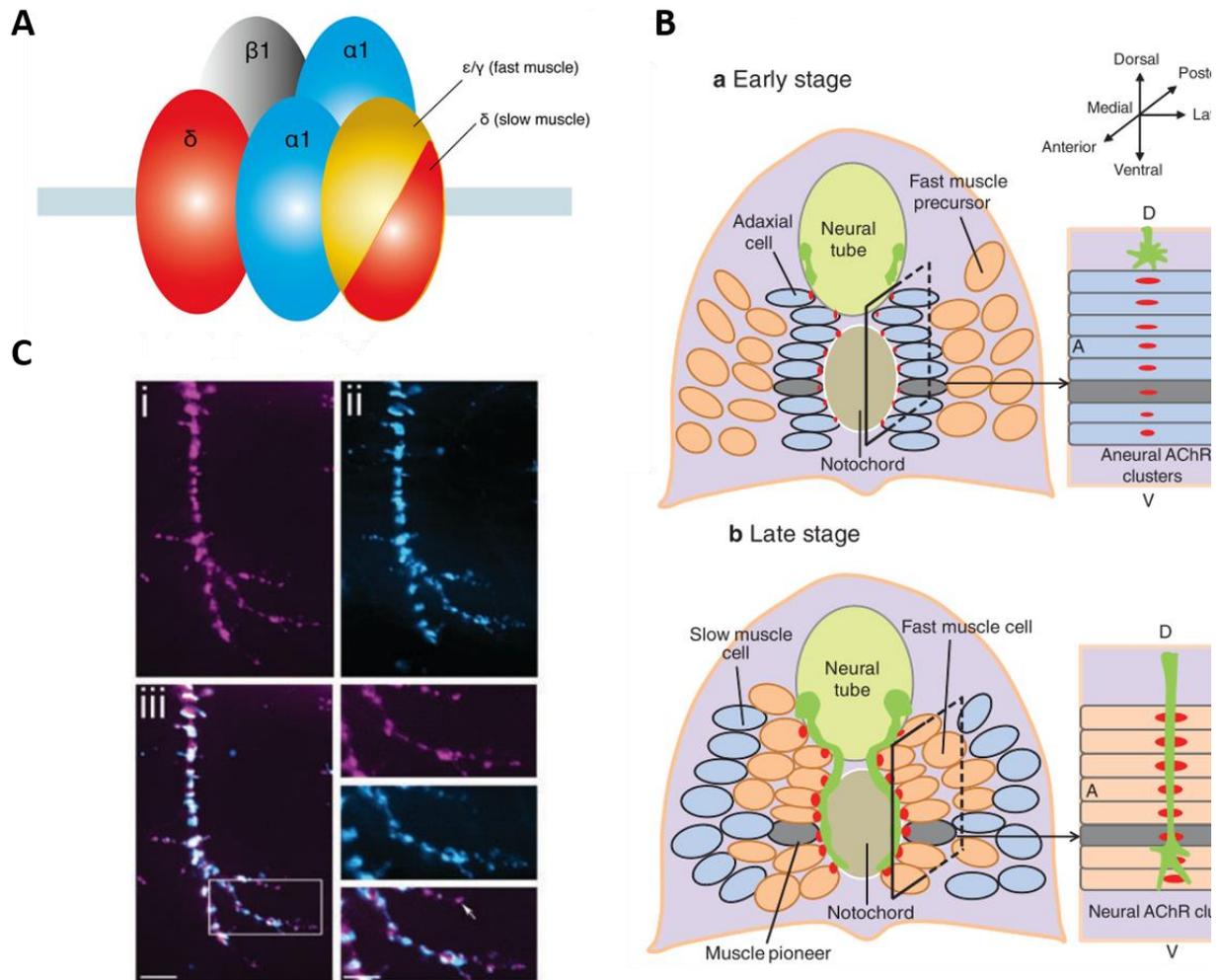


Figure 8: AChR pentamers and NMJ formation in zebrafish (derived from Daikoku et al., 2015; Wu et al., 2010; Armstrong and Drapeau, 2013). (A) The five subunits constituting an AChR, depending on the muscle type (Daikoku et al., 2015). (B) NMJ formation in zebrafish development through aneural cluster incorporation (Wu et al., 2010). (C) Example of fluorescent labelling of pre- (by anti-ZNP1; purple) and post- (by anti-bungarotoxin; cyan) synaptic components of the NMJ of zebrafish (Armstrong and Drapeau, 2013).

3.3. NMJ degeneration in ALS

Numerous studies focused on describing anterograde MN degeneration during ALS pathogenesis and to provide evidence on NMJ disassembly. However, few studies focused their work on the defects occurring at the motor axon terminals at the NMJ. Thus, our understanding of the events leading to NMJ degeneration is still incomplete.

Among the studies that explored the dying-back hypothesis, it was reported that SOD1^{G93A} mouse models presented defects at the NMJ and the motor nerve endings before the appearance of a clinical phenotype (Clark, Southam, Blizzard, King, & Dickson, 2016). Furthermore, a study suggested that, through the ROS sensitivity of nerve terminals, oxidative stress and impaired mitochondria accelerate the presynaptic decline in NMJ and affects the release of neurotransmitters (Pollari, Goldsteins, Bart, Koistinaho, & Giniatullin, 2014). Others noted the reduction of pre-synaptic vesicle density in mutant SOD1 mice (Cappello et al., 2012), as well as a reduction of the size of the synaptic vesicles pool as a consequence of altered vesicle transport in the motor axons (Pun, Santos, Saxena, Xu, & Caroni, 2006).

Despite the predominant use of rodent models for studying pathomechanisms and potential therapeutic targets in ALS, the use of smaller animal models, like *Drosophila melanogaster* and zebrafish (*Danio rerio*) allowed to unravel a significant number of clues about the toxic effects of ALS-associated protein mutations on the development and maintenance of the NMJ thanks to its similar structure and formation, as well as better accessibility for analyses on these organisms (Menon, Carrillo, & Zinn, 2013; H. Wu et al., 2010). Their advantages lie in their fast development allowing quick generation of lines, their availability and the ease in manipulating gene expression and in drug screening. In drosophila, studies showed locomotor defects, reduced life span, and anatomical defects at the NMJ, causing impairments in synaptic transmission, in loss and gain of function models of TARDBP (Diaper et al., 2013; Feiguin et al., 2009). Zebrafish studies have highlighted gain and loss of function mechanisms for TARDBP and FUS, demonstrating shorter axonal projections from MNs, premature and excessive branching, impaired synaptic transmission at the NMJ leading to swimming defects (Kabashi, Bercier, et al., 2011; Kabashi et al., 2009a).

3.4. The role of skeletal muscle in ALS-related NMJ degradation

The muscle contribution in ALS development is still a matter of debate with limited information gathered on this theme of research. However, increasing evidence points to the critical role of muscle integrity in the early stage of the disease in ALS patients and a variety of animal models have permitted important advances into exploring this hypothesis.

Like for MNs, mitochondria evaluation is one of the strongest indexes of the defects potentially found in the muscle fiber. Thus, sALS patients and transgenic mouse models displayed structural and functional defects in muscle and in mitochondrial dynamics that were observed at presymptomatic stages or appearing as the disease progresses (Luc Dupuis & Loeffler, 2009; Echaniz-Laguna et al., 2006; O Pansarasa, Rossi, Berardinelli, & Cereda, 2014), suggesting an affection independent of MN degeneration. Also, by overexpressing PGC-1 α , a transcription coactivator that boosts mitochondrial biogenesis, in mutant SOD1 mouse models, mitochondrial and muscle functions were found to be restored (S. Da Cruz et al., 2012).

Other studies demonstrated that mutant SOD1 expression would cause progressive muscle atrophy as well as muscle strength reduction and defects in contraction machinery (Dobrowolny, Aucello, Molinaro, & Musarò, 2008; M. Wong & Martin, 2010). In addition, in SOD1^{G93A} mice, defects in muscle metabolism and muscle mass reduction were reported before any evidence of central neuron degeneration (Marcuzzo et al., 2011).

These reports highlighted the importance of muscle implication in ALS progression. Other investigations provided critical clues more directly linked with NMJ denervation. Indeed, studies reported that overexpression of mutant SOD1 in muscles caused different ALS phenotypes, among which NMJ alteration and MN degeneration are the most noticeable (M. Wong & Martin, 2010). Also, a correlation was made between decreased levels and secretion

of muscle FGF1, a growth factor binding protein involved in cell proliferation, found in ALS and altered NMJ stability (Taetzsch, Tenga, & Valdez, 2017).

Cholinergic activity is also a good indicator of NMJ assembly and stability in ALS pathogenesis. Neuromuscular transmission of mutant SOD1 mice were found to be impaired in a subset of NMJs in the late stages of the disease (Rocha, Pousinha, Correia, Sebastião, & Ribeiro, 2013). Other studies focused their investigation at pre- and post-synaptic levels by analyzing the spatiotemporal expression of the choline acetyltransferase (ChAT) and vesicular ACh transporter (VACHT), involved in the packaging of ACh inside the synaptic vesicles in the MNs. They found that patient tissues and mice models presented a significant downregulation of these two molecules, suggesting that ACh management might play a role in MN distal degeneration (Campanari et al., 2016).

Initial studies also revealed a reduction in AChE levels in ALS patient muscle biopsies (Rasool, Chad, Bradley, Connolly, & Baruah, 1983), and other reports showed a link between this reduction and a significant increase in the plasma level of the circulating enzyme (Fernandez & Inestrosa, 1976; Wilson, Linkhart, Walker, & Nieberg, 1973), highlighting a possible link between protease activity in the synaptic cleft and ALS pathogenesis. Indeed, further data showed matrix metalloproteinases (MMP) activity to be increased in central nervous system and in muscles (Schoser & Blottner, 1999) and plasma of ALS patients (Demestre, Parkin-Smith, Petzold, & Pullen, 2005). MMPs are endopeptidases that are able to digest components of the extracellular matrix at the synaptic cleft, like collagen, proteoglycan and laminin (Vincenti & Brinckerhoff, 2007) in reaction to changes in neuronal activity (Reinhard, Razak, & Ethell, 2015). A study on SOD1^{G93A} mice strengthened the implication of MMPs in ALS by demonstrating an improvement of lifespan through early treatment of an MMP inhibitor (Lorenzl et al., 2006). However, it is worth noting that muscles are not the only sources of AChE. MNs produce and release AChE too, and Schwann cells also express

butyrylcholinesterase (BChE) that interferes with plasma AChE level assessment (García-Ayllón, Millán, Serra-Basante, Bataller, & Sáez-Valero, 2012; García-Ayllón et al., 2010), making it difficult to identify clearly the actor of the tripartite synapse responsible for the reported defects (Campanari et al., 2016).

In all these processes characterizing cholinergic activity, AChRs have also been reported to be implicated in NMJ alteration related to ALS pathology. Indeed, studies demonstrated that AChRs from ALS patients presented similar pharmacological and electrophysiological profiles with AChRs from denervated muscles of non-ALS individuals, except for a significant decrease in ACh affinity and a notable augmentation of the expression of the $\alpha 1$ subunit of the receptor compared to denervated non-ALS, in addition to the typical increase of the γ subunit in denervated muscle. It suggested that changes at the NMJ induced by ALS-associated denervation of muscles are different from the ones found in denervated muscles due to trauma or other pathologies (Palma et al., 2011, 2016b).

Denervation is widely studied to characterize the pathways leading to degeneration, but reinnervation as a compensatory process is also a phenomenon worth focusing on and found in some types of patients, that is, the ones with a slow disease progression and prolonged survival. A report demonstrated the implication of muscle histone deacetylase (HDAC4) and its regulator microRNA-206 in compensatory reinnervation and disease progression in a mutant SOD1 mouse model (Cohen et al., 2009). Later, another study focused on ALS patients with survival differences and they found that the proportion of reinnervated neuromuscular junctions was significantly higher in long-term survivors than in patients with rapidly progressive disease (Bruneteau et al., 2013). By assessing the expression of muscle candidate genes involved in reinnervation, they demonstrated that HDAC4 upregulation was significantly greater in patients with rapidly progressive disease and was negatively correlated with the extent of muscle reinnervation and functional outcome (Bruneteau et al., 2013), suggesting that muscle

expression of HDAC4 might play a significant role in muscle reinnervation and disease progression in ALS patients.

All this evidence supports the idea that impairments in muscle can have an impact on MN terminals, contributing to NMJ denervation. This highlights the fact that muscle fibers play an important role in ALS pathogenesis and progression, and potential therapies could be developed by targeting muscles.

4. The gene FUS and its implication in ALS

As introduced before, the gene FUS is a major gene associated with ALS pathogenesis and the characterization of its molecular mechanisms is crucial to the understanding of the disease and neurodegeneration in general. In this section, I will review the genetic features of FUS mutations in patients and animal models as well as the known cellular and molecular mechanisms of wild type and mutant FUS proteins.

4.1. FUS structure and properties

FUS was initially identified in 1993 as an oncogene that undergoes chromosomal translocation in myxoid liposarcoma (Croizat et al., 1993; Rabbitts, Forster, Larson, & Nathan, 1993). In these original studies, it was shown that the N-terminal transcriptional activation domain of FUS is fused to a transcription factor, CAAT enhancer-binding homologous protein (CHOP), that is part of the C/EBP family and is a growth arrest and DNA-damage inducer (Croizat et al., 1993; Rabbitts et al., 1993). Other studies also demonstrated that FUS was an activator of ETS-related gene (ERG) in acute myeloid leukemia (Ichikawa, Shimizu, Hayashi, & Ohki, 1994; Panagopoulos et al., 1994) and in Ewing's sarcoma tumors (Shing et al., 2003).

The FUS gene is located on chromosome 16 (Åman et al., 1996a) and encodes a 526 amino acid protein that is part of a family of FET RNA binding proteins, including FUS, Ewing's sarcoma RNA binding protein 1 (EWSR1) and Tata-binding protein-associated factor 2N (TAF-15). These proteins are structurally characterized by the presence of an N-terminal transcription activation domain SYGQ-rich region, a C2/C2 zinc finger (ZnF) motif and one or more arginine-glycine-glycine (RGG)-repeat sequences, or Gly-rich regions (Guerrero et al., 2016; Morohoshi et al., 1998).

The human FUS protein can be divided into the QGSY-rich region and a Gly-rich region (RGG1) at the N-terminus, also known as low complexity domain, as well as an RNA recognition motif (RRM), a nuclear export signal (NES) and two other RGG-repeat sequences (RGG2 and 3) separated by a ZnF region followed by a non-conventional nuclear localization signal (NLS) at the C-terminus (**Figure 9**) (C. Chen, Ding, Akram, Xue, & Luo, 2019). Furthermore, bioinformatics analysis identified two prion-like domains in FUS structure, one at the N-terminus on the low complexity domain (QGSY-rich region and RGG1) and the other on the RGG2 region (Z. Sun et al., 2011).

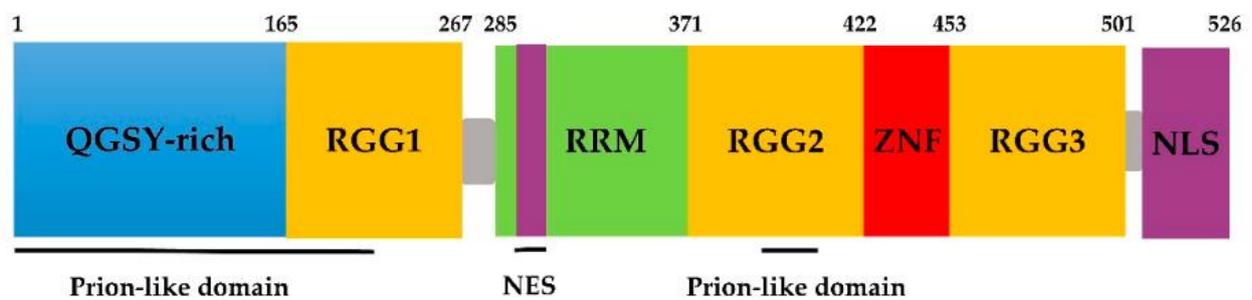


Figure 9: FUS protein domains (derived from Chen et al., 2019)

These domains are responsible for the different roles that FUS play at transcriptional and post-transcriptional level through protein-RNA and protein-protein interaction (Ratti & Buratti, 2016). Some of these activities are reviewed here and resumed in **Figure 10**.

RNA-binding property: After its identification, FUS has been further studied to decipher its biochemical properties and reports found an RNA-binding property allowing splicing regulation (Shang & Huang, 2016). Indeed, previous studies showed through UV cross-linking that FUS can bind to RNA with its ZnF motif (Iko et al., 2004; H Zinszner, Sok, Immanuel, Yin, & Ron, 1997b). Another important feature identified for FUS was its ability to form stable complex with different members of the heterogeneous ribonuclear protein (hnRNP) family in the nucleus (Calvio, Neubauer, Mann, & Lamond, 1995; H Zinszner et al., 1997a; Helene

Zinszner, Albalat, & Ron, 1994). These proteins are the most abundant nuclear proteins and they participate in pre-mRNA processing like splicing and are key actors in mRNA export, localization, translation and stability (Chaudhury, Chander, & Howe, 2010; Dreyfuss et al., 2002).

Several reports have demonstrated a direct binding of FUS to specific RNA by identifying a common GGUG motif in RNA oligoribonucleotides that bind to recombinant FUS protein (Lerga et al., 2001). Through FUS antibody immunoprecipitation and cross-linking immunoprecipitation (CLIP)-RNA sequencing (CLIP-Seq), studies could show that mouse and human FUS proteins bind to RNA possessing enriched GUGGU motifs but different from the GU-rich binding sequence reported for TDP-43 (Lagier-Tourenne et al., 2012b; Polymenidou et al., 2011b; Shang & Huang, 2016; Tollervy et al., 2011a). Other studies suggested that FUS binding sites in RNA tend to form stable secondary structures, like the stem-and-loop structure (Colombrita et al., 2012; Hoell et al., 2011; Ishigaki et al., 2012; Rogelj et al., 2012), or showed that FUS interacted with short RNA repeats (UUAGGG) in the G-quadruplex telomeric repeat-containing RNA (TERRA) by forming unique secondary and tertiary structures (Takahama et al., 2013). Another study found that FUS was able to bind to the previously reported motifs but also other RNA without these repeats and with the same affinity (Xueyin Wang, Schwartz, & Cech, 2015). This shows that the binding property of FUS has a large action on different nucleic acids and is dependent on the secondary or tertiary structure of RNA (Shang & Huang, 2016). However, consensus on RNA binding sequences for FUS was not met among researchers using CLIP-seq technology.

RNA splicing and transcription: With its RNA binding ability, reports quickly linked this property with another key feature of FUS, RNA splicing. Studies demonstrated a binding between FUS and components necessary to constitute the spliceosome both in the cytoplasm and the nucleus, namely SMN protein, U1 small nuclear ribonucleoprotein (snRNP) and Sm-

snRNP complex (Gerbino, Carrì, Cozzolino, & Achsel, 2013; Ratti & Buratti, 2016; Yamazaki et al., 2012). Also, FUS was identified as an interacting partner with SC35 and serine/arginine-rich splicing factor 10 (SRSF10), which are splicing factors (Behzadnia et al., 2007; Wahl, Will, & Lührmann, 2009; L Yang, Embree, Tsai, & Hickstein, 1998).

Due to its splicing and RNA binding activity, FUS is a major actor in transcription. Indeed, a report showed that FUS directly regulates the activity of RNA-pol II by influencing its phosphorylation during transcription (Schwartz et al., 2012). FUS also binds to nascent pre-mRNAs and play the role of molecular mediator between RNA-pol II and U1-snRNP for splicing (Yu & Reed, 2015). In addition, it was demonstrated that FUS regulates the alternative polyadenylation signals of different transcripts in neuronal cells (Masuda et al., 2015) depending on the position of FUS binding to nascent pre-mRNAs (Masuda et al., 2015). Also, the formation and maintenance of nuclear gems, which are nuclear bodies involved in snRNP modification and assembly, are dependent on the action of FUS (Yu & Reed, 2015). In mutated conditions, the mislocalization of FUS to the cytoplasm provoke altered interactions with SMN and different snRNPs, causing a loss of nuclear gems that impacts splicing processes and could contribute to neurodegeneration (Groen et al., 2013; Yu & Reed, 2015).

Another clue that confirms the role of FUS as a transcriptional regulator is its association with the transcription factor IID (TFIID) complex (Bertolotti, Lutz, Heard, Chambon, & Tora, 1996a). FUS is also able to directly bind to single stranded DNA (ssDNA) elements in gene promoters and regulates their expression (A. Y. Tan, Riley, Coady, Bussemaker, & Manley, 2012). It was found that FUS chromatin binding, responsible for transcriptional regulation, depended on the low-complexity domain of FUS at the N-terminus (Liuqing Yang et al., 2014), and FUS point mutations were shown to alter these processes of transcription (Kwiatkowski et al., 2009; C. Vance et al., 2009).

The study of disease models involving FUS allowed to identify different splicing targets of FUS (Colombrita et al., 2015; Hoell et al., 2011; Honda et al., 2014a; Ishigaki et al., 2012; Lagier-Tourenne et al., 2012b; Rogelj et al., 2012). Studies also showed that most RNAs that bind to FUS contain intronic sequences, and this binding is realized in a saw-tooth manner in neuronal cells (Lagier-Tourenne et al., 2012b; Rogelj et al., 2012). One of the neuronal splice targets of FUS is the microtubule-associated binding protein Tau (MAPT) pre-mRNA, which presents multiple exonic and intronic sites that can bind to FUS (Orozco et al., 2012a). Through loss of FUS function, reports showed that there is a skipping of exon 3 and 10 as well as increased expression of 4-repeat Tau that can be linked to FTD and other neurodegenerative pathologies (Hutton et al., 1998). FUS was also shown to autoregulate its own levels by binding to its own pre-mRNA, inhibiting exon 7 splicing, thus repressing the degradation of the exon 7-skipped splice variant by non-mediated decay (Lagier-Tourenne et al., 2012b; Y. Zhou, Liu, Liu, Öztürk, & Hicks, 2013a). Another reported clue concerning FUS splicing activity is the properties of mutant FUS proteins to change their binding affinity consistently due to their abnormal localization in the cytoplasm (Hoell et al., 2011). All these findings highlight the complexity to define FUS transcriptional regulation processes and their association with ALS and neurodegeneration.

miRNA processing and lncRNAs expression: FUS, along with TDP-43, was found to be associated with the Microprocessor complex component Drosha, a complex involved in the early steps of microRNA (miRNA) processing (Gregory et al., 2004). These types of non-coding RNA function in RNA silencing and post-transcriptional regulation of gene expression (Ambros, 2004; DP, 2004). Specifically, FUS recruits Drosha to sites of active transcription, therefore facilitating miRNA biogenesis (Morlando et al., 2012). Also, reports showed that a consistent number of analyzed miRNAs are down-regulated when FUS is depleted, including miR-9, miR-125b and miR-132 that play key roles in neuronal function and synaptogenesis

(Morlando et al., 2012). Another study demonstrated that FUS also mediates the processing of miR-141 and miR-200a that bind to the 3'UTR of FUS mRNA, causing a downregulation of FUS protein synthesis, which sets an autoregulatory feed-forward loop that regulates FUS levels (Dini Modigliani, Morlando, Errichelli, Sabatelli, & Bozzoni, 2014).

Another type of non-coding RNA that were found to interact with FUS are the long non-coding RNAs (lncRNAs), which can regulate gene expression by recruiting chromatin modifiers or other proteins to sites of transcription or specific chromosomal territories (Batista & Chang, 2013; Ederle & Dormann, 2017). It was demonstrated that FUS interacts with a fraction of lncRNAs, including MALAT1 and NEAT1, both implicated in nuclear splicing speckles and paraspeckle formation (Lagier-Tourenne et al., 2012b; Lourenco, Janitz, Huang, & Halliday, 2015; Tollervey et al., 2011b). From this, studies confirmed the importance of FUS, with TDP-43, in the assembly of nuclear paraspeckles (Nishimoto et al., 2013; Shelkovernikova et al., 2013), as well as their dysfunction due to FUS ability to sequester them in the cytoplasm which might contribute to ALS/FTLD pathogenesis (Shelkovernikova et al., 2013).

mRNA stability: Reports demonstrated that FUS played a role in other processes that affect mRNA, including their stability or decay, translation and transport (Colombrita et al., 2012; Hoell et al., 2011; Rogelj et al., 2012). Udagawa and colleagues reported that when FUS is depleted in primary neurons, there is a down-regulation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor subunit GluA1, and that FUS binds to GluA1 mRNA near the 3' terminus and controls poly (A) tail maintenance, suggesting a regulation of GluA1 mRNA stability (Udagawa et al., 2015). More precisely, they found that FUS formed a complex with different proteins, PAN2, PABPC1 and CPSF6, involved in the control of mRNA 3'-end processing and polyadenylation. When FUS was depleted, they saw a disruption of the complex associated with a decrease in the poly (A) tail length and in the stability of GluA1 mRNA, causing a down-regulation of GluA1 proteins (Udagawa et al., 2015). Another recent

study showed that FUS has an impact on the mRNA stability of a significant number of neuronal transcripts (Kapeli et al., 2016). However, the specific process of FUS mRNA stabilization is not well-understood still needs to be investigated.

mRNA trafficking: In neurons, proteins that play a role in axons or dendrites need to be transported there or locally synthesized from mRNAs that are transported to these parts of the cells (Ederle & Dormann, 2017). For this, they travel in messenger ribonucleoprotein (mRNP) granules, also named neuronal transport granules, and FUS was found to be localized in these particles in dendrites and axon terminals of hippocampal neurons (Belly, Moreau-Gachelin, Sadoul, & Goldberg, 2005; Ederle & Dormann, 2017; Schoen et al., 2015), suggesting an active role of FUS in mRNA trafficking along axons and dendrites.

Studies demonstrated the role of FUS in the transport of Nd1-L mRNA, encoding for an actin-stabilizing protein, into dendrites and dendritic spines (Ederle & Dormann, 2017; Fujii et al., 2005; Fujii & Takumi, 2005). More specifically, when neurons are stimulated, mRNP granules gathering FUS, Nd1-L and β -actin mRNA were found to be moving into dendritic spines and FUS being enriched at postsynaptic densities (PSDs) (G. Zhang, Neubert, & Jordan, 2012). Other reports went further and identified an association of FUS with microtubules and also actin filaments and the molecular motor myosin-V (that sorts RNA granules into dendrites) in order to ensure FUS transport into dendrites (Fujii & Takumi, 2005; Yoshimura et al., 2006).

mRNA translation: The evidence for FUS to be involved in translational control is not clear-cut, but reports confirmed its implication in translation by finding FUS to co-localize with the APC protein in mRNP granules in cell protrusions and to promote translation of several protrusion-localized mRNAs such as Kank2, Ddr2 and Pkp4 (Yasuda et al., 2013). They also showed that FUS mutants impaired translation processes in APC-positive granules (Yasuda et al., 2013). However, FUS role in mRNA translation remains elusive and needs to be further evaluated.

Response to DNA damage: Evidence for the implication of FUS in DNA repair was demonstrated early, in 1999 from Baechtold and colleagues, with the observation of D-loop formation and homologous recombination during DNA double-strand break repair promoted by FUS (Baechtold et al., 1999). Later, other studies reported increased chromosome breakage and genomic instability in FUS knock-out mice (G G Hicks et al., 2000), and also a phosphorylation of serine residues at the N-terminus of FUS by two protein kinases ATM and DNA-PK when DNA damage was induced (Gardiner, Toth, Vandermoere, Morrice, & Rouse, 2008). It was also demonstrated that FUS was involved in DNA repair through direct interactions with Poly-ADP-ribose (PAR) polymerase and Histone deacetylase 1 (HDAC1) protein at the site of DNA damage. When FUS is mutated, these interactions are impaired and this resulted in a reduced ability to repair DNA (Mastrocola, Kim, Trinh, Rodenkirch, & Tibbetts, 2013; S.L. et al., 2014; W. Y. Wang et al., 2013). In addition, when DNA damage occurs, it was found that FUS bind to different ncRNAs specifically transcribed from the Cyclin D1 gene and this would result in a FUS protein conformational change that facilitates its binding with the CREB-binding protein/p300 histone acetyltransferase, resulting in repression of Cyclin D1 transcriptional activities (Xiangting Wang et al., 2008).

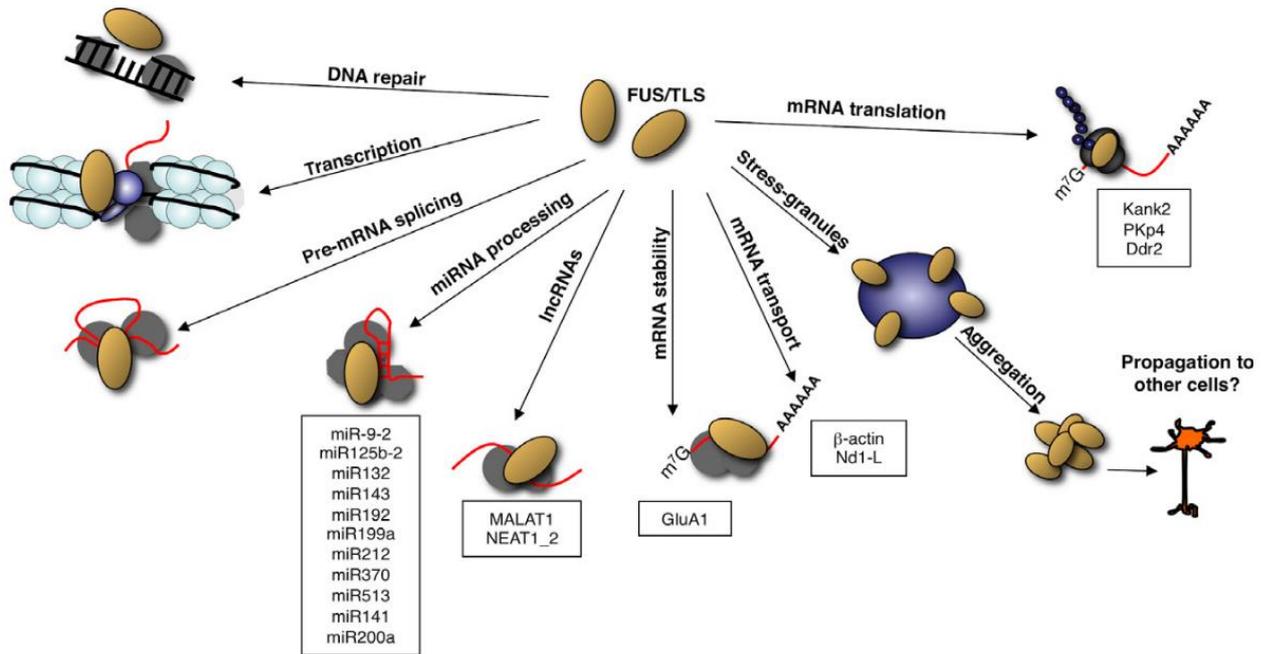


Figure 10: FUS known cellular functions (derived from Ratti et al., 2016)

4.2. ALS-FUS pathology

As mentioned earlier, FUS is part of the major genes which mutations are implicated in ALS pathogenesis. Indeed, like for TARDBP, FUS mutations account for around 5% of fALS cases (Cirulli et al., 2015). However, a peculiarity reported in fALS caused by FUS is the significantly younger age of disease onset than the ones due to other genes, with FUS mutations accounting for 35% of fALS cases that are under 40 years old (Millecamps et al., 2012). Meta-analyses further confirmed this feature with the assessment of different samples of ALS cases with FUS mutations that show a disease onset around 44 years (**Figure 11A**) (H. Deng, Gao, & Jankovic, 2014; Lattante et al., 2013; Yan et al., 2010). In addition, other studies reported that 60% of ALS patients presenting FUS mutations are diagnosed before 45 years old, with a significant fraction considered as juvenile ALS cases, meaning presenting a disease onset at adolescence and early 20's (**Figure 11B**) (Bäumer et al., 2010; E. J. Huang et al., 2010).

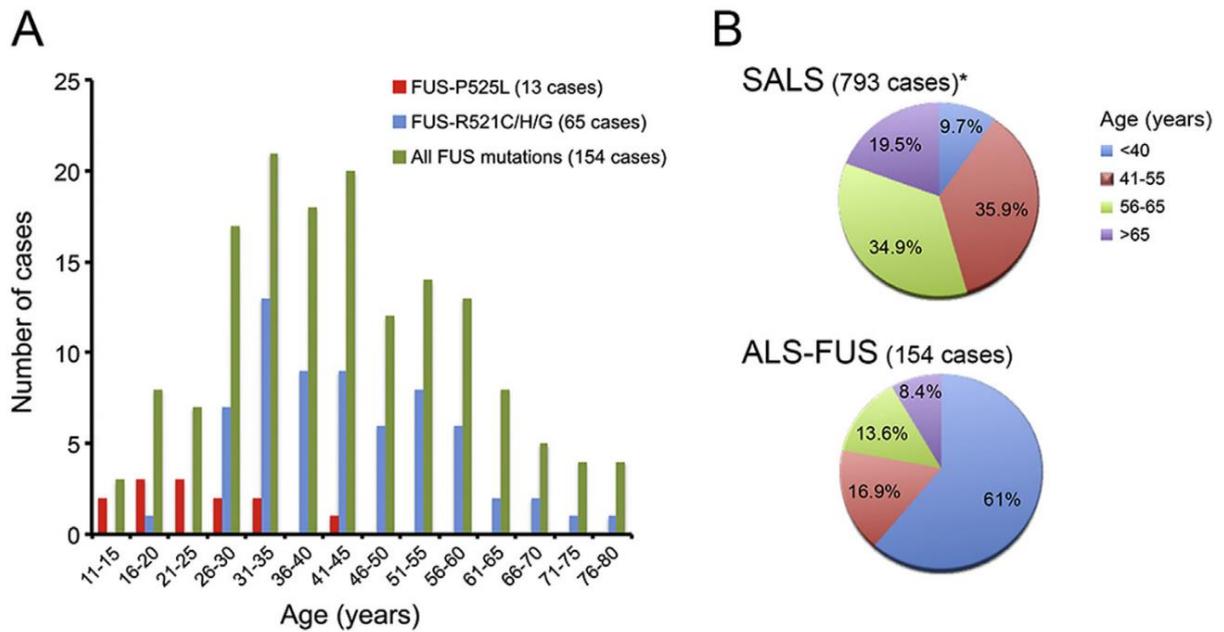


Figure 11: Age of disease onset in fALS-FUS patients (from Shang et al., 2016). (A) Meta-analyses on fALS patients showing proportions of different ages of disease onset. (B) Difference in age of disease onset between sALS patients and ALS-FUS patients.

More than 50 mutations were identified in fALS cases, a majority being missense mutations and some being in-frame deletions (H. Deng et al., 2014; Nolan, Talbot, & Ansorge, 2016). Most of them are clustered in the C-terminal region of the FUS protein, including the NLS and the RGG domains, but a significant number of mutations is also found at the N-terminus in the low complexity domain (**Figure 12**) (Shang & Huang, 2016). Also, reports found that some mutations, including the common P525L mutation or the recently identified Y526C mutation, are associated with juvenile onset and severe disease progression (Bäumer et al., 2010; Corcia et al., 2017; E. J. Huang et al., 2010; Mochizuki et al., 2012).

Researchers mostly focused their work on possible FUS nuclear import defects as the majority of the mutations found are at the C-terminus, near the NLS domain. A study investigated several mutations occurring at the C-terminal region, including R521G/H/C, R522G, R524S and P525L. They expressed these mutations in HeLa cells and they observed through immunostaining that the P525L mutation causes severe nuclear import defect, but the others

would only show moderate phenotype and mild cytoplasmic localization (Dormann et al., 2010). They also found that FUS P525L significantly accumulates in the cytoplasm of neurons from rat hippocampus and frontal cortex, and in zebrafish eggs (Dormann et al., 2010), that was later confirmed by other reports (Niu et al., 2012; Y. Zhou, Liu, Liu, Öztürk, & Hicks, 2013b).

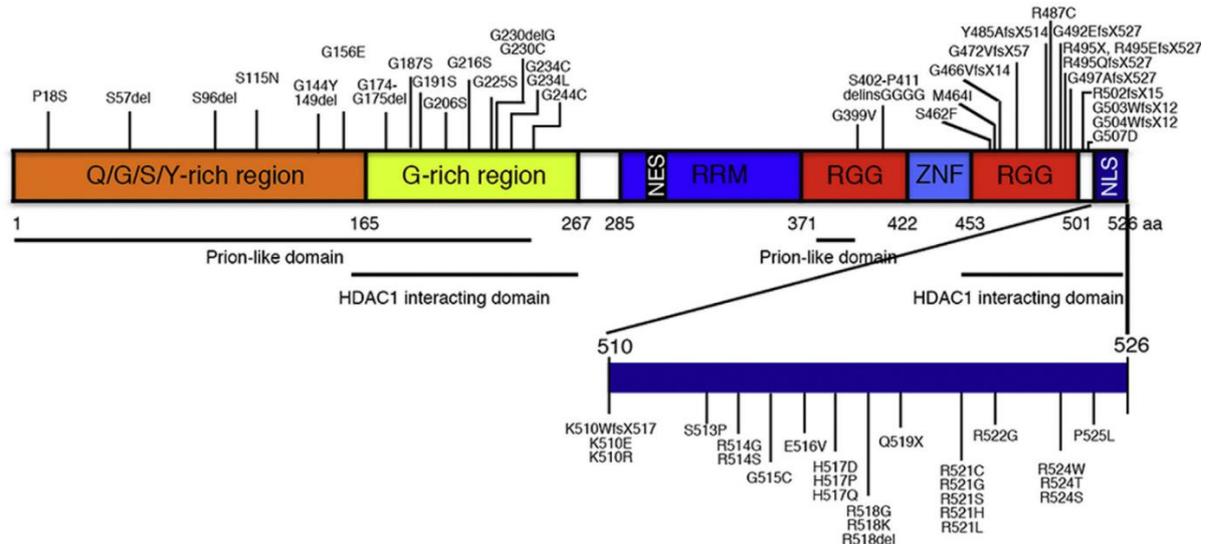


Figure 12: FUS mutations identified in ALS and their localization on the functional domains of FUS protein (derived from Shang et al., 2016)

FUS has also been associated with FTD, but always in cases of co-occurrence with ALS (H. Deng et al., 2014; I. R. A. Mackenzie, Rademakers, & Neumann, 2010; Manuela Neumann et al., 2009; Snowden et al., 2011) and no case of pure clinical FTD related to FUS has been reported (Nolan et al., 2016), which is strengthened by genome-wide association studies (GWAS) on FTD patients that did not find significant links FUS (Ferrari et al., 2014). However, FUS inclusions were still detected in some frontotemporal lobar degeneration cases (FTLD), and they are now categorized as atypical FTLD-U, neuronal intermediate filament inclusion disease (NIFID) or basophilic inclusion body disease (BIBD) (I. R. A. Mackenzie et al., 2010; Yokota et al., 2008) (reviewed in Nolan et al., 2016).

At the pathobiological level, like TDP-43, the common feature of FUS in ALS disease is characterized by its protein aggregation in the cytoplasm of neurons (S. C. Ling, Polymenidou, & Cleveland, 2013; Ratti & Buratti, 2016). Depending on the severity of the disease or the mutation, FUS pathology differs, with early-onset ALS cases being characterized by basophilic inclusions and round neuronal cytoplasmic FUS inclusions, whereas tangle-like FUS-containing inclusions define the pathology of late-onset ALS cases in neurons and glial cells (S. C. Ling et al., 2013; I. R. A. Mackenzie et al., 2011).

FUS aggregation has been suggested to be caused by defects in nuclear import/export processes through an imbalanced accumulation of FUS in the cytoplasm (Dormann & Haass, 2011). To shuttle between the nucleus and the cytoplasm, FUS relies on Transportin 1 (or Importin β 2) (Dormann et al., 2010), but also by protein arginine methyltransferase 1, which mediates the arginine methylation of the RGG domain of FUS causing a reduction in Transportin 1 binding (Dormann et al., 2012; Tradewell et al., 2012; Yamaguchi & Kitajo, 2012a).

Several factors have been described to explain FUS aggregation, including mutations, lower efficiency of the autophagic/proteasome pathways or small increases in endogenous expression (Shelkovernikova et al., 2013), but one of the factors that has been studied thoroughly and that provided significant understanding in aggregation process is SG formation. Indeed, FUS was identified as a SG component in its mutated form (Bosco, Lemay, et al., 2010), and other studies later confirmed this observation with FUS overexpression that resulted in a colocalization with SGs in the cytoplasm in response to oxidative and osmotic stress (Blechingberg et al., 2012; Sama et al., 2013; Yamaguchi & Kitajo, 2012b), as well as induction of SG formation (Gal et al., 2011). Another study found that a particular mutation of FUS (R495X) altered the dynamic properties of SGs, delaying their assembly, making them more dynamic, larger and more abundant when formed (Baron et al., 2013). Knowing the important role SGs play in cell survival through translational interruption of house-keeping proteins, the induction of aberrant

SG formation and persistence through FUS mutations that are unable to be removed by the protein quality system is a major factor in disease pathogenesis.

4.3. Models of FUS in ALS pathology

With FUS being linked to ALS and its multiple phenotypes, researchers made significant progress in trying to model FUSopathies associated with FUS mutations and which develop ALS-like phenotypes. The first FUS models were knock out (KO) mouse models created to investigate its original role as a fusion oncoprotein in the development of round cell liposarcomas and human myeloid leukemias as well as the effects of haploinsufficiency (Geoffrey G. Hicks et al., 2000; Kuroda et al., 2000). These models have different genetic backgrounds and present different features, with inbred KO mice presenting perinatal death with defects in the immune system, as well as chromosomal aberrations (Geoffrey G. Hicks et al., 2000), and outbred KO mice showing developmental impairments, however with the ability to reach adulthood (Kuroda et al., 2000). This suggests that the inbred background lacks the necessary genes to compensate for the effects of FUS KO (Ishigaki & Sobue, 2018).

At the neuropathological level, the first evidence came from a report from Tolino and colleagues who investigated neuron morphology from embryos of inbred FUS KO mice. They demonstrated in primary hippocampal neurons strong irregularities in dendrite branching, as well as processes related to immature axons (Tolino, Köhrmann, & Kiebler, 2012). After that, different research teams developed transgenic FUS models expressing human FUS mutations (R495X, R521C/H/G, R518K and others) in different organisms like *Drosophila*, zebrafish or mice (Lanson et al., 2011). In *Drosophila*, they found that different FUS mutations caused severe neurodegeneration characterized by disorganized ommatidia and loss of mechanosensory bristles in the eyes. In zebrafish, not every mutant showed FUS cytoplasmic

accumulation in cells, but all of them presented SG formation when under heat shock stress (Lanson et al., 2011; Lanson & Pandey, 2012). Other models targeting the NLS domain of FUS presented ALS-like features such as loss of spinal MNs and peripheral nerve fibers or lower MN populations in the brainstem (Shelkovnikova et al., 2013). Another model was developed with the RRM domain lacking and carrying the human R522G mutation, which presented neuronal proteinopathy and shorter lifespan (Robinson et al., 2015).

Overall, many models, with different systems, were created to investigate the multiple features associated with FUSopathies in the spectrum of ALS (**Table 2**), but most of them could not provide a strong consensus on FUS pathomechanism in ALS. Indeed, most reports suggest that FUS mutations work in a gain-of-function manner, with loss-of-function being insufficient to contribute to MN degeneration in ALS (Lebedeva, de Jesus Domingues, Butter, & Ketting, 2017; Scekcic-Zahirovic et al., 2016; Sharma et al., 2016b), but many studies provided solid evidence of the implication of FUS deficiency in ALS and FTLD pathogenesis, such as neuronal cell death, shorter lifespan, reduced motor activity and MN degeneration in models of *Drosophila* and zebrafish (G. A. B. Armstrong & Drapeau, 2013; Frickenhaus, Wagner, Mallik, Catinozzi, & Storkebaum, 2015; Kabashi, Bercier, et al., 2011; Sasayama et al., 2012), as well as behavioral impairments in mice (Kino et al., 2015; Udagawa et al., 2015) reminiscent of FTLD phenotypes. It led the scientific community to suggest that one of the possibilities of ALS pathogenesis is a combination of both gain- and loss-of-function effects, possibly acting at different timeframes (Halliday et al., 2012; E. B. Lee, Lee, & Trojanowski, 2012; Ratti & Buratti, 2016), but these discrepancies reveal the limitations of the animal models and the need for additional models to better understand the role of FUS in ALS pathogenesis.

Study	Mutation	Species	Targeting strategy	Phenotype
Hicks et al., 2000	LOF	Mouse	KO	- Perinatal death - Defective immune system - Genomic instability
Kuroda et al., 2000			KO	- Male sterility - Chromosomal aberrations
Sharma et al., 2016			Conditional KO	- No phenotype in adult neurons
Wang et al., 2011		Drosophila	Caz gene deletion	- Pupation and eclosion defects - Adult caz mutants show reduced survival and locomotor defects
Lebedeva et al., 2017		Zebrafish	KO by CRISPR-Cas9 system	- No distinctive phenotype - Normal locomotor activity
Kabashi et al., 2011	LOF/GOF	Zebrafish	AMO knockdown	- Axon & behavioral defects - Rescue by WT FUS but not R521C or R521H - Toxic GOF phenotype in R521H
Armstrong & Drapeau, 2013			AMO	- Impaired motor activity - Reduced NMJ synaptic transmission - R521H reduces synaptic fidelity
Bosco et al., 2010	GOF	Zebrafish	mRNA injection (WT FUS, FUS-H517Q, R521G, R495X, G515X)	- Cytoplasmic mislocalization - Stress granule formation
Seekic-Zahirovic et al., 2016	GOF	Mouse	Knockin by conditional ablation of FUS exon 15 (lack of NLS)	- Perinatal death - Respiratory insufficiency - Reduced body weight and length - Altered transcriptomic profile - Reduced MN number - Increased MN apoptosis
	LOF		KO	- Perinatal death - Respiratory insufficiency - Reduced body weight and length - Altered transcriptomic profile - No MN phenotype
Sharma et al., 2016	GOF		WT FUS, R521C and P525L transgenic expression	- Modest loss of spinal MNs - Loss of NMJ synapse - Astrogliosis and microgliosis - No phenotype in WT FUS transgenic mice
Mitchell et al., 2013			Mouse PrP promoter WT human FUS	- ALS-like phenotypes in homozygotes - FUS+/ubiquitin-cytoplasmic inclusions
Shelkovnikova et al., 2013, 2014			Thy1 promoter FUS 1-359	- Cytoplasmic FUS aggregates - MN loss - Disruption of paraspeckles
Qiu et al., 2014			FLAG-FUS-R521C transgenic expression	- ALS-like phenotypes with moderate loss of spinal MNs
Sephton et al., 2014			WT FUS or R521G transgenic expression by ubiquitous Cre promoter	- ALS-like phenotypes in R521G transgenic mice - Severe dendritic defects - Neuroinflammation - Dysregulation of synaptic proteins
Huang et al., 2011		Rat	Tet-inducible system WT FUS and FUS-R521C	- ALS-like phenotype - Early postnatal death
Huang et al., 2012			Camk2a-tTA inducible WT FUS and FUS-R521C	- Dendrite defects in neurons in the entorhinal cortex
Lanson et al., 2011 Shahidullah et al., 2013		Drosophila	Global or neuronal expression of human FUS	- Severe eye degeneration - Pupal mortality & locomotor defects - Cytoplasmic mislocalization - Stress granule formation - Synapse defects precede neurodegeneration
Machamer et al., 2014 Sasayama et al., 2012	LOF/GOF	Drosophila	Conditional overexpression/knockdown by UAS-GAL4 system	- Decreased synaptic transmission - Reduced number of presynaptic active zones, - Altered postsynaptic glutamate receptor subunit composition at the NMJ, - MN degeneration - Impaired motor behavior

Table 2: Summary of ALS-associated FUS mutant models (optimized from Shang et al., 2016). LOF: loss-of-function; GOF: gain-of-function; KO: knockout; AMO: antisense morpholino; PrP: prion

4.4. FUS pathogenicity

Dendritic and synaptic defects:

The characterization of FUS mutations consequences in the nervous system went through the creation of different models that presented consistent neurodegenerative phenotypes. Studies reported that rodent models expressing the R521C mutation showed several ALS-like symptoms including hindlimb paralysis, muscle wasting and impaired innervation at the NMJ (C. Huang et al., 2011; Qiu et al., 2014; Sharma et al., 2016b). These were associated with reductions in dendritic arborization and synaptic density in the spinal MNs and cortical neurons in the sensorimotor cortex of transgenic mice (Qiu et al., 2014) and of Cre-inducible transgenic mice (C. F. Sephton et al., 2014), as well as in the entorhinal cortex of Camk2a-tTA transgenic rats (C. Huang et al., 2012). This phenotype was also observed in cultured cortical neurons and the use of the growth factor BDNF could partially rescue it (Qiu et al., 2014).

Other model organisms revealed a pool of evidence regarding FUS implication during development and its disruption contributing to neurodegeneration. A research team studied the homolog of FUS in *Drosophila*, the cabeza (*caz*) gene, expressed in neurons, glia and muscle cells, and they showed through its loss-of-function that only a small fraction of these mutant reached adulthood, albeit with a severely reduced survival, and associated with a strong motor phenotype (J. W. Wang, Brent, Tomlinson, Shneider, & McCabe, 2011). These pathological features could be rescued partially by transgenic expression of *caz* and human wild-type FUS, but not with human FUS mutants (R522H and P525L) (J. W. Wang et al., 2011). Furthermore, other studies used also the *Drosophila* to express different mutant human FUS proteins in the eye, MNs or the nervous system and they demonstrated eye degeneration, defects in motor activity and decreased survival rate (Lanson et al., 2011). In a more in-depth analysis of the locomotion defects, Shahidullah and colleagues demonstrated that mutant FUS proteins caused

synaptic impairments before MN degeneration, characterized by the disorganization of the protein brunchpilot, which is a presynaptic active zone protein, as well as reduced quantal contents and miniature presynaptic currents, and reduced postsynaptic currents in the muscle cells (Shahidullah et al., 2013). Other reports were in line with these results and showed that gene deficiency and overexpression of FUS caused decreased synaptic transmission, reduced number of presynaptic active zones, altered postsynaptic glutamate receptor subunit composition at the NMJ, MN degeneration and impaired motor behavior (Machamer, Collins, & Lloyd, 2014; Sasayama et al., 2012)

In the zebrafish, FUS synaptic functions were investigated with the use of antisense morpholino (AMO) to cause a knockdown of the gene. This resulted in motor behavioral defects and significant impairments in the branching and motor axon length (Kabashi, Bercier, et al., 2011). In addition, another study observed that AMO knockdown of FUS and the expression of human mutant FUS proteins in zebrafish caused defects in synaptic transmission at the NMJ by reducing presynaptic quantal contents (G. A. B. Armstrong & Drapeau, 2013).

Impairments in DNA damage response and repair

In the section 4.1 (FUS structure and properties), I briefly reviewed some studies that underscored the role of FUS in DNA damage response and repair. In the case of ALS, it is interesting to note that fALS-FUS mutations do not affect the recruitment of mutant FUS proteins to DNA damage foci (Shang & Huang, 2016), but rather FUS mechanism to regulate the repair process is dependent on FUS ability to interact with HDAC1, which is fundamental for DNA repair and genomic stability (Shang & Huang, 2016). Previous studies reported that the loss of HDAC1 caused hypersensitivity to DNA damage in neurons and an induction of aberrant cell cycle re-entry, whereas the overexpression of HDAC1 has a protective effect in

neurons from genotoxic agents (Dobbin et al., 2013; D. Kim et al., 2008). This was confirmed with HDAC1 mutant neurons and neurons with a FUS deficiency that presented increased DNA damage after being treated with etoposide, an anti-cancer drug causing errors in DNA synthesis, underlying the interactive feature between FUS and HDAC1 and its role in maintaining genome stability (Dobbin et al., 2013; W.-Y. Wang et al., 2013). Structure-function analyses on FUS identified two regions in its structure, the first Gly-rich domain (156-262) and the C-terminal region (450-526), that are necessary for FUS-HDAC1 interaction (**Figure 12**) (W.-Y. Wang et al., 2013), and which contain most of the fALS mutations. Additionally, they showed in cells that FUS mutations in these domains, including R244C, R514S and R521C caused an altered interaction with HDAC1 associated with DNA damage response and repair defects (W.-Y. Wang et al., 2013).

Another study, in line with these results, showed a lack of complex formation between HDAC1 and FUS mutant R521C (Qiu et al., 2014), with a total interference of the interaction between wild type FUS and HDAC1, highlighting a dominant-negative effect (Qiu et al., 2014). They also observed the presence of DNA damage on NR2A and GluR2, both implicated in synaptic transmission, and BDNF, a dendritic growth factor, that could lead to neurodegeneration.

All this evidence supports the idea that FUS mutations play a major role in defective DNA damage response and repair and is a pathological feature associated with ALS pathogenesis and neurodegeneration in general.

RNA splicing defects

With FUS having an impact on multiple aspects of RNA metabolism, neurodegeneration mediated by FUS mutations could be explained by transcriptome deterioration. Therefore, genome-wide surveys through RNA sequencing (RNA-seq) were performed to identify

possible effects of FUS mutations on the transcriptomic profile of genes (Qiu et al., 2014; Robles & Baier, 2012; Sanes & Yamagata, 2009). Qiu and colleagues used RNA-seq on the spinal cord of FUS-R521C transgenic mice and showed an altered transcription and RNA splicing of specific genes involved in dendritic growth and synaptic functions (Qiu et al., 2014). Other studies realized the same RNA-seq analysis and demonstrated alterations in the expression or splicing of genes involved in the organization of extracellular matrix, like members of the collagen and cadherin gene families that regulate the specificity of axonal projection and target innervation (Robles & Baier, 2012; Sanes & Yamagata, 2009). Similar results were showed with a *Xenopus* model with FUS knocked down by AMO (Dichmann & Harland, 2012). In addition, RNA-seq analyses showed that among the target genes, some of them are transcriptional targets of DNA damage response gene, namely Cockayne syndrome (CSB) and HDAC1 (Newman, Bailey, & Weiner, 2006), further supporting the evidence that FUS is critical for DNA damage repair.

In the previous part, I already reported that FUS interacts with SMN and U1 snRNP, components of the spliceosome, and that FUS KO or expression of fALS-FUS mutations altered the organization of Gem bodies, thus impacting the splicing process (S. Sun et al., 2015; Yamazaki et al., 2012; Yu & Reed, 2015). Another evidence of FUS mediating the spliceosome organization is its implication in the integrity of subnuclear structures that regulate gene expression by holding RNA in the nucleus, known as paraspeckles (Bond & Fox, 2009). Among the paraspeckle proteins, splicing factor, proline- and glutamine-rich (SFPQ) has been shown to interact with FUS in the nuclei of neurons to regulate RNA metabolism, and specifically alternative splicing of the MAPT gene (Ishigaki et al., 2017) (further reviewed in section 5 Beyond FUS). In another report, cytoplasmic aggregates of another paraspeckle protein, P45NRB/NONO, were found in spinal MNs of transgenic mice expressing a truncated version of FUS (Shelkovernikova et al., 2013). Finally, RNA-seq analyses on spinal cord from RUS-

R521C transgenic mice showed altered transcriptomic profile of NEAT1, which is required to maintain the integrity of paraspeckles (Qiu et al., 2014). More recently, FUS mutant cell lines generated by CRISPR/Cas9 were shown to present accumulation of NEAT1 isoforms associated with compromised structure and functionality of paraspeckles, suggesting that FUS confers both loss (through dysfunctional paraspeckles) and gain of function (through excessive NEAT1) in the nucleus (H. An et al., 2019).

Mitochondrial defects and oxidative stress:

Susceptibility of FUS to oxidative stress has been demonstrated in a previous study (Caroline Vance et al., 2013) and could be associated with its co-localization with SGs in patients and cellular models (Y. R. Li, King, Shorter, & Gitler, 2013), that are formed when cells go under different cellular stress, including oxidative stress. With reports observing prolonged oxidative stress in ALS MNs, partially due to mitochondrial dysfunction, the link can be made between oxidative stress and impaired RNA metabolism through SG formation contributing to FUS aggregation (Bozzo et al., 2017). This is further supported by a study on neuron-like cells where they increased the level of the Oxidative Stress Resistance Protein 1 (Oxr1), a protein essential to protect cells from oxidative stress and shown to bind FUS as well as being up-regulated in spinal cords of ALS patients. They demonstrated that this protein level increase caused a decrease in FUS cytoplasmic localization and aggregation. On the contrary, silencing Oxr1 induces an increase in FUS inclusions under conditions of oxidative stress (Finelli, Liu, Wu, Oliver, & Davies, 2015). Other reports also observed that when FUS inclusions are formed, there is an increase in mitochondrial damage, highlighting the existence of a feed-forward loop (J. Deng et al., 2015). It was also demonstrated that FUS, with TDP-43, colocalized with mitochondrial markers in different ALS models, suggesting their involvement in altering

mitochondrial function (J. Deng et al., 2015; Magrané, Cortez, Gan, & Manfredi, 2013; W. Wang et al., 2013a).

Another line of research followed to explain mitochondrial damage through FUS is its possible impaired interaction with other non-mitochondrial proteins that can have an impact on mitochondrial homeostasis. For example, it was demonstrated that FUS interacts with PGC-1 α , a transcriptional co-activator that mediates the expression of genes involved in energy metabolism, including genes coding for MnSOD and catalase, which have a protective role against oxidative stress (Herrmann & Riemer, 2014). It was also reported that levels of PGC-1 α were decreased in the brain stem and the spinal cord of mutant FUS mice (Bayer et al., 2017), and the induction of PGC-1 α through the use of an agonist could rescue dendrite loss and spatial memory in rats expressing mutant FUS (C. Huang et al., 2012). However, their involvement in ALS pathogenesis still needs further investigation.

With the help of specific chaperones and proteases, mitochondria are able to control protein folding or degradation of misfolded proteins. Among these chaperones, Hsp60 has been reported to interact directly with FUS in the cytosol and in mitochondria (J. Deng et al., 2015). From these results, it has been hypothesized that in pathological conditions, FUS aggregates sequester the chaperones, causing alterations in protein folding control (Bozzo et al., 2017). Chaperones also bind newly synthesized mitochondrial proteins to keep them unfolded in order to facilitate their import in mitochondria (Beddoe & Lithgow, 2002). Therefore, since Hsp60 also acts in mitochondria, it was suggested that its trapping by FUS could have a direct pathological impact on mitochondrial functions (Bozzo et al., 2017).

Overall, since the discovery of FUS in 2009, a decade of research allowed to gather major information on the molecular basis of ALS in relation to this gene, and all these results promote the fact that FUS is a major element of study to better understand the consequent complexity of the disease. One challenge is to unravel common pathways in ALS and other neurodegenerative

diseases, as they share common alterations involving common genes, thus highlighting the need for additional models and new leads for therapeutic strategies.

5. Beyond FUS: RNA targets and their implication in ALS/FTD

As discussed before, FUS plays a role in different steps of RNA metabolism, and transcriptome alteration through FUS mutations is considered one of the major causes for neuronal toxicity. Following this hypothesis, numerous studies assessed the FUS-related transcriptome profiles to identify genes of interest that could contribute to neurodegeneration (Honda et al., 2014a; Ishigaki et al., 2012; Lagier-Tourenne et al., 2012b; Rogelj et al., 2012). From this, it was speculated that impairments to FUS functionality could result in a partial effect by altering expression levels of these genes, and that neurodegeneration might result only after transcriptional disruption caused by loss of FUS functionality reaches a critical point (Ishigaki & Sobue, 2018).

To better understand this mechanism, we focused our work on specific genes possibly related to FUS and that are the most likely to be disease-associated: the MAPT gene and the CHCHD10 gene, which will be further reviewed here.

Microtubule-Associated Protein Tau (MAPT): The protein tau was originally studied in the 1970's when it was described as an essential actor for microtubule assembly (Weingarten, Lockwood, Hwo, & Kirschner, 1975). The MAPT gene encoding for tau was then identified years later (Drubin, Caput, & Kirschner, 1984; M Goedert, Wischik, Crowther, Walker, & Klug, 1988), and it was reported that tau localizes to axonal microtubules in the healthy brain, whereas it is unusually concentrated in a hyperphosphorylated form in the soma and dendrites

of neurons under pathological conditions (Kosik, Joachim, & Selkoe, 1986; Wood, Mirra, Pollock, & Binder, 1986). It was also demonstrated that these hyperphosphorylated forms of tau would be the major components of the neurofibrillary tangles (NFTs) found in Alzheimer's disease (AD) brains, as well as Pick bodies found in Pick's disease (Grundke-iqbal et al., 1986). Further studies were then able to link MAPT mutations with familial forms of FTLT (Hutton et al., 1998; Poorkaj et al., 1998; M. G. Spillantini, Crowther, Kamphorst, Heutink, & Van Swieten, 1998), highlighting the importance of alternative splicing in neurodegeneration. Indeed, it was observed in FTLT-tau patients that MAPT mutations around exon 10 alter its splicing, leading to tau aggregation and impairing the axonal function of tau (M. Hong et al., 1998). Today, FTD is accepted as a highly heterogeneous disorder affecting behavior, language, memory and motor functions (Bodea, Eckert, Ittner, Piguet, & Götz, 2016), thus constituting with ALS a same spectrum of disease (Renton et al., 2014).

The first reports associating FUS and MAPT were derived from CLIP analyses that identified an increase in MAPT exon 10 inclusion under the condition of a loss of FUS (Ishigaki et al., 2012; Lagier-Tourenne et al., 2012b; Orozco et al., 2012b; Rogelj et al., 2012). Other studies also reported increased inclusion of exon 2 and 3 upon FUS knockdown (Lagier-Tourenne et al., 2012b; Orozco et al., 2012b), which suggests possible alterations in the regulation of tau alternative splicing. Indeed, tau presents a complex alternative splicing regulation acting on N-terminal (exon 2 and 3) and C-terminal different domains (exon 10), which leads to six different isoforms (**Figure 13**) (Bodea et al., 2016; Michel Goedert & Spillantini, 2011), distinguished in two types, the ones containing 4 tubulin-binding repetitions (4R), allowing microtubule binding, or the ones containing 3 tubulin-binding repetitions (3R). During splicing events, the inclusion of exon 10 allows the insertion of a fourth tubulin-binding repetition, which increases the affinity of 4R-tau to microtubules compared to 3R isoforms (Buée, Bussièrre, Buée-Scherrer, Delacourte, & Hof, 2000). In healthy conditions, there is a balance of expression between 4R-

tau and 3R-tau isoforms, but the ratio has been reported to be disrupted in different tauopathies, including FTL, progressive supranuclear palsy and corticobasal degeneration (M. Hong et al., 1998; Umeda et al., 2013; Yoshida, 2006).

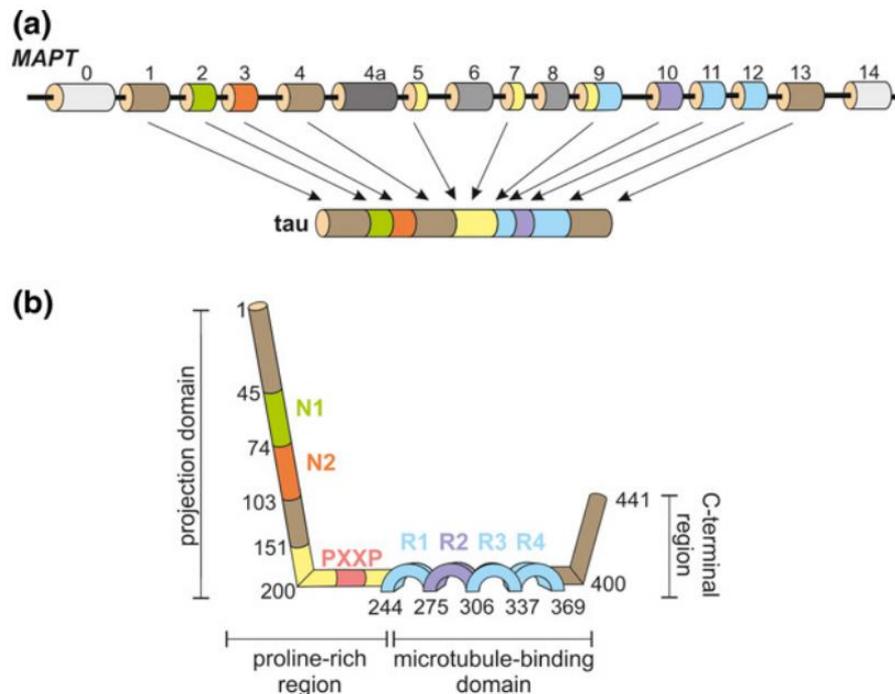


Figure 13: Structure of human MAPT gene and protein (derived from Bodea et al., 2016). Different splicing events will induce the expression of 6 different isoforms differing by the number of acidic regions (N1/2) at the N-terminus and tubulin-binding repetitions (R1/2/3/4).

Recently, a study from Ishigaki and colleagues demonstrated that the interaction between FUS and SFPQ regulates alternative splicing of MAPT exon 10, and that when FUS or SFPQ is silenced in mice there is an increase in 4R-tau expression leading to FTL-like behaviors, reduced adult neurogenesis, phosphorylated tau accumulation and neuronal loss (Ishigaki et al., 2017). This report, along with another identifying a familial FTL case presenting a FUS mutation associated with altered tau isoform ratios (Isidre Ferrer et al., 2015), suggest the importance of impaired tau isoform ratio as a pathogenic factor of ALS/FTD and other tauopathies.

Coiled-Coil-Helix-Coiled-Coil-Helix Domain 10 (CHCHD10): The CHCHD-containing proteins are nucleus-encoded small mitochondrial proteins (Z. D. Zhou, Saw, & Tan, 2017), with nine members of this member being already identified, and only CHCHD10 has been so far linked with ALS and FTD (Bannwarth et al., 2014b; Funayama et al., 2015; Z. D. Zhou et al., 2017).

Structurally, CHCHD10 has one CHCH domain (**Figure 14**), constituted by a twin CX(9)C motif (two cysteine separated by 9 amino acids), which is the signature of proteins involved in assembly of cytochrome c oxidase and maintenance of mitochondrial structures and function (Cavallaro, 2010; Z. D. Zhou et al., 2017). CHCHD10 also contains other functional domains like the mitochondrial targeting sequence (MTS) and a central α -helix domain (**Figure 14**) (Z. D. Zhou et al., 2017). Previous reports demonstrated that CHCHD10 is a soluble mitochondrial protein located in the intermembrane space (IMS; the space between the outer and the inner membranes) of mitochondria (J. An et al., 2012; Bannwarth et al., 2014b; Chacinska et al., 2008; Darshi et al., 2011; Hofmann et al., 2005), and is considered to be part of the mitochondrial contact site (MICOS) complex, which mediates mitochondrial crista ultrastructure stabilization (Friedman, Mourier, Yamada, Michael McCaffery, & Nunnari, 2015; Zerbes et al., 2012). Cristae integrity in mitochondria is paramount to stability and assembly of respiratory chain supercomplexes that are responsible for the efficiency of mitochondrial respiration as well as cell viability (Cogliati et al., 2013).

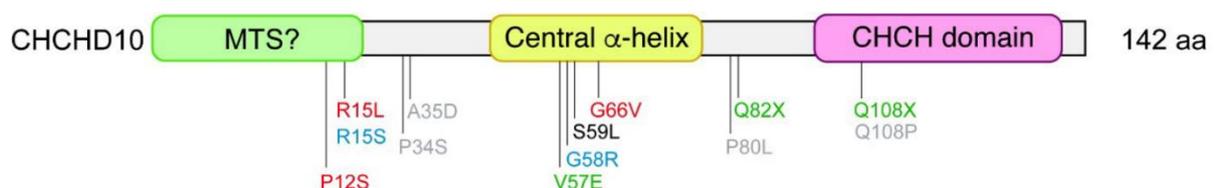


Figure 14: Structure of CHCHD10 protein presenting mutations of different neurodegenerative diseases (derived from Imai 2019). In green, coding variants in FTD patients; in red, patients with ALS; in black, patients with ALS-FTD and MN disease; in blue, patients with mitochondrial myopathies; in grey, potential risk variants found in ALS, FTD, Parkinson's disease, Alzheimer's disease and ALS-FTD patients (reviewed in Imai 2019).

CHCHD10 was first linked to ALS in a French family, through the identification of the S59L mutation, with a complex phenotype of ALS, FTD, cerebellar ataxia and mitochondrial myopathy, due to mitochondrial DNA (mtDNA) alterations (Bannwarth et al., 2014b). In this study, fibroblasts from patients showed abnormal crista structure associated with deficiencies in respiratory chain components and reduced respiration activity (Bannwarth et al., 2014b). In addition, it was demonstrated in this study that overexpression of mutant CHCHD10 in HeLa cells led to fragmentation of the mitochondrial network and ultrastructural abnormalities, including loss, disorganization and dilatation of cristae (Bannwarth et al., 2014b).

From these reports, two recent studies developed mutant CHCHD10 mouse models to investigate the pathogenic mechanisms of CHCHD10. Both are knock-in (KI) mice, carrying the S59L mutation (Genin et al., 2019) or an equivalent, S55L mutation (Anderson et al., 2019a). Genin and colleagues demonstrated with their S59L KI model multiple pathological features, including fatal mitochondrial cardiomyopathy before 14 months old associated with increased mitophagy, as well as NMJ and MN degeneration with hyper-fragmentation of the motor end plate and MN loss in lumbar spinal cord at the end stage of the disease (Genin et al., 2019). They also showed oxidative phosphorylation deficiency in muscle at 3 months of age in the absence of neuron loss in spinal cord, promoting the idea that the pathological effects of the S59L mutation target muscle before NMJ and MN (Genin et al., 2019).

Anderson and colleagues show similar results with their S55L mutation model, with progressive motor deficits, myopathy and cardiomyopathy as well as survival deficits (Anderson et al., 2019a). They also showed CHCHD10 aggregation with its paralog CHCHD2, leading to aberrant organelle morphology and function, and inducing mitochondrial integrated stress response (mtISR) through mTORC1 activation, with elevation of stress-induced transcription factors, secretion of myokines, upregulated serine and one-carbon metabolism, and

downregulation of respiratory chain enzymes (Anderson et al., 2019a). They also studied the effects of CHCHD10 ablation and they observed no particular disease pathology or active mtISR, suggesting an absence of loss-of-function effect in disease pathogenesis.

Based on the available knowledge concerning CHCHD10, there is no published evidence of a link between FUS and this gene. However, reports indicated in different model organisms a direct interaction of CHCHD10 with TDP-43 after translocation from the mitochondria in conditions of mitochondrial stress, exerting a protective role in mitochondrial and synaptic integrity as well as in the retention of nuclear TDP-43 (Woo et al., 2017). With TDP-43 sharing multiple roles with FUS in RNA metabolism and having common RNA targets (Guerrero et al., 2016; S. C. Ling et al., 2013), as well as both colocalizing with mitochondrial markers in different ALS models ((H. Deng et al., 2014; Magrané et al., 2013; W. Wang et al., 2013a), there is a significant possibility that FUS and CHCHD10 are linked, constituting a strong lead in the investigation of ALS pathogenesis.

Overall these new models will provide key elements for the understanding of ALS/FTD and mitochondrial pathologies in general, but they also highlight the importance of developing additional models to decipher the mechanisms involving CHCHD10 in disease pathogenesis and its possible interaction with other key actors of ALS like FUS.

6. Working hypothesis and objectives

While significant progress has been made on the understanding of FUS mechanisms in ALS pathogenesis, there is still an increasing need to unravel the still unknown and elusive cellular and molecular features of FUS mutations, as well as solve the discrepancies surrounding its pathomechanisms, notably in terms of loss- and gain-of-function. ALS being a complex and heterogeneous disease, the development of novel models is crucial to improve our knowledge and possibly develop efficient therapeutic strategies. Therefore, the main objective of our work presented here was to provide new key elements in the understanding of ALS and FTD by characterizing at all scales (morphological, behavioral, cellular, molecular) two novel genetic mutant models presenting a link with these diseases. One of the main objectives was to characterize a new genetic knock-out mutant of FUS in zebrafish, simulating a loss-of-function, by identifying possible phenotypic traits associated with ALS, through assessment of survival, locomotion, NMJ, MN and muscle development and stability, as well as its effects on transcription. Its link with MAPT gene is also investigated in order to find common links between these two genes and their common implication in ALS and FTD pathogenesis. We also aimed to decipher possible features involving mitochondrial pathology in ALS through investigation of possible mitochondria impairments in my FUS mutant model, as well as characterization of another genetic knock-out mutant zebrafish model of CHCHD10 and identification of a possible link between FUS and CHCHD10 that could explain these mitochondrial phenotypic features.

CHAPTER 2 – RESULTS

1. Functional characterization of the novel FUS mutant zebrafish model

1.1. Generation and characterization of the model

As stated before, numerous animal models featuring depleted or reduced FUS have been generated and analyzed including vertebrate and invertebrate models. However, the major phenotypic features obtained in these models are complex and often controversial.

In order to study the consequences of *fus* loss of function in zebrafish, we obtained a mutant genetic line for *fus* from the Zebrafish Mutation Project (reference: sa15506, mutation c.155T>A; p.Y52X, Sanger Institute) (Kettleborough et al., 2013). In this study, they generated the mutant line through *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis, which is considered the most appropriate technique to generate point mutations by chemical mutagen (Kettleborough, Bruijn, Eeden, Cuppen, & Stemple, 2011; Mullins, Hammerschmidt, Haffter, & Nüsslein-Volhard, 1994; Solnica-Krezel, Schier, & Driever, 1994). The mutants were then identified with a reverse genetic approach known as TILLING, which allows the identification of point mutations in a specific gene, and proved to be efficient in ENU-mutagenized zebrafish (Wienholds, Schulte-Merker, Walderich, & Plasterk, 2002; Wienholds et al., 2003). The mutation carriers were crossed and the homozygous generation was sent for Sanger high-precision sequencing, which confirmed the presence of the point mutation (**Figure 1A**). The mutation leads to a translation termination (stop) codon at the beginning of exon 3. Since exon 3 is responsible for the prion-like domain generation at the N-terminus of *fus* protein (**Figure 1B**), this model should lead to an inactive *fus* gene. To ascertain that this mutation leads to *fus* KO animals, we used Western blot analyses to determine the levels of *fus* in this model. At 48 hours post-fertilization (hpf), *fus* homozygous embryos showed a drastic reduction of *fus* protein level compared to WT (**Figure 1C**). Coherently, the decrease in the protein corresponds

to an increase of *fus* transcript, measured by RT-PCR (**Figure 1D**). Indeed, a feed-back *FUS* autoregulation was proposed in human cells (Lagier-Tourenne et al., 2012b; Y. Zhou et al., 2013a) and mouse models (Mitchell et al., 2013). Briefly, *FUS* is a repressor of its own exon 7, leading to alternative splicing and NMD (nonsense-mediated decay). Since this mechanism was unexplored in zebrafish, we checked for levels of *fus* transcript with primers for the exon 1 and observed a significant increase (**Figure 1D**). Taken together, these results indicate that the *fus*^{-/-} mutant line generates an inactive *fus* transcript.

The morphological aspects and main phenotypic features of WT and *fus*^{-/-} embryos were assessed at 2 days post-fertilization (dpf). Compared to WT embryos, *fus*^{+/-} and *fus*^{-/-} embryos showed no major morphological deficits with absence of body and head malformations (**Figure 1E**). To exclude developmental delays of the locomotor system, we assessed the spontaneous twitching of the body axis, which comprises the first movements that zebrafish embryos begin to display around 18 hpf (Saint-Amant & Drapeau, 1998). Both homozygous and heterozygous larvae did not show signs of impaired motility at this stage compared to the wild-type condition (**Supplementary Material, Figure S1**). Despite the normal development, the viability of *fus*^{-/-} embryos is severely reduced (**Figure 1F**). In fact, starting from day 6, more than 80% of homozygous embryos died. Importantly, no major difference was observed between the WT and *fus*^{+/-}.

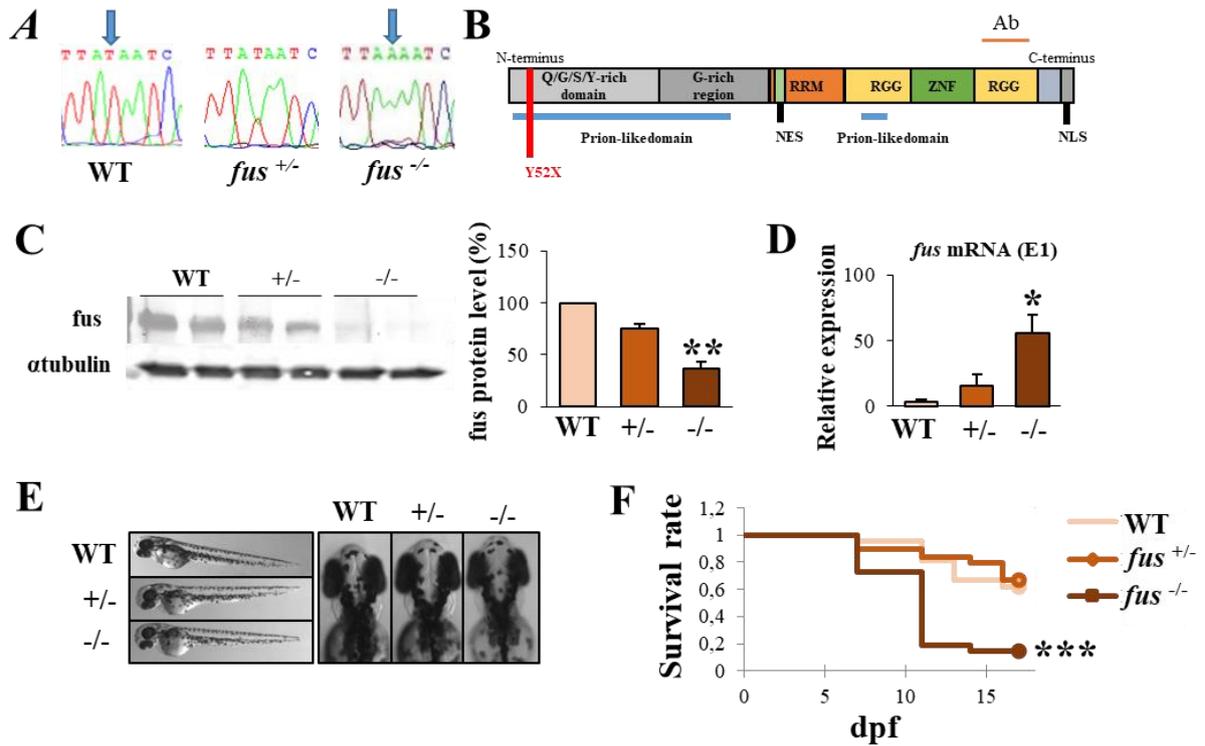


Figure 1: Characterization of the zebrafish *Fus* knock-out model. **A**, Sanger sequencing results with arrows showing the mutation target site. Compared to WT larvae, homozygotes do not exhibit the peak corresponding to T bp. **B**, schematic representation of zebrafish *Fus* protein showing the site of mutation at the N-terminus and the antigen recognized by Santacruz antibody at the C-terminus. **C**, *Left*, immunoblot analysis of *fus* protein in total extract from WT, hetero and homozygous embryos at 48hpf using a C-terminal antibody. The band detected is around 77 KDa. *Right*, densitometry quantification of *fus* protein level normalized to α tubulin. (One-way ANOVA with Bonferroni post-hoc test. $**p < 0, 01$). **D**, RT-PCR analysis of *fus* messenger (One-way ANOVA with Bonferroni post-hoc test. $*p < 0, 05$). **E**, Morphological phenotype of the different tested conditions at 2 dpf. **F**, Kaplan-Meier survival curves for WT, *fus*^{+/-} and *fus*^{-/-}. Time is shown in days post fertilization (dpf). An *n* of 21 embryos was used for WT, while 48 have been used for both hetero and homozygous mutants (*Log-rank test*, $*p < 0, 0001$).

1.2. *fus* inactivation causes locomotion defects in zebrafish

The zebrafish is an excellent model to study neuronal organization characterizing motor function. In fact, by 48 hpf, it exhibits stereotyped swimming behavior that can be efficiently quantified under controlled conditions. To analyze swimming features, we utilized the *touch-evoked escape response* (TEER) assay. Similar to previous studies (Kabashi, Bercier, et al., 2011; J. W. Wang et al., 2011), we induced embryos locomotor activity at 2 dpf by gently

touching the tail with a glass pipette (**Figure 2A, left**). For the WT condition (*light brown*), it resulted in a powerful rectilinear swim away from the stimulus (**Figure 2A, right**). Also, in heterozygous fish (*orange*), the locomotion is not affected, being similar to WT. However, homozygous embryos (*dark brown*) presented an aberrant locomotion characterized by early fatigue and, in some cases, paralysis. Using a tracking system to monitor swimming behavior, we measured the distance, the time and the velocity for each animal (**Figure 2B**). We found that in *fus*^{-/-}, the locomotor abilities are significantly reduced, confirming an important role for *fus* in the proper development of the motor circuitry (Gerlai, Lahav, Guo, & Rosenthal, 2000; Sasayama et al., 2012). Despite the low degree of similarity at the gene sequence alignment [human *FUS* (NM_004960), zebrafish *fus* (NM_201083), 14.94%], the human and zebrafish Fus proteins share a high degree of similarity (79,58%), which suggests functional analogy. Thus, we tested the ability of human *FUS* to rescue the motor defects in *fus*^{-/-}, injecting a plasmid containing the human *FUS* gene at one cell stage. Indeed, the expression of human *FUS* was able to restore embryos motility, increasing swimming distance and speed (**Figure 2C**). These results are in concordance with functional rescue previously observed in KD *fus* model in zebrafish (Kabashi, Bercier, et al., 2011) and *Drosophila* (J. W. Wang et al., 2011).

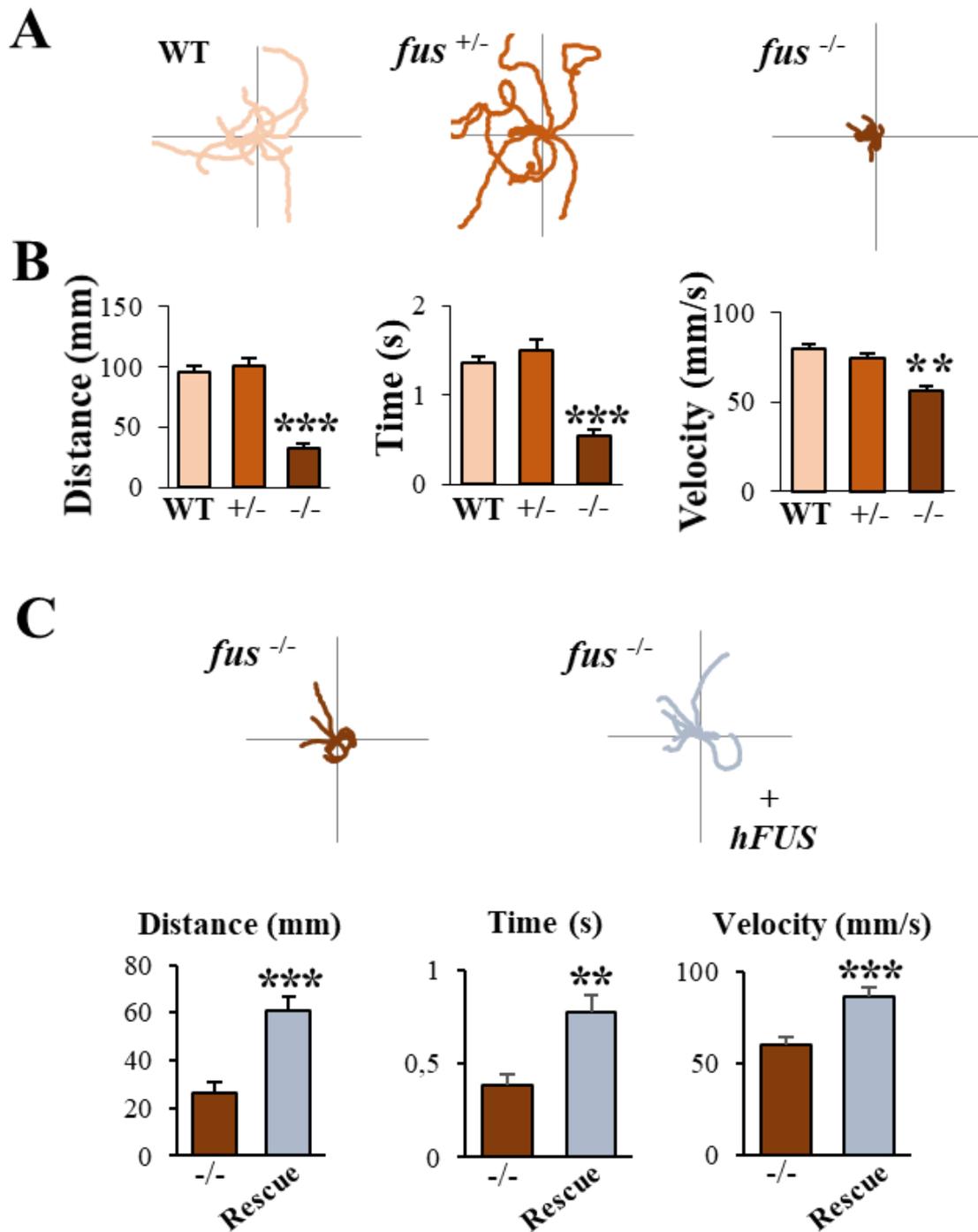


Figure 2: Zebrafish *fus*^{-/-} shows locomotion defects at TEER. **A**, traces representing the swim response of heterozygous and homozygous embryos compared to WT controls during the touch-evoked escape response assays (TEER). Briefly, 2 dpf zebrafish embryos are gently touched on the tail with a glass pipette and their escape behavior is assessed and recorded. **B**, Quantitative analyses of TEER. *fus*^{-/-} (dark brown) showed a significant decrease in the distance travelled, time spent and velocity of swimming (One-way ANOVA with Bonferroni post-hoc test. **p*<0, 05; ***p*<0, 01; ****p*<0, 001). **C**, Rescue of the major swim deficits in *fus*^{-/-} co-injected with human *FUS* gene (*Fus*^{-/-} n=30, *Fus*^{-/-} + hFUS n=25; unpaired t-test. **p*<0, 05; ***p*<0, 01; ****p*<0,001).

1.3. *fus* depletion causes behavioral impairment.

Zebrafish are diurnal animals, meaning that they are particularly active during light exposition, where predator, food finding and sexual behaviors are evident (Neuhauss, 2003). It also means that their visual system is refined (Champagne, Hoefnagels, De Kloet, & Richardson, 2010) and essential for their proper development and survival. For these reasons light stimuli tests are widely used in zebrafish research to assess their motor performances independently from vision defects (Champagne et al., 2010; Panzer et al., 2005b). Since vision develops rapidly in zebrafish over the course of 2 days (**Supplementary Material, Figure S2A**), we determined whether the motor impairment in homozygous zebrafish exists and persists at 3, 4 and 5 dpf after light stimulation. Using the ZebraLab platform, we designed a behavior tracking protocol in which single zebrafish larvae, placed in each well of a 96-well plate for a period of 7 minutes, are simultaneously stimulated by light after 5 minutes of adaptation in darkness (**Figure 3A**). With this test, we have been able to track the larval displacement, which is severely compromised in *fus*^{-/-}, (**Figure 3B**) and the global activity over time (**Figure 3C, D**). At day 3, homozygous embryos showed a significant decrease in both spontaneous and induced (startle) motor activity with respect to the WT and heterozygous fish (**Figure 3C**). Interestingly, at day 4, the *fus*^{+/-} presented a reduced startle response similarly to homozygous larvae (**Supplementary Material, Figure S2B, C**). At this stage, the behavioral impairment was also present during the freezing response, where a sudden delayed motor activity is present for both *fus*^{+/-} and *fus*^{-/-}. At day 5, augmented movement in the well by approximately 15-fold represents the complex spontaneous motor behavior of larvae that is being refined (**Figure 3D**). Similarly, at 5dpf, *fus*^{+/-} displayed spontaneous locomotion impairment that is comparable to *fus*^{-/-} zebrafish larvae. Taken together, these locomotion impairments suggest that *fus* is necessary for motor development in zebrafish. Also, the progressive motor impairment developed by heterozygous zebrafish indicates a cumulative effect of *fus* partial depletion.

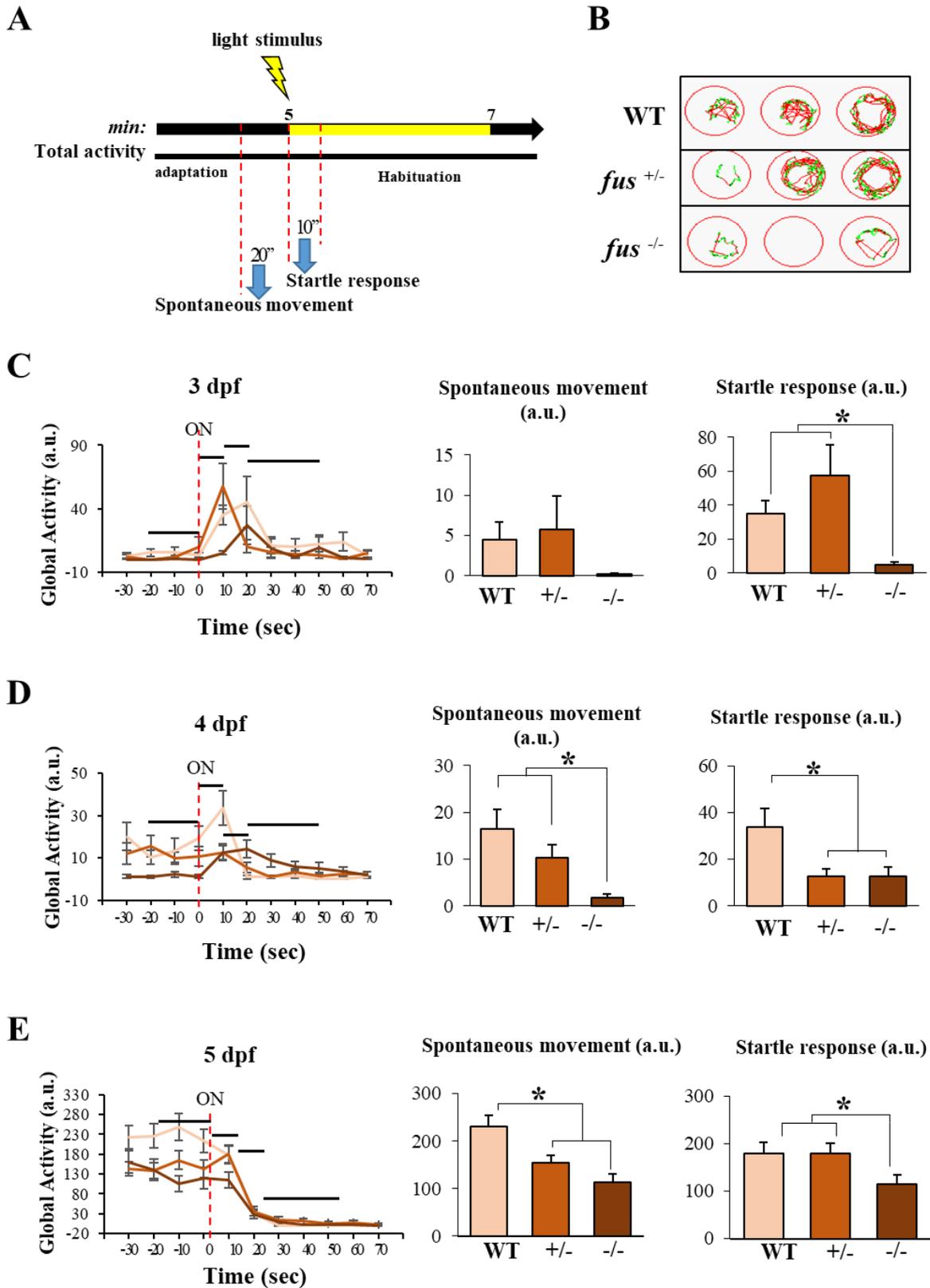


Figure 3: Behavioral activity in dark/light stimulation. A, automatized protocol used at the Viewpoint Zebrafish system. Light stimulation is preceded by a period of five minutes of darkness, called *adaptation period*. This period represents the favorite environment for zebrafish, probably mimicking a protective area from predators, in which we can measure an

increased locomotion activity that we call *Spontaneous movements*. An abrupt stimulation by light, the *startle response*, is then applied and the fish responds with a brief period of elevated activity, where anxiety increases. The total experiment duration is of 7 minutes and it is repeated over time until day 5. **B**, Representative examples of the swimming tracks in WT, *fus*^{+/-} and *fus*^{-/-} embryos at 3dpf. **C, D and E, Left panels** are representative graphs from global locomotor activity data for each condition at 3, 4 and 5 dpf respectively (*light brown*: WT, *orange*: *fus*^{+/-}, *dark brown*: *fus*^{-/-}). Black bars indicate the 4 periods considered for global activity quantification. *Red dashed line* represents the light application. **Right panels** are quantification for activity during darkness (spontaneous movement) and light (startle response) (One-way ANOVA with Bonferroni post-hoc test. *p<0, 05).

1.4. *fus* loss-of-function causes defects at the zebrafish NMJs

To better refine the phenotypic features in the homozygous line, we performed an immunolabeling analysis for NMJ components both at the pre-synaptic and post-synaptic terminals. We used an anti-SV2 antibody to mark the presynaptic vesicles and the α -bungarotoxin to mark the acetylcholine receptor (AChR) clusters at the post-synaptic level. By 48 hpf, in WT embryos, the caudal primary motor neuron had extended branches and formed synapses along the length of individual muscle fibers (**Supplementary Material, Figure S3A**). To evaluate the correct conformation of the NMJ, we used the Imaris software “spot” detection in conjunction with the colocalization function in 3D reconstructions of confocal imaging (**Supplementary Material, Figure S3B**). At 48 hpf, presynaptic terminals are almost always precisely opposed to AChR clusters (**Figure 4Ai**). A similar immunostaining pattern was observed in *fus*^{+/-} embryos as compared to WT controls (**Figure 4 ii**). In contrast, in *fus*^{-/-} pre- and post-synaptic clustering were impaired (**Figure 4iii**). We measured an increasing number of both α BTX and SV2 orphans (**Figure 4B, C**), suggesting the presence of disconnected motor neurons terminals. Moreover, the α BTX staining showed the appearance of smaller and diffused AChR clusters (**Figure 4a, b, c**), reminiscent of those seen during the early stage of

development, prior to any motor innervation (Panzer et al., 2005b). We also observed shorter axons (**Figure 4D**) in *fus*^{-/-} embryos compared with WT.

Next, we analyzed whether the aberrant phenotype at the motor and NMJ levels was caused by denervation or collateral reinnervation of previously denervated muscle fibers. Functional AChR in adult muscle is an heterooligomeric membrane protein composed of α , β , ϵ and δ subunits in which the α subunits bear the acetylcholine binding sites (Merlie, Sebbane, Gardner, Olson, & Lindstrom, 1983) (**Figure 4E**). In mammals and fish, the γ subunit is part of the pentamers during development, before being replaced by ϵ (Gu & Hall, 1988; Walogorsky et al., 2012). In ALS patients, where muscle denervation and abnormal reinnervation are pathological hallmarks of the disease, both subtypes exist (Palma et al., 2011, 2016a). For these reasons, abnormal levels of subunit α (*chrn α 1*), γ (*chrn γ*) and ϵ (*chrn ϵ*) are normally considered as markers of denervation/reinnervation together with muscle histone deacetylase 4 (HDAC4) level which is dramatically induced upon denervation in ALS mice (Cohen et al., 2007) as well as in rapidly progressive patients with ALS (Bruneteau et al., 2013). In *fus*^{-/-} at 2 dpf, we observed an important transcript induction of AChR γ and AChR ϵ , with a slight but not significant increase of AChR α as compared to age-matched *fus*^{+/-} and WT controls (**Figure 4E, bottom**). Reversely, *hdac4* level is decreased compared to controls. These results suggest the coexistence of denervation and reinnervation processes in this zebrafish model and are consistent with a recent study that highlighted an altered expression of AChR subunits associated with denervated neuromuscular endplates in iPSC-derived myotubes from patients with *FUS*-ALS (Picchiarelli et al., 2019).

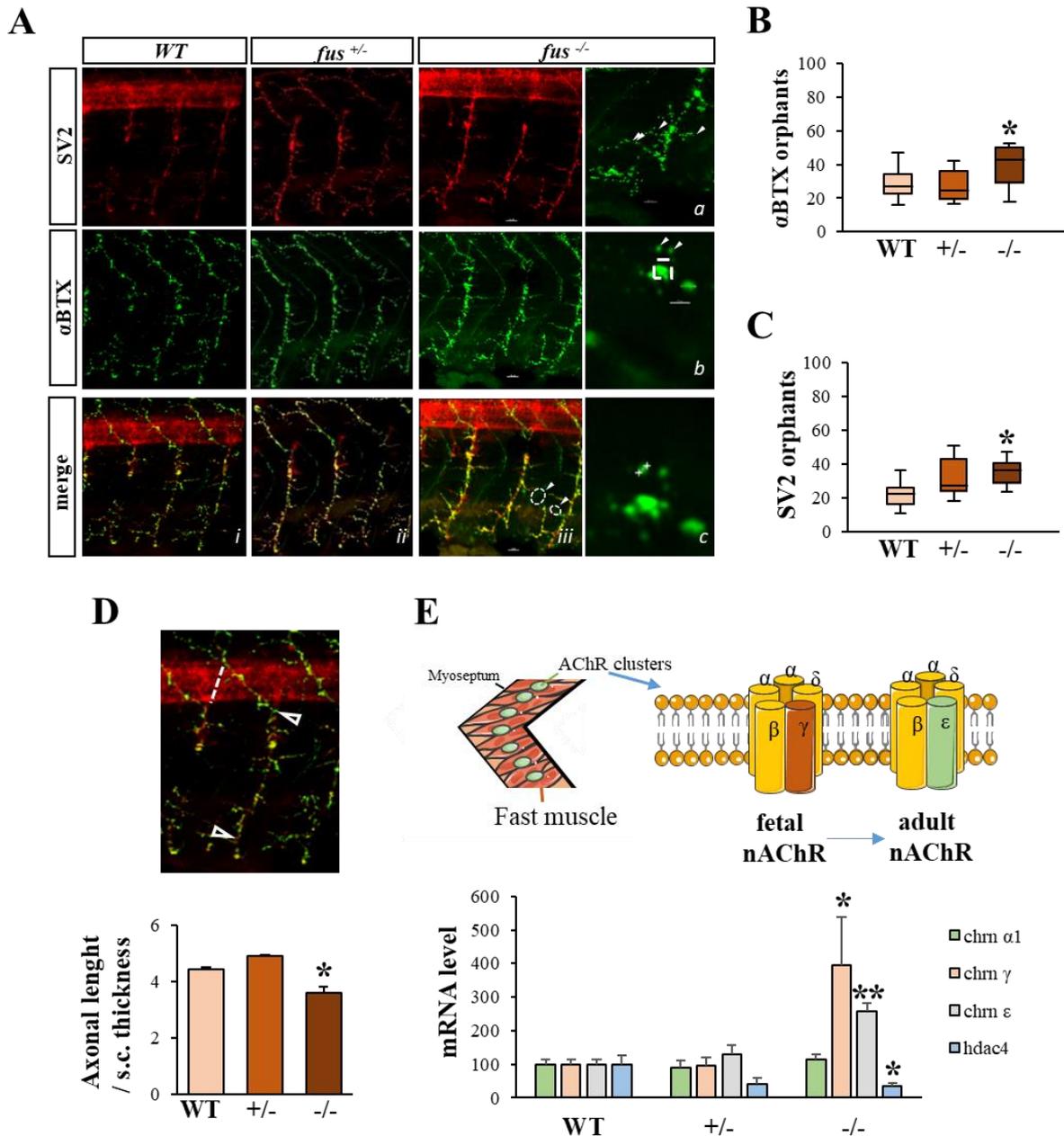


Figure 4: *fus* loss-of-function causes defects at the NMJs. **A**, Primary motor axon (Cap) of WT and mutant *fus* embryos stained immunofluorescently with the antibody against SV2 (red) and AChR (green). At 48 hpf, WT and heterozygous motor axons have grown along the lateral myosepta and axon branches are present in all muscle fiber layers. Functional synapses, made of innervated NMJs are well colocalized (*i* and *ii*, yellow). In *fus*^{-/-} embryos, motor neurons displayed a more elaborated branching, parallel to an increasing staining of uncolocalized red and green puncta (*iii*). AChR morphology changes and several smaller green spots appear to be diffused on muscle membrane (arrows in *a*, *b*, *c*). **B**, spots quantification with Imaris software of uninervated AChR (One-way ANOVA with Bonferroni test, * $p < 0, 05$). **C**, quantification of orphaned SV2 pre-synaptic marker (One-way ANOVA with Bonferroni test, * $p < 0, 05$). **D**, in the upper figure is shown a representative motor neuron used for axonal length measurement. Total length was taken between the represented empty arrows and length was normalized to the spinal cord (s.c.) thickness (dashed line). In the upper part the total length quantification showed a decrease in *fus*^{-/-} motor neurons size (One-way ANOVA with Bonferroni test, * $p < 0, 05$). **E**,

schematic representation of zebrafish myotome segment. Fast muscles fibers, which project with a 30 degree angle with respect to the axial structure, present round, punctated AChRs distributed along the length of each fiber, forming a vertical line in the center of the somite (Behra et al., 2002; Codina, Li, Gutiérrez, Kao, & Du, 2010; Panzer et al., 2005b). AChRs in the fast muscle undergo molecular changes during development, where the γ subunits are substituted by ϵ , as described in the text. *Bottom*, Total RNAs were extracted from WT, *fus*^{+/-} and *fus*^{-/-} and RT-PCR was performed to determine the transcript level of subunit α , γ , ϵ and HDAC4 (One-way ANOVA with Bonferroni test, *p<0, 05, **p<0, 01).

1.5. TDP-43 overexpression does not restore *fus*^{-/-} phenotype

Previous studies using animal models and primary cortical neurons have described that the gene *TARDBP*, that encodes for the protein TDP-43, and *FUS* are part of a common pathogenic mechanism (Kabashi, Brustein, et al., 2011; J. W. Wang et al., 2011). Furthermore, these important factors for ALS pathogenesis, share common transcriptome profiles (Colombrita et al., 2012), suggesting a collaboration of these two genes in mRNA maturation and transportation (Honda et al., 2014b). In the same way, *FUS* share structural and functional features with a set of RNA-binding proteins, such as TATA box-binding protein (TBP)-associated factor 15 (*TAF15*) and with Ewing sarcoma breakpoint region 1 (*EWSR1*) (I. R. A. Mackenzie & Neumann, 2012).

Considering the proved link between these genes and their close resemblance, we measured *tardbp* and *taf15* expression, in order to rule out a possible phenomenon of compensation or addition in the homozygous mutants. Transcript levels of *tardbp* and *taf15* did not show any significant difference between WT and mutant conditions (**Figure 5A**). Moreover, tdp-43 protein levels were not altered in both *fus*^{-/-} and *fus*^{+/-} conditions compared to WT controls (**Figure 5B**). Finally, overexpression of human *TARDBP* was not able to rescue the phenotypic features of the *fus*^{-/-} genetic line (**Figure 5C**). These results are consistent with previous studies presenting TDP-43 upstream of FUS in a common pathway and suggest no compensatory or

additional effect due to TAF15 or TDP-43 expression in the *fus*-related phenotypes observed in this novel zebrafish model of disease.

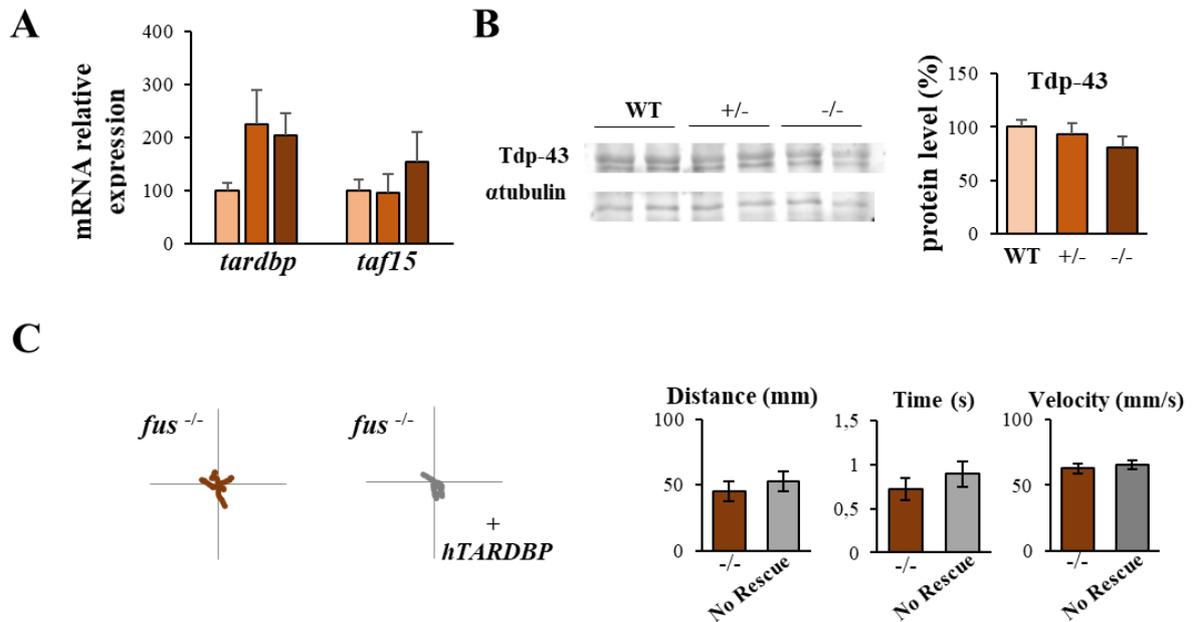


Figure 5: *Fus*^{-/-} phenotype is not due to compensatory pathways. **A**, qPCR analysis of *Tardbp* and *Taf15* expression in WT, hetero and homozygous embryos at 48 hpf. No significant changes were measured. **B**, Protein levels of endogenous TDP-43, assessed by Western Blot, do not change among conditions. **C**, Overexpression of human *TARDBP* did not rescue the motor phenotype observed in the *Fus* homozygous mutants.

1.6. *Tau* isoforms imbalance in *fus*^{-/-} zebrafish

To better explore the cause of the motor deficit seen in *fus*^{-/-} embryos, we checked the transcript expression of genes that play a central role in neuronal development and synaptic plasticity. The *Muscle blind like splicing regulator 1* (MBNL1) gene, which promotes muscle differentiation by regulating alternative splicing and sub-cellular localization of specific transcripts (Pascual, Vicente, Monferrer, & Artero, 2006). Consistent with the regulatory role of that protein, *Drosophila* and mouse *mbnl1* loss-of-function mutants show a clear musculature

phenotype (Artero et al., 1998; Kanadia et al., 2003). The *fragile X mental retardation 1 (FMR1)* gene, which is involved in the normal development of dendritic structures by regulating the metabolism of several mRNAs (Antar, Li, Zhang, Carroll, & Bassell, 2006; Braun, 2000; A. Lee et al., 2003; Nimchinsky, Oberlander, & Svoboda, 2001). The *Ca²⁺/calmodulin-dependent protein kinase IIa (CaM-KIIa)*, which is a multifunctional kinase that transduces neuronal Ca²⁺ signals in the brain, highly enriched in postsynaptic densities and key regulator of activity-induced structural plasticity (Jourdain, Fukunaga, & Muller, 2003). The *microtubule-associated protein tau (MAPT)* gene, which is implicated in the outgrowth of neural processes and in the development of neuronal polarity (R Brandt, 1996). In zebrafish, two paralogues of MAPT have been annotated *mapta* and *maptb* (M. Chen, Martins, & Lardelli, 2009).

At 48 hpf, only *maptb* transcript is significantly increased in *fus*^{-/-} embryos (**Figure 6A**) among the genes measured. Interestingly, MAPT has been identified as a physiological splicing target of FUS (Orozco et al., 2012b), with FUS loss-of-function disrupting tau isoform equilibrium and inducing FTL-like behavioral impairments and tauopathy in rodents (Ishigaki et al., 2017). Therefore, we analyzed the complex pattern of alternative splicing of the *mapta* and *maptb* transcripts using the primers previously described in Moussavi Nik et al., 2014, designed to amplify all zebrafish tau isoforms (**Figure 6B**). Normally, at 48 hpf, the expression level of small and longer Tau isoforms are similar (M. Chen et al., 2009). However, we found an increase in the 4R and 3R isoforms of *mapta* and *maptb* respectively (**Figure 6C, D**) in *fus*^{-/-} embryos. Although the *mapta* 6R and *maptb* 4R expression do not change, the increase of the smallest transcripts, causes an overall decrease of 6R/4R and 4R/3R ratios of Tau transcripts (**Figure S4**).

This is consistent with the increase of the transcript levels of *tra2b* (**Figure 6E**), a splicing regulator which putative binding sites have been detected in exon 8 of *mapta* and exon 9 of

maptb (Moussavi Nik et al., 2014). Thus, *tra2b* may contribute to 3R and 4R isoforms levels by increasing the exon 8 and 9 exclusion, as previously described for the corresponding human TRA2B and the tau exon 10 (Y. Wang et al., 2005). The data from the Light Stimulation assay showed a temporal decline of motor responses in *fus*^{+/-} larvae comparable to *fus*^{-/-} zebrafish. For that reason, we checked for *mapta* and *b* isoforms at 4 dpf. At this stage, the expression level of *mapta* isoforms appeared to be similar among conditions (**Figure 6F**). Contrarily, the 3R form of *maptb* show a certain level of decrease in both *fus*^{+/-} and *fus*^{-/-} larvae compared to control (**Figure 6G**). Interestingly, *tra2b* transcript also decreases, confirming the role of this factor on *mapt* splicing (**Figure 6H**).

Finally, we performed western blot analysis to check if *mapt* increase at 48 hpf was corresponding to an increase of the tau protein level. As expected, the total amount of tau is higher in *fus*^{-/-} zebrafish compared to WT control (**Figure 6I**).

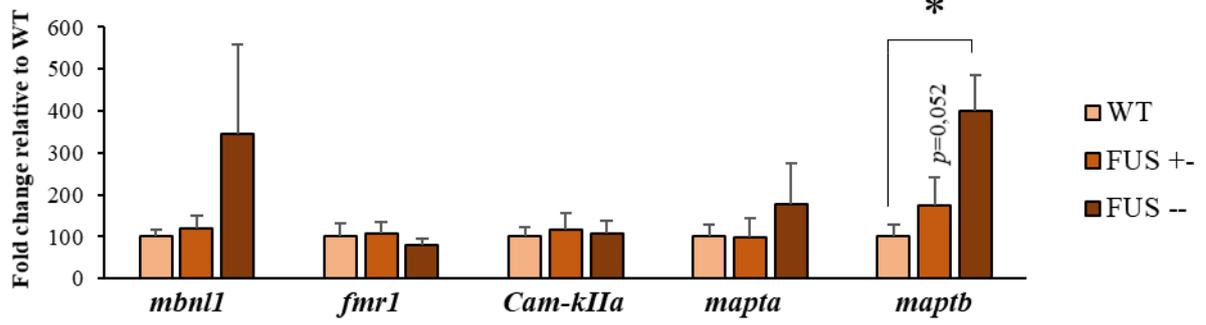
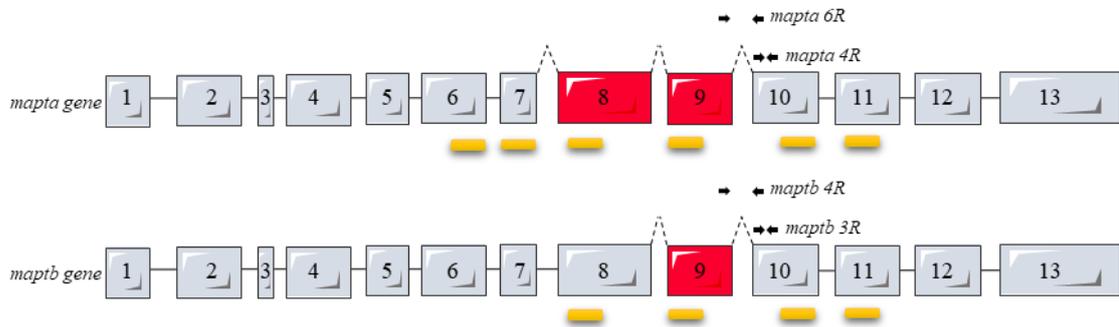
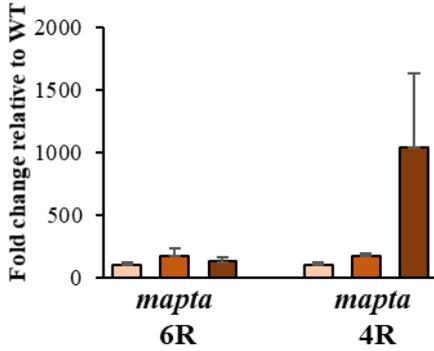
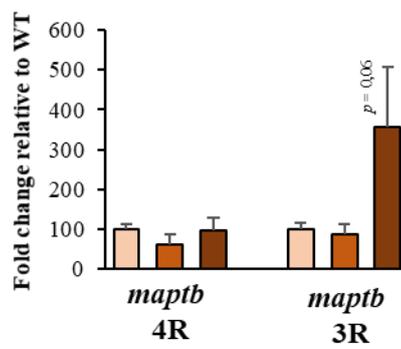
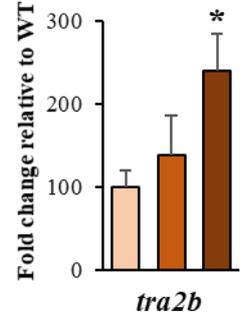
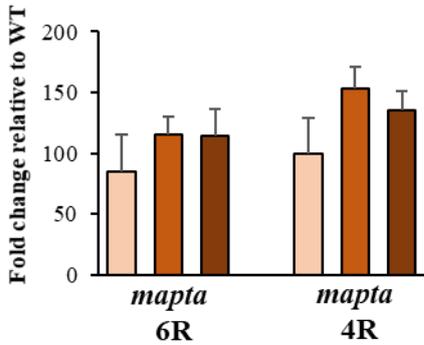
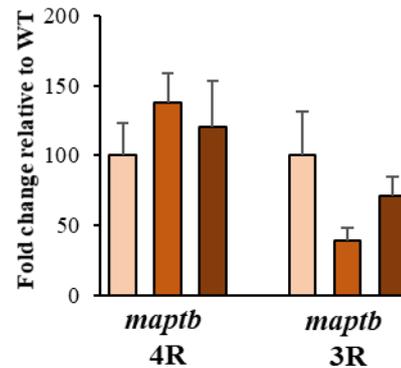
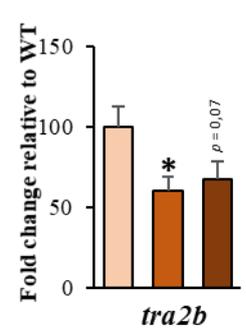
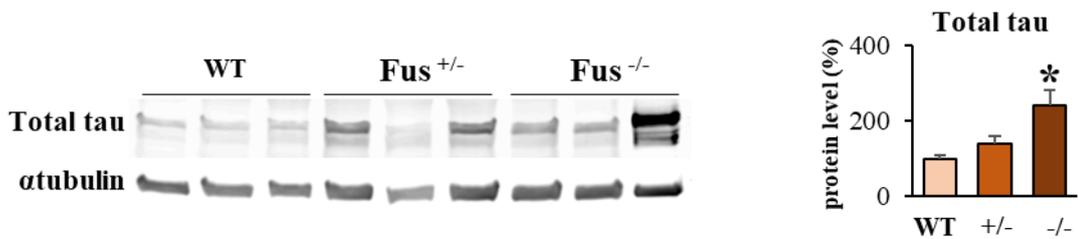
A**B****C****D****E****F****G****H****I**

Figure 6: *Fus* deletion causes changes in *mapta* and *maptb* balance. **A**, qPCR analysis of zebrafish *mbnl1*, *fmr1*, *cam-kIIa*, *mapta* and *maptb*, all fundamental genes during development of muscle and neurons. Among the genes measured in this study, *maptb* transcript is increased in *Fus*^{-/-} embryos at 48 hpf. **B**, splicing isoforms of *mapta* and *maptb* mRNA transcripts. Exon 8 and 9 for *mapta*, and only exon 9 for *maptb*, are susceptible to splicing events, causing a switch between short and long tau isoforms. *mapta* lead to 4R or 6R-tau translation while splicing of exon 9 in *maptb* lead to either 3R or 4R-tau translation. Grey boxes represent constitutive exons, the red ones being subject to alternative splicing. The tubulin binding motifs are indicated by yellow bars. Arrows indicate the primers binding sites. **C, D and E**, *mapt* isoforms transcript measurement by qPCR at 2 dpf. The results show a general abundance of smaller *mapt* isoforms (*mapta* 4R and *maptb* 3R). **F, G and H**, *mapt* isoforms transcript measurement at 4 dpf. At this stage the *maptb* 3R isoform decreases in both *fus*^{+/-} and *fus*^{-/-} larvae. **I**, western blot analysis shows an increase of tau protein in homozygous embryos at 2 dpf.

1.7. Kinase expression changes in *fus*^{-/-} zebrafish

In motor neurons, microtubules are crucial structures for maintaining cellular organization and motility. In this context, tau plays a central role and its binding to microtubule largely depends on phosphorylation (Roland Brandt, Lee, Teplow, Shalloway, & Abdel-Ghany, 1994; Tucker, 1990). The phosphorylation of tau negatively regulates tau-microtubule interactions (Bramblett et al., 1993; J. H. Cho & Johnson, 2003), inducing microtubules destabilization (J.-H. Cho & Johnson, 2004) and in certain cases aggregation and diseases (Ballatore, Lee, & Trojanowski, 2007; T. Guo, Noble, & Hanger, 2017). tau is phosphorylated at many sites via several protein kinases, and the most characteristic phosphorylation is at the Ser/Thr residues in the Proline rich sequences, which are targeted by proline-directed protein kinases such as GSK3 β (Hernández, Lucas, Cuadros, & Avila, 2003), Cdk5 (Kimura, Ishiguro, & Hisanaga, 2014), and the extracellular regulated kinases 1 and 2 (ERK1/2) (I Ferrer et al., 2001). Although its precise role is still not well understood, a number of reports have described the possible implication of Leucine-rich repeat kinase 2 (LRRK2) in Tau phosphorylation (Araki, Ito, & Tomita, 2018). We decided to check the expression of these enzymes due to the significant role they have in tau phosphorylation and the existence of their gene in unduplicated forms in zebrafish.

At 2 dpf, *fus*^{-/-} embryos have an augmented transcript rate of *cdk5* and *lrrk2* (**Figure 7A**). An opposite tendency is measured for *erk2* which decreases, whereas no significant change is visible for *gsk3β*. Interestingly, kinase levels are not maintained over time, and a change in their transcript level is measured at 4 dpf. In fact, *cdk5* and *erk2* return to a normal level comparable to WT fish (**Figure 7B**) while *gsk3β* transcript decreases drastically and *lrrk2* continues to be increased in *fus*^{-/-} larvae. As *lrrk2* showed consistency in its transcriptomic profile change between 2 and 4 dpf, we went further and assessed its protein level by Western blot in the *fus* mutant model. In line with the increase of *lrrk2* mRNA transcripts, there is an increase of *lrrk2* protein levels in *fus*^{+/-} and *fus*^{-/-} embryos (**Figure 7C**).

These results suggest a possible alteration in kinase activity in the absence of *fus*. This change could lead to alterations in tau phosphorylation and probably disturb or compensate microtubule dynamics during zebrafish development. Importantly, this disequilibrium amongst tau kinase activities is believed to play a central role in the development of tauopathies (I Ferrer et al., 2005).

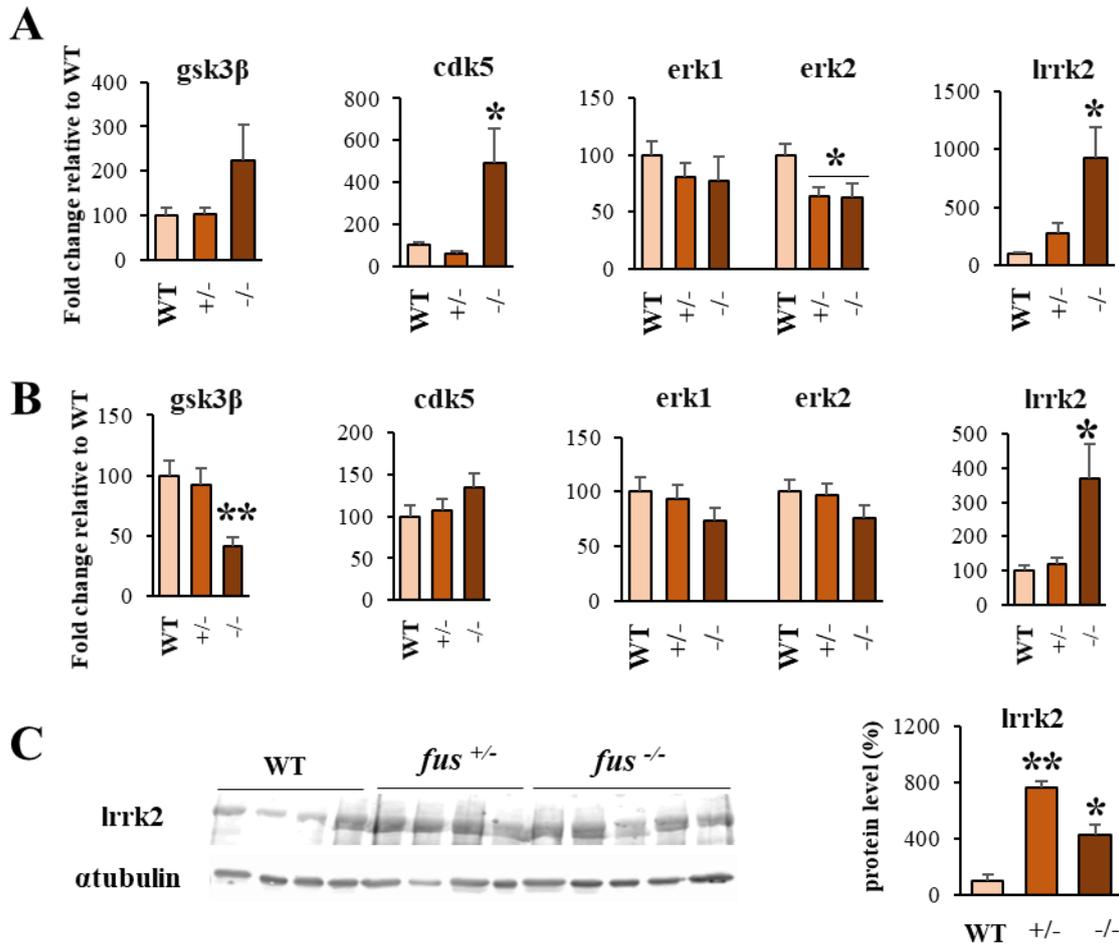


Figure 7: Disequilibrium among tau kinases in *Fus*^{-/-}. QPCR analysis of zebrafish *Gsk3b*, *Cdk5*, *Erk 1 and 2*, and *Lrrk2*. **A**, expression profile at 2 dpf. **B**, expression profile at 4 dpf. **C**, Western blot analysis showing an increase of *Lrrk2* protein levels in the *fus* mutants (One-way ANOVA with Bonferroni post-hoc test. * $p < 0,05$; ** $p < 0,01$).

1.8. Discussion

We demonstrated here, through the generation and the phenotypic characterization of a stable mutant zebrafish line for the unique FUS orthologue, that loss of *fus* drastically reduces the lifespan in zebrafish and leads to locomotor disabilities that persist over time, whether the movement is spontaneous or in condition when locomotion is induced by touch or light stimulation. The motor impairments were significantly restored upon expression of the human

FUS, but not *TARDBP*, and we did not observe any dysregulation of *tardbp* and *taf15* transcripts, indicating the specificity of the motor phenotype due to *fus* knockout as well as the functional conservation between human *FUS* and zebrafish *fus*. To extend this characterization, we associated these phenotypic features with the observation of aberrant axonal projections from motor neurons and disorganized NMJ morphology. Furthermore, we described here a significant dysregulation of several AChR subunits associated with a decrease of *hdac4*, highlighting possible denervation and reinnervation processes in our model. At the molecular level, we demonstrated transcriptomic and protein expression changes for the genes encoding for different isoforms of the protein tau in the model, as well as changes in the expression of kinases acting on tau phosphorylation, suggesting a link between *fus* and altered tau function and phosphorylation that could play a role in the development of tauopathies.

These results indicate that the orthologue of *FUS* in zebrafish is necessary for the proper development of motor circuits. These results are in clear contrast with the *fus*-CRISPR/Cas9 zebrafish model where no motor deficits were described (Lebedeva et al., 2017). It is quite possible that the genetic background could have an important influence on gene expression and the resulting phenotypic display. Indeed, in the *fus* mutant generated by CRISPR/Cas9, the authors used an outbred zebrafish (AB x TU) line to generate the *fus* knockout mutants (Lebedeva et al., 2017). Here, we obtained a pure, inbred AB zebrafish line in which the *fus* point mutation was generated and subsequently maintained. Indeed, genetic compensations have been described in CRISPR-generated mutants in zebrafish as compared to conditions when genes are knocked down by morpholinos (Rossi et al., 2015).

Furthermore, we speculated the existence of functional compensatory pathways that could mask the phenotypic features due to reduced levels of *fus*, as previously seen in a *Fus* knockout mouse model where increased levels of TAF15 and EWS were observed (Kino et al., 2015). *FUS*, together with TAF15 and EWS, belongs to the FET family of RNA-binding proteins, with

ubiquitous nuclear expression and implicated in regulation of gene expression, maintenance of genomic integrity and mRNA/microRNA processing, (Andersson et al., 2008b; Bertolotti, Lutz, Heard, Chambon, & Tora, 1996b; A. Y. Tan & Manley, 2009) and their expression may be finely regulated to guarantee their mutual compensation. Interestingly, some FUS-positive pathology can be also labeled for TAF15 and EWS, with cells showing a reduction in the normal nuclear staining of all FET proteins (I. R. A. Mackenzie & Neumann, 2012; Manuela Neumann et al., 2009), thus confirming the close relationship among these factors. Since zebrafish has one homolog for *taf15*, and two for *ews1*, we checked whether *taf15* expression could be implicated in our *fus*^{-/-} phenotype, but no particular change was measurable, underlining the specificity of the homozygous phenotype. However, it is still plausible that upregulation of other transcripts and proteins could be necessary to compensate for the loss of *fus* and transcriptomic as well as proteomic studies could unravel important alterations in this novel *fus* zebrafish model.

We showed that the locomotor impairment can be rescued by human *FUS*, revealing a remarkable conservation of protein function during evolution. In contrast, excess production of human *TARDBP* does not have the ability to rescue the homozygous phenotypes, as well as *fus* depletion does not induce an increase of tdp-43 expression. These data are consistent with a genetic relationship in which *fus* is downstream of *tardbp* as previously established in zebrafish (Kabashi, Bercier, et al., 2011) and drosophila (J. W. Wang et al., 2011). FUS protein is highly and predominantly expressed in the CNS of zebrafish from developmental stages to adulthood (Kabashi, Bercier, et al., 2011) and its depletion induces locomotion deficits as well as reduction of lifespan, both features of ALS pathology. Thus, these results demonstrate that *fus* activity is necessary to regulate locomotive behaviors and viability in vivo and indicates that the neurological problems observed in ALS patients may not be restricted to the late formation of insoluble FUS aggregated within the cytosol but also could be partially due to the early loss of

FUS function in the nucleus. These data are in line with the increasing evidence of an early involvement of FUS in ALS development, where the progressive and additive defects at the mRNA and DNA processing could play an important role in the onset and severity of the disease (Kuroda et al., 2000; Mallik et al., 2018; Scekcic-Zahirovic et al., 2016). This hypothesis is supported by the decreasing locomotor behavior that we could see in the *fus*^{+/-}. Thus, it would be interesting to examine motor and cognitive features of *fus*^{+/-} animals, as well as the *fus*^{+/-} survival, at later stages during juvenile and adult age. In particular, this model may provide an interesting tool to test genetic and chemical modifiers that could restore FUS activity.

Zebrafish is particularly well suited for a detailed analysis of the NMJ due to its transparency, its well-defined spinal network architecture and similarity to higher vertebrates. As previously described (G. A. B. Armstrong & Drapeau, 2013), by double-labeling of a pre-synaptic marker and post-synaptic acetylcholine receptors, we could count an increased number of uncolocalized NMJ structures suggesting the presence of denervated endplates. However, based on the transcriptional results, we have reasons to think that both denervation and reinnervation phenomena coexist in the homozygous model at 48 hpf. In fact, both *chrn γ* and *chrn ε* are elevated whereas the expression of the *hdac4* transcript was decreased. Very recently, a study highlighted a direct link between NMJ disruption and *FUS* in mouse models and patient samples. In this report, they found reduced endplate surface area and progressive endplate denervation preceding MN loss that was associated with altered expression of several *Chrn* genes, suggesting a transcription regulation of these genes by FUS (Picchiarelli et al., 2019). This is line with the NMJ dysfunctions and altered transcript levels we show in our *fus* mutant model. Furthermore, Hdac4, which is normally upregulated in aged SOD1G93A and SMARD1 mice, both examples of neurogenic muscle disease as well as in WT denervated mice, shows no significant elevation in myogenic muscular dystrophies (Cohen et al., 2007). These results suggest that Hdac4 is specifically induced in response to neural inactivity as opposed to muscle

dysfunction (Cohen et al., 2007). Hdac4 is responsible for the coordinated induction of synaptic genes upon denervation, and its upregulation is associated with lower muscle reinnervation ability, particularly visible in patients with rapidly progressive ALS (Bruneteau et al., 2013; Pradat et al., 2012). In the *fus*^{-/-} zebrafish embryos, the expression of the *hdac4* transcript is significantly reduced as compared to controls suggesting a reinnervation effect on muscles, as previously seen in knockout mice for *Hdac4*, where mutant muscles were reinnervated more rapidly than those from the controls after nerve injury (Pigna et al., 2018; Williams et al., 2009). Therefore, *fus*^{-/-} zebrafish may recapitulate certain hallmarks of ALS degeneration observed in human muscle biopsies (Bruneteau et al., 2013; Pradat et al., 2012) and thus, it could represent a good model where early NMJ dysfunctions can be studied.

The identification of expression changes of *mapt* genes and kinases responsible for tau phosphorylation in this model is interesting as it follows multiple evidence linking ALS and FTD. Indeed, it is estimated that up to 50% of ALS patients present signs of altered behavior and cognitive impairment with approximately 15% of them reaching the diagnostic criteria of FTD (Ringholz et al., 2005; Robberecht & Philips, 2013). The same has been reported for FTD patients with up to 15% of them having ALS motor dysfunctions (Burrell, Kiernan, Vucic, & Hodges, 2011; Van Langenhove et al., 2017). Another important information is the report of the association between neurodegeneration and an imbalance in the ratio of expression of tau isoforms (Hutton et al., 1998; M. G. Spillantini et al., 1998). In fact, human MAPT transcripts undergo alternative splicing to produce 3R- and 4R-tau isoforms in a one-to-one ratio to maintain microtubule dynamics and this ratio has been found to be altered in different neurodegenerative disorders in a disease-specific manner (reviewed in Goedert & Spillantini, 2011; Maria Grazia Spillantini & Goedert, 2013). Additionally, it was demonstrated that FUS was involved in tau splicing by skipping exons 3 and 10 (Orozco et al., 2012b), and that loss of FUS and its partner SFPQ in mice could cause an increase in 4R-tau expression and tau

hyperphosphorylation leading to neurodegeneration and FTD-like behaviors (Ishigaki et al., 2017). We show in this *fus* KO zebrafish model similar data through the regulation of expression of *mapta* and *maptb*, which are two paralogues of human *MAPT* that derived from a duplication of an ancestral *MAPT* orthologue (M. Chen et al., 2009). Like in humans, these genes show complex alternative splicing regulations, with *maptb* encoding 3R- and 4R-tau isoforms and *mapta* encoding 4R- and 6R-isoforms, and mRNA from *maptb* has been shown to be detected before mRNA from *mapta*, which is consistent with the predominance of 3R isoforms in human and mouse fetal stages (McMillan et al., 2008). However, at 48 hpf, *maptb* and *mapta* expression levels appear to be similar (M. Chen et al., 2009), and their expression pattern is similar to the one found in mice, indicating conserved function during evolution (M. Chen et al., 2009). Thus, zebrafish can be considered a useful model to study MAPT and its implication in neurodegeneration.

To be functional, tau proteins go through different post-translational modifications, with phosphorylation being the most abundant one (Buée et al., 2000), and hyperphosphorylation of tau, leading to its aggregation, has been identified as a major factor in the development of tauopathies (reviewed in Götz, Halliday, & Nisbet, 2019). Furthermore, extensive evidence demonstrates the implication of tau-specific kinases in the rise of Alzheimer's disease (AD) and other tauopathies. GSK3 β has been shown to induce tau hyperphosphorylation and consequently neurodegeneration when overexpressed (Lucas et al., 2001), as well as other features related to tauopathies (Martin et al., 2013). Cdk5 induces tau hyperphosphorylation and accumulation of neurofibrillary tangles (NFTs; aggregates of hyperphosphorylated tau) when overexpressed (J. C. Cruz, Tseng, Goldman, Shih, & Tsai, 2003; Noble et al., 2003) and its silencing was shown to reduce NFT formation (Piedrahita et al., 2010). As for Erk1 and 2, they phosphorylate tau at different sites that were also found to be phosphorylated in AD brains

(Ekinici & Shea, 1999; Guise, Braguer, Carles, Delacourte, & Briand, 2001), and abnormal tau phosphorylation was associated with an increased Erk1/2 activity (Pei et al., 2002).

LRRK2 has been mostly involved in the pathogenesis of Parkinson's disease (PD) with one of its mutation being the most frequent PD-causing mutation (Araki et al., 2018). It was reported that in pathological conditions, there is an increase in LRRK2 kinase activity, which is believed to contribute to PD pathology (Araki et al., 2018), and some reports showed that tau can be involved in PD pathogenesis besides LRRK2 (Nalls et al., 2014; Simón-Sánchez et al., 2009). Actually, a number of studies described that LRRK2 promotes tau phosphorylation in different mouse models of PD, whether directly (Bailey et al., 2013) or in the presence of tubulin or microtubules (Hamm et al., 2015; Kawakami et al., 2012). Another interesting information is that LRRK2 has been proposed to directly interact with GSK3 β , enhancing its kinase activity, which was correlated with higher levels of tau phosphorylation (Kawakami et al., 2014; Ohta et al., 2015). It was also found that LRRK2 binds to tau and is co-immunoprecipitated with tau and Cdk5, suggesting a role of scaffold protein from LRRK2 to facilitate Cdk5 phosphorylation of tau (Shanley et al., 2015). All this evidence encouraged us to explore the possibility that tau in the *fus* zebrafish model might be hyperphosphorylated. The first step that was envisaged was to assess directly the phosphorylation levels of tau through Western Blot analyses. However, although there was an increase in tau phosphorylation, it was not significant and could be correlated to the observed increase of total tau protein levels. The transcriptomic profile alterations of the tau-specific kinases in the *fus* model is interesting and suggests disrupted kinase activities that may impact tau function. However, depending on the developmental stage, the transcript profiles change, with significant modifications at 2 dpf that are back to normal levels at 4 dpf for *cdk5* and *erk1/2*. This could be explained by the particular developmental processes the embryos go through, facilitating plasticity of the cytoskeleton to ensure their development. However, despite this supposed delay, the NMJs are formed and found

denervated and reinnervated in the embryos. Reinnervation process generates the sprouting of new branches from motor axons (Cappello & Francolini, 2017), which puts them in a situation of extensive axonal plasticity. Thus, in this context, we can speculate that microtubules might be in an enhanced dynamic that is associated with enhanced tau function, which may explain the changes in expression of tau and related kinases. Furthermore, this process occurring only in motor axons, the changes in tau and kinases expression might be specific to MNs and not present in other parts of the organism. Thus, checking the mRNA and protein levels of tau and its related kinases in MNs specifically might give us additional insights on their expression profile in *fus* mutants.

Yet, it is important to note that *lrrk2* levels were consistent in their increase in *fus*^{-/-} embryos and supported by the increase in *lrrk2* protein levels. This suggests that *lrrk2* is involved in tau accumulation and this phenotypic feature can be linked to *fus* depletion. Thus, it would be the first molecular link identified between FUS, LRRK2 and tau that could be related to ALS-FTD, as well as tauopathies and neurodegeneration in general.

The characterization of this novel *fus* model has provided interesting information on ALS hallmarks and possible links with tau and *lrrk2* suggesting FTD features. However, a solid connection between the phenotypic features and the expression changes of tau and *lrrk2* could definitely link these genes to ALS pathogenesis. Therefore, we performed different drug tests that would modulate tau-isoforms or *lrrk2* expression and assessed a possible phenotypic rescue. We first used 2 different drugs to modulate the expression of tau isoforms: Epigallocatechin gallate (EGCG) is a *dyrk1a* inhibitor that promotes 4R tau expression in human and mouse. EGCG has been found to rescue dysregulated 4R/3R tau ratio and improved the impaired general behaviors in Ts65Dn mice (X. Yin et al., 2017). Tubastatin A is a HDAC6 inhibitor that restores microtubule stability and reverses tau phosphorylation (Ma, Huo, Jarpe, Kavelaars, & Heijnen, 2018). It was also found to reverse axonal transport defects in motor

neurons derived from FUS-ALS patients (W. Guo et al., 2017). Unfortunately, both these drugs did not rescue the motor phenotype found in our model (data not shown).

We also used CZC-25146, a LRRK2 inhibitor, with 2 different concentrations to counteract the expression increase observed in the *fus* mutant and we found that locomotion in *fus*^{-/-} had a tendency to improve (**Supplementary Material, Figure S5**). However, we were not able to reproduce these results. Thus, further investigation needs to be pursued in this direction and should provide new insights for therapeutic strategies.

Overall, these data demonstrate that normal expression of *fus* is essential for maintenance of NMJ structure in zebrafish and its depletion is sufficient to develop pathogenic changes, even in the absence of cytosolic aggregates. This zebrafish model recapitulates multiple features of ALS FUS-related pathology, including locomotor deficits, motor neurons and NMJ involvement and decreased viability. It also displays molecular links with major factors of tauopathies, which should make it valuable for studying the function and mechanism of this protein in human neurodegenerative disorders and help in the discovery of future treatments for these prevalent diseases.

1.9. Material and methods

Animal Care: Adult and larval zebrafish (*Danio rerio*) were maintained at the ICM (Institut du Cerveau et de la Moelle épinière, Paris) zebrafish facility and bred according to the National and European Guidelines for Animal Welfare. All procedures were approved by the Institutional Ethics Committees at the Research Centers of ICM and Imagine.

Screening of mutant lines: The *fus* mutant zebrafish line was obtained from the European Zebrafish Resource Center (EZRC) (reference: sa15506) and originates from the mutagenesis screen performed at the Wellcome Trust Sanger Institute (Stemple Laboratory). The mutation (c.155T>A; p.Y52X) in the *fus* genetic line is a *nonsense* point mutation at the exon 3 of *fus*. Heterozygous parents and homozygous offspring were screened through high-precision sequencing (GATC Biotech, Eurofins Genomics). DNA was extracted from by cutting a part of the fin from adult zebrafish. Prior to fin-clipping, the adult zebrafish were anesthetized in tricaine (160 µg/mL). A part of their caudal fin was cut and put in 75 µL of extraction solution (25mM NaOH, 0,2 mM EDTA). The samples were then incubated at 98°C for 1 hour and 75 µL of a Tris-HCl (40 mM, pH 5.5) solution was added to the tubes before centrifugation at 4000 rpm for 3 minutes. The samples were amplified by PCR using Taq polymerase Master Mix (Thermo Scientific K1071) and the following primers: CTGCCCAGAACTACAGTCAG (forward primer) and GCCACCAGAGCTATACGACT (reverse primer). The PCR products were then sent to GATC Biotech for sequencing.

Survival assay: Embryos were maintained according to the procedures of the ICM fish facility. Larvae were checked daily and maintained in petri dish in a 28.5°C incubator for the first 5 days post-fertilization (dpf). After 5 dpf, the fish were transferred in tanks with continuous water flux. They were then fed regularly and the number of dead larvae was scored each day for 20 days by a blinded observer.

Western Blot: 30 ug of proteins from zebrafish lysates (equal amount of protein in each lane) were separated by sodium dodecylpolyacrylamide gel electrophoresis. Samples were denatured at 98°C for 7 minutes. The separated proteins were transferred to nitrocellulose membranes (0.45µm, Life Sciences) and probed with the following primary antibodies: c-terminal FUS antibody (sc-47711, Santacruz), C-terminal TDP-43 antibody (12892-1-AP, Proteintech), recombinant anti-LRRK2 antibody (ab133475, abcam). A α-tubulin antibody

(T5168, Sigma-Aldrich) was used as a loading control. To assess tau protein levels, we used a homemade antibody targeting the C-terminus kindly sent by Valérie Buée-Scherrer and her team. The blots were incubated with the corresponding fluorescent secondary antibody and the signal was detected using the ODYSSEY® CLx. The intensity of bands in each of the lanes from the Western blots were measured by ImageJ.

RNA isolation and analysis of transcripts by q-PCR: Total RNA was isolated from fish using TRIzol Reagent (Sigma) according to the manufacturer's protocol. First-strand cDNAs were obtained by reverse transcription of 1 µg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Roche), according to the manufacturer's instructions. Quantitative PCR amplification was performed with SyBer2X Gene Expression Assays using the primers listed in **Table S1**. Data were analyzed transforming raw Cq values into relative quantification data using the delta Cq method.

Locomotion assessment: *Touch-evoked escape response (TEER):* 2 days post-fertilization (dpf) zebrafish embryos were tested for behavior under a stereomicroscope (Zeiss, Germany) by inducing a light touch-stimulus at the level of the tail with a tip and were scored for the distance travelled, the time spent swimming and speed in a 15-cm diameter petri dish. Their responses were recorded with a Grasshopper digital camera (Point Grey) at a rate of 30 frame/s and quantified using ImageJ. *Spontaneous locomotion:* 3 dpf-zebrafish larvae were maintained in a 96-well plate and assayed for total locomotor activity in ZebraBox (Viewpoint Life Sciences, Lyon, France). The embryos were tested for 10 min in a 5 min dark, 2 min light (100%), 3 min dark paradigm, repeated 3 times. The experiment was done on the same fish at 4 and 5 dpf. Total swim activity was analyzed using ZebraLab V3 software (Viewpoint Life Sciences, Lyon, France).

Immunohistochemistry: Animals were fixed in 4% paraformaldehyde for 2 hours at RT. After fixation the larvae were rinsed several times with PBS and then incubated in PBS containing 1 mg/ml collagenase (20 min) to remove skin. The collagenase was washed off with PBS Triton X-100 (PBST; 1 h) and heads were cut away. After an incubation of 30 min in blocking solution (1% BSA, 1% triton, PBS, 2% Goat serum), the embryos were incubated overnight at 4°C in synaptic vesicle 2 (SV2, 1:200; DSHB) antibody diluted in blocking solution. The embryos were then washed and incubated for 30 min in PBST containing α -bungarotoxin conjugated to Alexa 488 (α BTX, 1:1000; Abcam). The larvae were then rinsed several times with PBST and then incubated in freshly prepared block solution containing a secondary antibody (Alexa Fluor 568, 1:1000; Life Technologies) for 3h at RT before mounting on glass slide in 50% glycerol. The NMJs were visualized using a Spinning disk system (Intelligent Imaging Innovations, USA), an Examiner.Z1 upright stand (Carl Zeiss, Germany), a CSU-W1 head (Yokogawa, Japan), and an ORCA-Flash 4.0 camera (Hamamatsu, Japan). All images were captured at 40x and stained embryos were processed using Imaris Image Analysis software and ImageJ.

TDP-43 and FUS overexpression studies: Human WT TARDBP (WT TDP-43) and FUS mRNAs were transcribed from NotI-linearized pCS2 β using SP6 polymerase with the mMESSAGE Machine Kit (Ambion). Injections were then performed in 1–4 cell stage blastulae. Embryos were maintained at 28°C and manually dechorionated using fine forceps at 24 hpf. After behavioral test, 15 to 20 fish were deyolked and resuspended ice-cold extraction buffer: 50 mM Tris-HCl, pH 7.4/ 500 mM NaCl/ 5 mM EDTA/ 1% (w/v) Nonidet P-40/ 0.5% (w/v) Triton X-100 supplemented with a cocktail of protease inhibitors. Fish were then sonicated and centrifuged at 14000 \times g at 4 °C for 20 minutes. Supernatants were collected and frozen at -80°C until biochemical analysis.

Drug screening: zebrafish embryos were incubated at 24 hpf in different concentrations (1 and 10 μ M) of the following drugs: Tubastatin A hydrochloride (SML004, Sigma-Aldrich),

Epigallocatechin gallate (E4143, Sigma-Aldrich), CZC-25146 LRRK2 inhibitor (438194, Sigma-Aldrich). The embryos were tested the following days for locomotion through TEER and Viewpoint tests.

Statistical analysis: All data values for the zebrafish experiments are represented as average standard error of mean (SEM) with significance determined using one-way ANOVAs. Differences between groups were identified via post hoc comparisons, specified in the legend of each figure. All analyses were performed using Prism 5.0 (Graph Pad, CA). Significance level was set at $p < 0.05$.

1.10. Supplementary Material

	WT (n=37)	+/- (n=85)	-/- (n=107)
Coils/min (mean value)	14,642	9,615	11,890

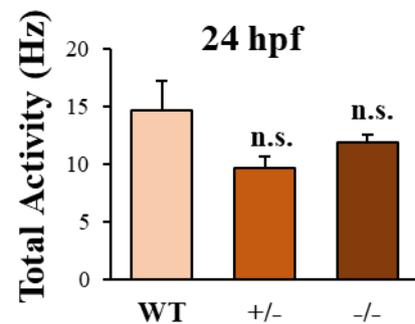


Figure S1: Coiling activity at 24 hours post-fertilization (hpf). Spontaneous premotor movement of the embryos inside the chorion was monitored and quantified. These correspond to stereotyped movement consisting of repetitive coils (complete rotations of the body in the chorion). No significant difference was observed between WT, +/- and -/- conditions (one-way ANOVA with Bonferroni post-hoc test), suggesting a normal development at early stages.

A

- The optic axon reach the tectum
- Motor and sensorial systems are formed

*Touch/EscapE response***48 hpf (2 days)**

- Beginning of retina formation

*Light stimulation (startle) response***62 hpf (2,5 days)**

- Complex visual system

*Visual behavior (hunting...)***72 hpf (3,5 days)**

- Fully functioning eye

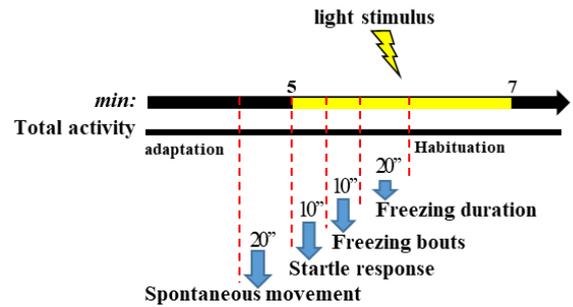
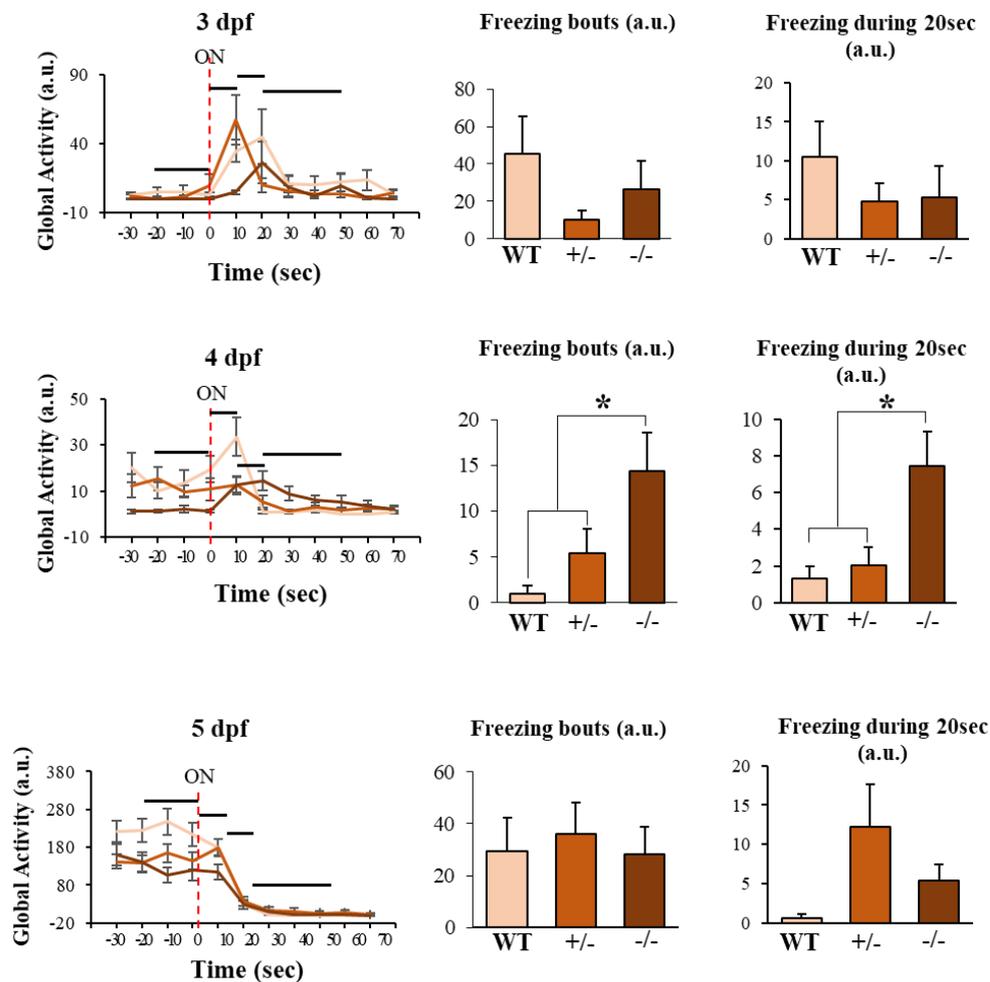
*Spontaneous locomotor repertoire***5 days****B****C**

Figure S2. **A**, schematic diagram of retina formation in zebrafish over time. **B**, in the automatized protocol used at the Viewpoint Zebbox system, after the abrupt stimulation by light (startle), the increased anxiety brings to a final reduction of locomotion, known as freezing response, which is characterized by a rapid fall of activity (freezing bouts) which is maintained over time (freezing duration). Normally, it is followed by a habituation response, where anxiety decreases and movements are reestablished. **C**, Left panels are

representative graphs from global locomotor activity data for each condition at 3, 4 and 5 dpf respectively. Black bars indicate the 4 periods considered for global activity quantification. Red dashed lines represent the light application. Right panels are quantification for activity after light stimulation, that we call Freezing bouts and Freezing duration (One-way ANOVA with Bonferroni post-hoc test. * $p < 0,05$).

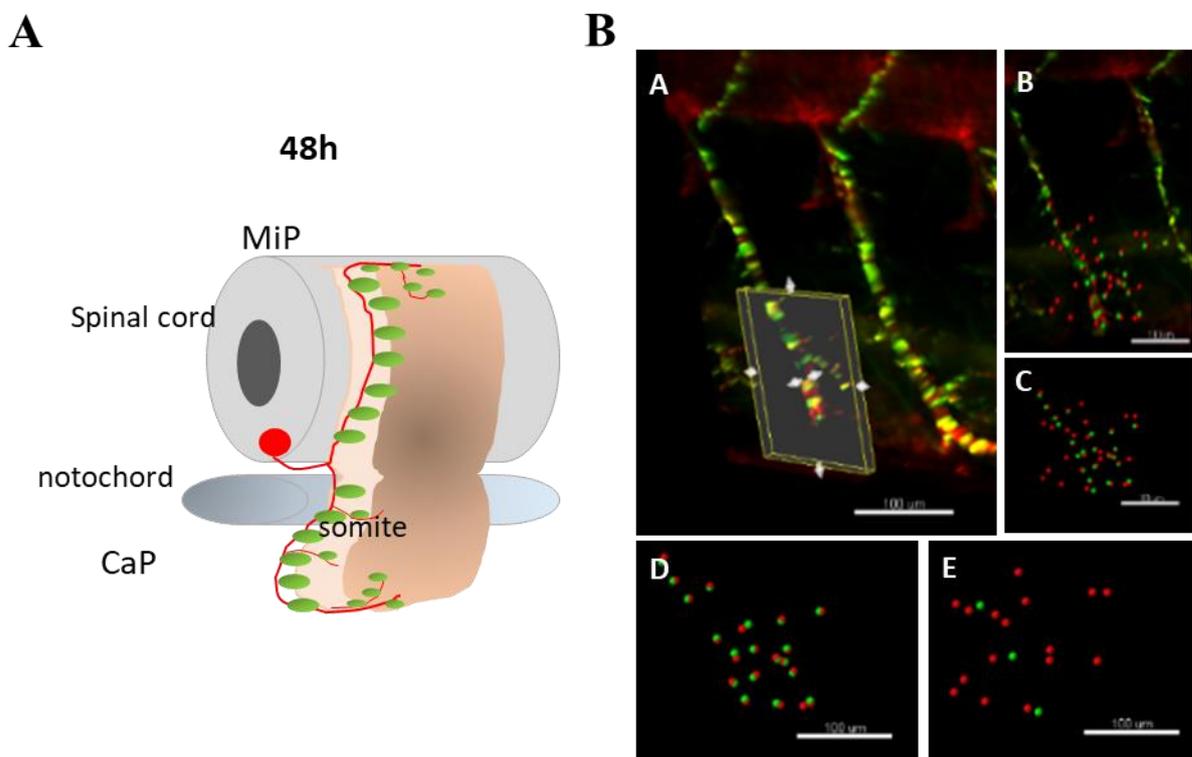


Figure S3: A, schematic representation of one spinal segment. At 48 hpf, the somitic muscle is innervated by two primary cholinergic motor neurons: the Caudal Primary (CaP) and the middle primary motoneuron (MiP). Later in development, another primary motor neuron appears, the rostral primary motoneuron. It innervates the middle part of the somite (not shown). At this moment, the density of innervating fibers is so high that it could be impossible to follow a single motor neuron. Green spots represent the classical distribution of AChR clusters along the axon. **B, Spot detection and colocalization analysis using Imaris.** 23 confocal z-stacks of $0,59 \mu\text{m}$ were used to build the motor neuron 3D structure with Imaris. A specific region (rectangle selection) corresponding to the ventral CaP axon extension, was used for analysis. Red and green circles are SV2 and αBTX respective staining built with spot detection algorithm. This procedure facilitates the analysis of synapses, the NMJ in this particular case. In fact, we can easily count the presence of colocalized (white D) and not-colocalized (white E) pre and postsynaptic puncta, called Orphaned αBTX and Orphaned SV2 respectively.

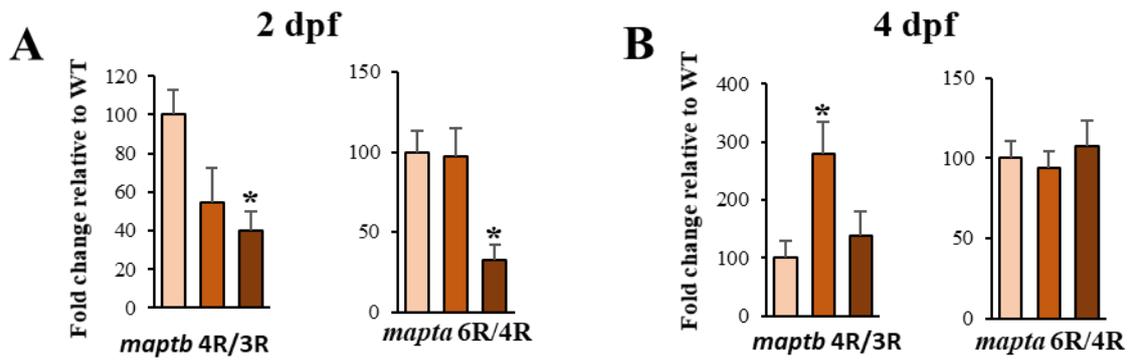


Figure S4: Quantification of tau isoforms ratio at 2 and 4 dpf

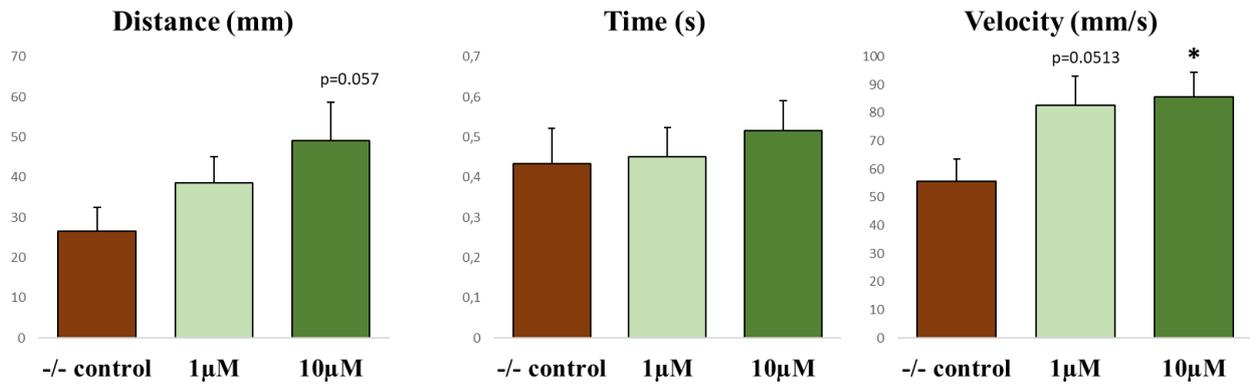


Figure S5: LRRK2 inhibitor treatment on *fus*^{-/-} mutants. Quantification of TEER analysis showing the distance, the velocity and the time spent for *fus*^{-/-} embryos that were untreated (dark brown) or treated with 1 μ M of drug (light green) or 10 μ M (dark green) (One-way ANOVA with Bonferroni post-hoc test. *p<0, 05).

hdac4 F	GATTATCTGGCAGCATTGAG
hdac4 R	GAAAACCTCATTCGCTATCGG
chrna1-F	TCGTCCTGTTAGCCATTTTA
chrna1-R	GATTAACCTCATCCACGCTG
chrne-F	TAGTTGCTATTGTGCTTCTG
chrne-R	CTCTTCATTCCCAGTGA
AChr gamma-F	GACACCTGAGCAGGAGGAAG
AChr gamma-R	GGACACACACTGCTGCACTT
Fus F	CGACTGTACGTATAACACCT
Fus R	CCATAATCATTTGACGCCAT
Taf15 F	TATGGTGGATCCCAGGGTTA
Taf15 R	CCACTTGACTGCTGTCGTA
Tdp-43 F	GGAAACCTAGTCTATGTGGT
Tdp-43 R	CATCGATCTCATCCATCTTC
mbnl1 F	GTGTATGGATTACATAAAGGGC
mbnl1 R	TGGAAGTATTTGCACTTGTC
fmr1 F	GAACGACAGATTTCTTTCCA
fmr1 R	TAGAGTACACCTCCA
CamkIIa F	GATTTTGGAGGCTGTA
CamkIIa R	TTCTGGCTTTAAGTCTCTGT

mapta 6R F	TCGTCACAAACCAGGTGGAG
mapta 6R R	GCTCACGGAACGTCAGTTG
mapta 4R F	CGGAGGTGGAAAATTGAGTCAC
mapta 4R R	CTCCTCCAGGGACACAATTCT
maptb 4R F	AAGATCGGCTCCACTGAGAACC
maptb 4R R	GATCCAACCTTTGACTGGGCTT
maptb 3R F	GGGAAGGGGTGGAAATGTC
maptb 3R R	GATCCAACCTTTGACTGGGCTT
Tra2b F	GCAGACGACATATTGGTGACC
Tra2b R	TGACTGCTGGTCGTACACAATG
Gsk3b F	GATTTAAGAACCGTGAGCTT
Gsk3b R	TGTTGCAGTGGTCTAATTTCC
Cdk5 F	CATGCAAAAGTATGAGAAGC
Cdk5 R	TACCATATGTACCCTCTCTCT
Erk1 F	CCCAAAGCAGACAACAAAGC
Erk1 R	GGCTCATCAGATGGGTCATAG
Erk2 F	CGCACACTGCGTGAGATTA
Erk2 R	CCATCAGGTCCTGTACGATGT
Lrrk2 F	GCTTAATTAACACCCTCTCC
Lrrk2 R	ACCTGATGCTGTTTAGTTTG

Table S1: List of primers used for qPCR analyses

2. FUS and CHCHD10 implication in muscle and mitochondria pathology

2.1. Objectives

Following the characterization of the *fus* mutant zebrafish model that displayed NMJ disorganization, a particular interest was directed towards the investigation of post-synaptic phenotypic features, including features in muscle and in mitochondria. Indeed, several studies revealed the implication of muscle and mitochondria pathology in ALS. Particularly, different SOD1 mutant models displayed reduced muscle volume and strength as well as mitochondrial dysfunctions alongside other hallmarks of ALS, like abnormal NMJs and MN degeneration, when mutant SOD1 was overexpressed (Wong & Martin, 2010) or when the expression of PGC-1 α , a transcription coactivator that boosts mitochondrial biogenesis, was altered in a SOD1 mutant (Da Cruz et al., 2012) (reviewed in Loeffler, Picchiarelli, Dupuis, & Gonzalez De Aguilar, 2016). Recently, a study revealed toxic effects from mutant FUS on muscles (Picchiarelli et al., 2019). Specifically, they showed that FUS is intrinsically toxic in myotubes derived from patients and also myogenic pathological activity in muscle biopsy samples (Picchiarelli et al., 2019).

Therefore, the second part of the thesis is focused on the identification of muscle and mitochondrial pathological traits in the *fus* model.

Also, to reinforce our investigation on mitochondria pathology, we put our interest in the characterization of a new zebrafish model of a CHCHD10 non sense mutant, implicated in mitochondria stability. Indeed, studies reported mutations of this gene to be associated with ALS patients (Bannwarth et al., 2014a). Recently, new models emerged to try to unravel the pathological mechanisms of CHCHD10 (Anderson et al., 2019a; Brockmann et al., 2018; Burstein et al., 2017; Genin et al., 2019). In all these models, different mutations of CHCHD10 displayed several ALS hallmarks, including mitochondrial abnormalities (Anderson et al.,

2019a), NMJ and MN degeneration (Genin et al., 2019), as well as abnormal myofibrillar structure and motility deficits in a zebrafish model (Brockmann et al., 2018).

Therefore, by investigating the mechanisms underlying mutated CHCHD10 through the characterization of a new model, we aim to provide new insights to better understand the implication of this gene in ALS pathogenesis.

2.2. *fus* KO causes slow muscle disorganization

Skeletal muscle is a major component that makes about 50% of the body mass of a human and around 80% of that of a fish (Jackson & Ingham, 2013), and, apart from locomotion, has a role in metabolic homeostasis (Jackson & Ingham, 2013). It is constituted by different types of fiber distinguished by their physiological and metabolic properties (Schiaffino & Reggiani, 2011). Type I or slow muscle fibers are characterized by their lower contraction velocity and high mitochondria concentration, thus being more efficient in oxygen consumption and ATP production, allowing robust endurance levels (Talbot & Maves, 2016). Type II or fast muscle fibers are more adapted to short bursts of strength or speed but are more prone to fatigue due to their high contraction velocity (Talbot & Maves, 2016).

In zebrafish, fast muscle fibers constitute the majority of the muscle fibers but they are covered on their outer surface by a subcutaneous layer of slow muscle fibers (**Figure 8**). These type I fibers are the first to contract spontaneously in the embryo starting at 17 hpf and are responsible for their coiling behavior (Saint-Amant & Drapeau, 1998). The type II fibers are functional hours later and mediate the characteristic fast movements of the embryo (Naganawa & Hirata, 2011).

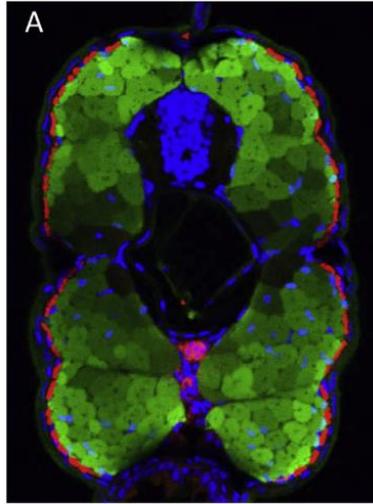


Figure 8: Transversal section of a 48 hpf zebrafish embryo at the trunk level showing fast muscle fibers (green) and slow muscle fibers (red) (derived from Jackson et al., 2013)

To assess the phenotypic features of muscle structure we first used a fluorescently labeled phalloidin, able to mark actin filaments, in 48-hpf *fus* mutant embryos. This labeling permitted us to reveal all types of muscles fibers that we could observe through confocal microscopy.

In WT and *fus*^{+/-} conditions, muscle fibers are well organized, displaying the typical parallel lines pattern in every somite (**Figure 9Ai, ii**). However, when we looked at *fus*^{-/-} embryos, we noted a disorganized muscle pattern, with non-parallel and curvy fibers (**Figure 9Aiii**). This suggests the existence of a disorganized muscle structure in the homozygous mutants.

To strengthen this observation, we then decided to assess the different muscle types specifically. We performed an immunolabeling analysis using specific antibodies for fast (F310; **Figure 9B**) and slow muscles (F59; **Figure 9C**). In all the conditions, the fast muscle fibers seemed unaltered and well organized in straight parallel lines (**Figure 9Bi, ii, iii**). In the case of slow muscles, *fus*^{+/-} presented the similar pattern of WT's embryos (**Figure 9Ci, ii**). Contrarily, *fus*^{-/-} fish displayed disorganized muscle pattern with aberrantly curvy slow muscle fibers (**Figure 9Ciii**). This observation suggests the implication of *fus* gene in muscle organization and

development in zebrafish. This is in line with a recent study that revealed several alterations in human *FUS*-ALS skeletal muscles as well as in mice carrying *FUS* mutated protein (Picchiarelli et al., 2019).

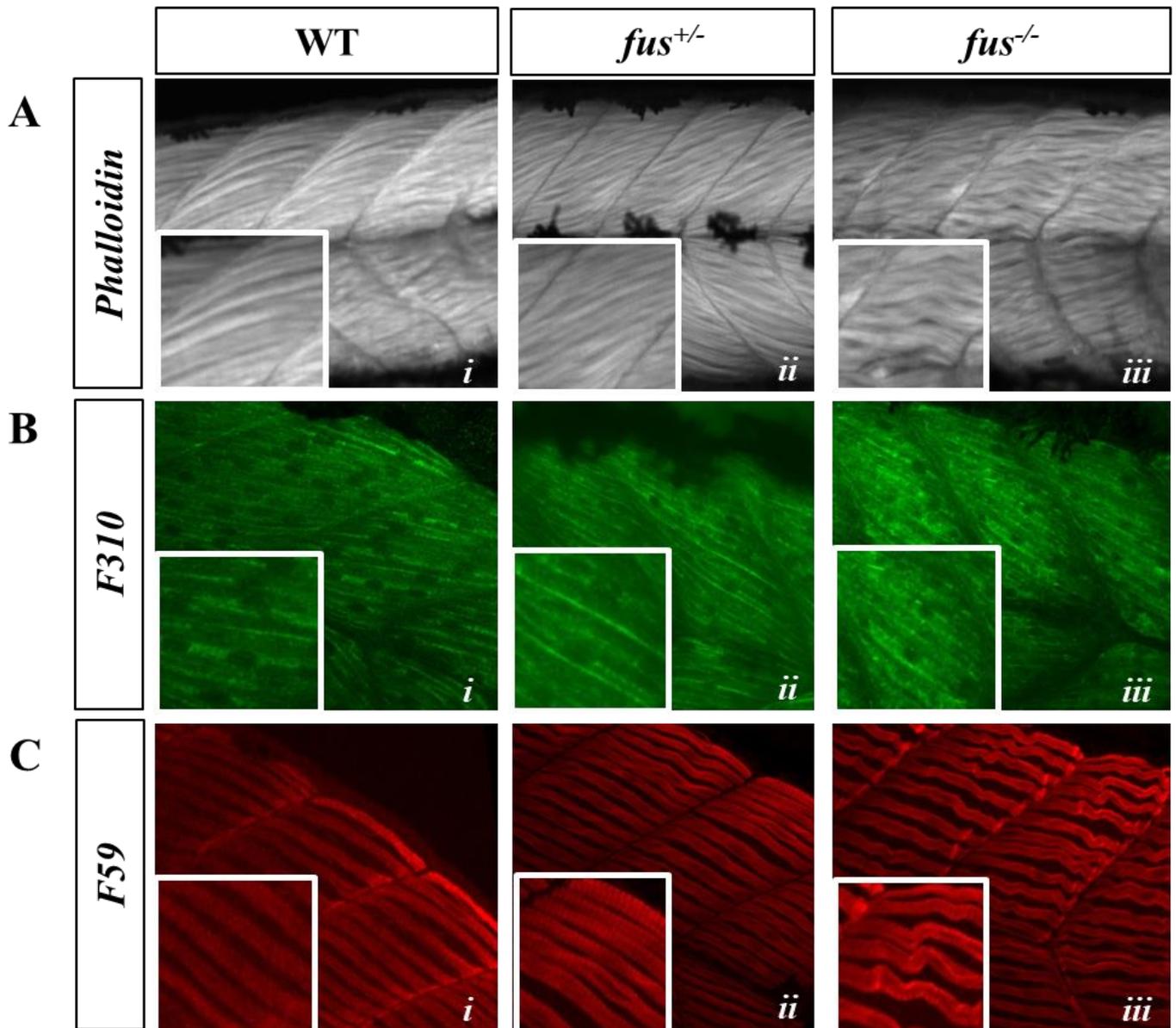


Figure 9: Muscle morphology in *fus* zebrafish KO mutants at 2 dpf. **A**, Phalloidin staining of WT (i) and *fus* mutant embryos (ii, iii). WT and *fus*^{+/-} conditions displayed well organized muscle structures with typical parallel line pattern. On the contrary, *fus*^{-/-} conditions showed a disorganized structure with curvy and non-parallel fibers. **B**, immunolabelling of fast muscle fibers with F310 antibody in WT (i) and *fus* mutant embryos (ii, iii). No significant difference was observed between WT and *fus* mutants. **C**, immunolabelling of slow muscle fibers with F59 antibody in WT (i) and *fus* mutant embryos (ii, iii). *fus*^{+/-} presents the similar pattern of WTs embryos, but *fus*^{-/-} displayed disorganized muscle pattern with aberrantly curvy slow muscle fibers.

2.3. *fus* KO impacts the mitochondrial network in the zebrafish embryos muscles

The skeletal muscle being a key factor in energy metabolism, its investigation is regularly associated with mitochondrial feature assessment (Gouspillou & Hepple, 2016).

Indeed, previous studies showed oxidative stress and mitochondrial dysfunctions in muscles of ALS patients and animal models (Loeffler et al., 2016). For example, mutant SOD1 muscles displayed increased levels of reactive oxygen species (ROS) before motor impairment (Halter et al., 2010), suggesting an aberrant oxidative metabolism (Capitanio et al., 2012). Also, altered expression of PGC-1 α has been shown to contribute to excessive ROS production and aberrant oxidative metabolism, and the increase of PGC-1 α in mutant SOD1 muscle was shown to maintain mitochondrial biogenesis and improved muscle function (Da Cruz et al., 2012), thus highlighting the involvement of mitochondrial dysfunction in ALS-linked muscle pathology (reviewed in Loeffler et al., 2016).

Since mitochondrial network form is a good indicator of mitochondrial function and stability, we decided to assess its morphology in *fus* mutant embryos.

To do so, we used two different staining techniques: an immunolabelling using an antibody targeting specifically the outer mitochondrial membrane (TOMM20), and a fluorescent MitoTracker probe that accumulates in active mitochondria (**Figure 10**). It last has been injected in the blood circulation at 2-dpf embryos, providing mitochondrial network visualization *in vivo* (**Supplementary Material, Figure S6**). The mitochondrial network was then reconstructed through Imaris “Surface” option.

Importantly, in both cases, mitochondrial network seems to be less elongated and more fragmented in *fus*^{-/-} embryos (**Figure 10Aiii and Biii**) compared to WT and heterozygous fish (**Figure 10Ai, ii and Bi, ii**).

This shape was particularly evident after 3D MitoTacker reconstruction (**Figure 10B, in yellow**). Altogether, these results suggest a major impact of *fus* loss-of-function on mitochondrial function in zebrafish muscles.

To strengthen these observations, we also assessed the production of ROS level in order to evaluate the presence of oxidative stress in 2 dpf embryos. For this, we used dihydrofluorescein diacetate (DHF), a molecule that becomes fluorescent when in contact with ROS. The qualitative observation of *fus*^{-/-} embryos showed higher levels of fluorescence in muscles (**Figure 10C**) compared to WT and *fus*^{+/-}, indicating aberrant levels of oxygen radicals. However, despite a trend of increase of the ROS levels, the quantification showed no significant difference between WT and *fus* mutants due to high variability (**Figure 10D**). Therefore, there is still a strong need to further this investigation on mitochondria through other techniques, including metabolic approaches to assess oxygen consumption and ATP production.

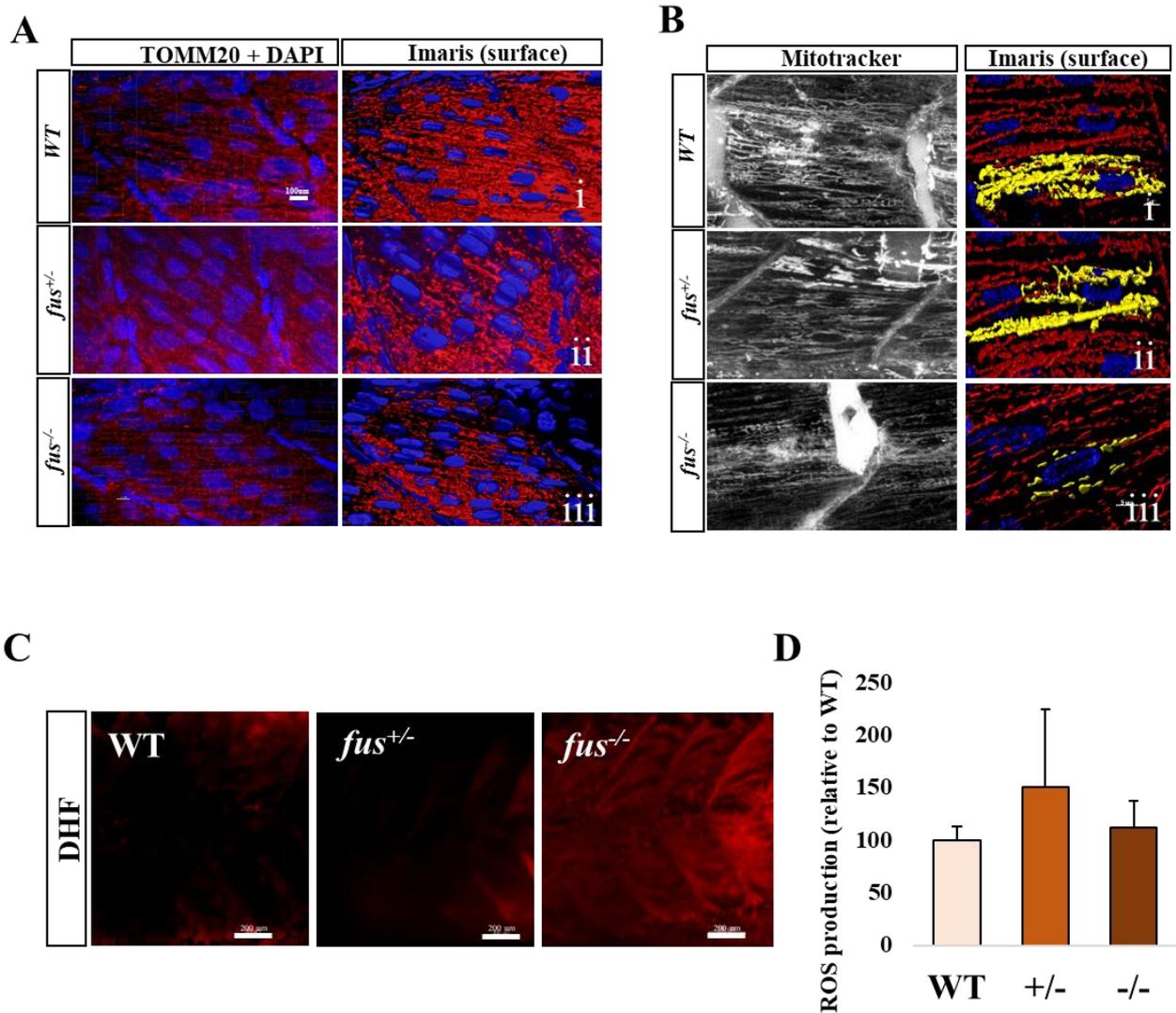


Figure 10: Mitochondrial network in *fus* KO 2 dpf embryos. Immunostaining (TOMM20) (A) and MitoTracker labelling (B) of WT (i), *fus^{+/-}* (ii) and *fus^{-/-}* (iii) conditions. In both cases, *fus^{-/-}* embryos displayed a less elongated and more fragmented mitochondrial network compared to WT and *fus^{+/-}* conditions. The nuclei were also marked with DAPI to better visualize the network in the muscle fibers. Confocal z-stacks of 0,15 μm were used to build the mitochondrial network 3D structure with Imaris. The “surface” option was used to create a 3D structure from the confocal microscopy data. This allowed us to identify relevant entities within the z-stacks to build the mitochondrial network in 3D. C, D, oxidative stress assessment with DHF showing ROS levels.

2.4. Expression of mitochondrial genes in *fus* mutants

We decided then to check for transcript expression of genes that play essential roles in mitochondrial DNA (mtDNA) maintenance and replication, as well as in the respiratory chain complexes biogenesis.

mtDNA is highly conserved in all vertebrates (Boore, 1999; Brown, Waring, Scazzocchio, & Davies, 1985), and its molecular machineries, are almost completely conserved during evolution (Artuso et al., 2012).

Thus, we decided to evaluate the expression of some genes previously described by Artuso and colleagues, particularly involved in mitochondrial functions.

The DNA polymerase γ (*polg1*) plays key roles in replication, recombination and repair of mtDNA (Copeland & Longley, 2003). Several studies showed aberrant decrease in mtDNA levels leading to lethality and severe respiratory chain deficiencies in different mutant models (Genga, Bianchi, & Foury, 1986; Iyengar, Roote, & Campos, 1999).

TWINKLE mediates mtDNA conformation and acts as mtDNA stabilizer during replication (Garrido et al., 2003).

Thymidine kinase (*tk2*) is an intramitochondrial pyrimidine nucleoside kinase that participate in the salvage pathway of deoxynucleotide synthesis in the mitochondria, considered essential for the replication of mtDNA (Johansson & Karlsson, 1997).

We also checked two genes implicated in the biogenesis of mitochondrial complexes: the cytochrome *c* oxidase subunit Va (*cox5ab*) (Rizzuto, Nakase, Zeviani, DiMauro, & Schon, 1988) and the ATP synthase mitochondrial F1 complex alpha subunit (*atp5a1*) (Akiyama, Endo, Inohara, Ohta, & Kagawa, 1994).

At 2 dpf, *twinkle*, *tk2* and *polg1* transcripts showed no significant change of expression in *fus*^{-/-} embryos (**Figure 11A**). The same thing has been observed for the genes involved in mitochondrial complexes–biogenesis (**Figure 11B**). However, an interesting tendency to decrease (**Figure 11A**) and increase (**Figure 11B**) in *fus* KO fish can be noted. Therefore, further measurements need to be performed.

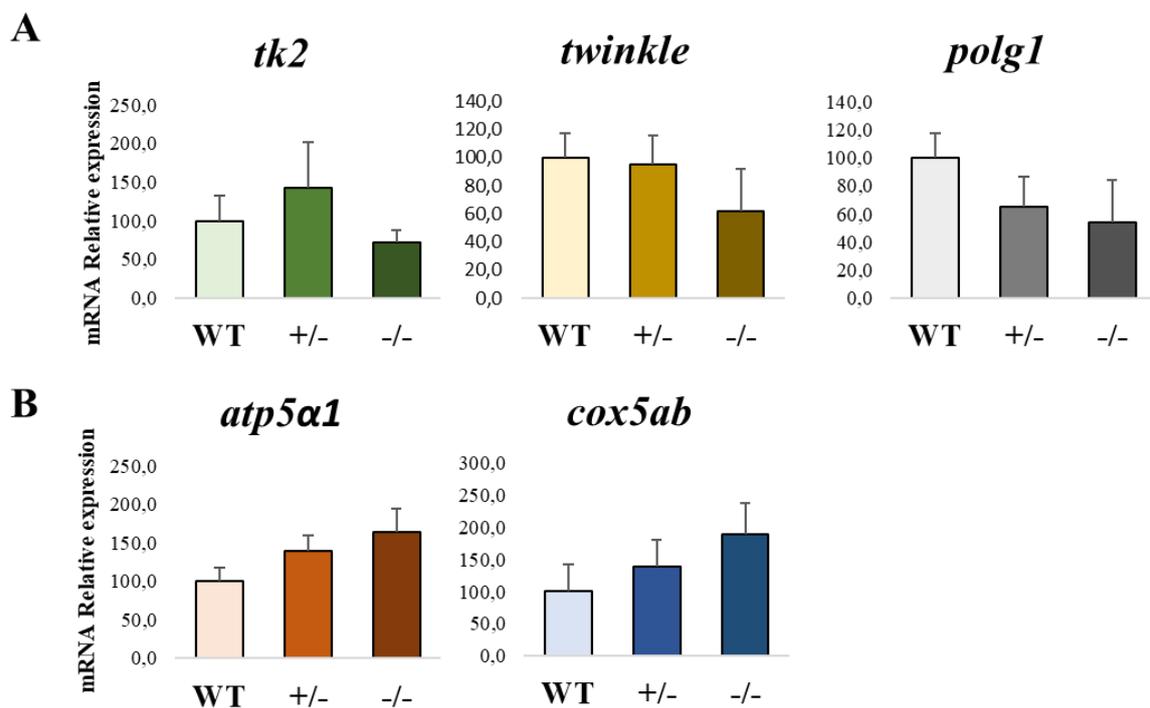


Figure 11: Mitochondrial gene expression in *fus* mutants. **A**, qPCR analysis of *twinkle*, *tk2* and *polg1*, genes involved in mtDNA maintenance and replication. **B**, qPCR analysis of *atp5α1* and *cox5ab*, genes involved in the biogenesis of mitochondrial complexes.

3. Functional characterization of the novel CHCHD10 mutant zebrafish model

To pursue our investigation of mitochondria pathology in ALS, we decided to characterize a novel zebrafish model presenting a non-sense mutation on the *chchd10* gene. This gene encodes for the mitochondrial protein chchd10 located in the intermembrane space and involved in mitochondrial cristae stabilization (Friedman, Mourier, Yamada, Michael McCaffery, & Nunnari, 2015; Hofmann et al., 2005) and linked to the disease (Bannwarth et al., 2014b).

3.1. Generation and characterization of the model

The CHCHD10 gene has been associated with ALS-FTD only recently (Bannwarth et al., 2014b) and highlighted the significant implication of mitochondrial alterations in the etiology of the disease. Few studies on model organisms followed this discovery and gathered additional information on CHCHD10 involvement in ALS pathogenesis (Anderson et al., 2019a; Genin et al., 2019; Woo et al., 2017). However, the phenotypic features observed in these studies are too complex and new models are still needed to enrich our understanding of the pathomechanisms that characterize this gene in ALS.

Similar to the *fus* KO zebrafish model, we obtained a mutant genetic line for *chchd10* from the Zebrafish Mutation Project (reference: sa18461, mutation c.266T>A; p.Y89X, Sanger Institute) (Kettleborough et al., 2013). The mutants were generated through ENU mutagenesis and identified by TILLING approach (reviewed above). After high-precision sequencing to confirm the presence of the point mutation (**Figure 12A**), we identified the mutation to be located before the CHCH domain of the protein (**Figure 12B**) and leading to a translation termination (stop) codon, thus simulating a loss of function through truncation of the functional domain of *chchd10*.

We determined the protein levels of *chchd10* by Western blot analyses at 48 hpf. The decreased amount measured in *chchd10*^{-/-} conditions compared to WT and *chchd10*^{+/-} (**Figure 12C**) confirmed the efficacy of the nonsense mutation. We also observed a decrease tendency in the mRNA expression of *chchd10* in the mutants compared to WT conditions, though without reaching significance (**Figure 12D**). In addition, no major morphological and developmental deficits were noticed in this model and the viability of the *chchd10* mutants is comparable to WT controls (**Figure 12E**).

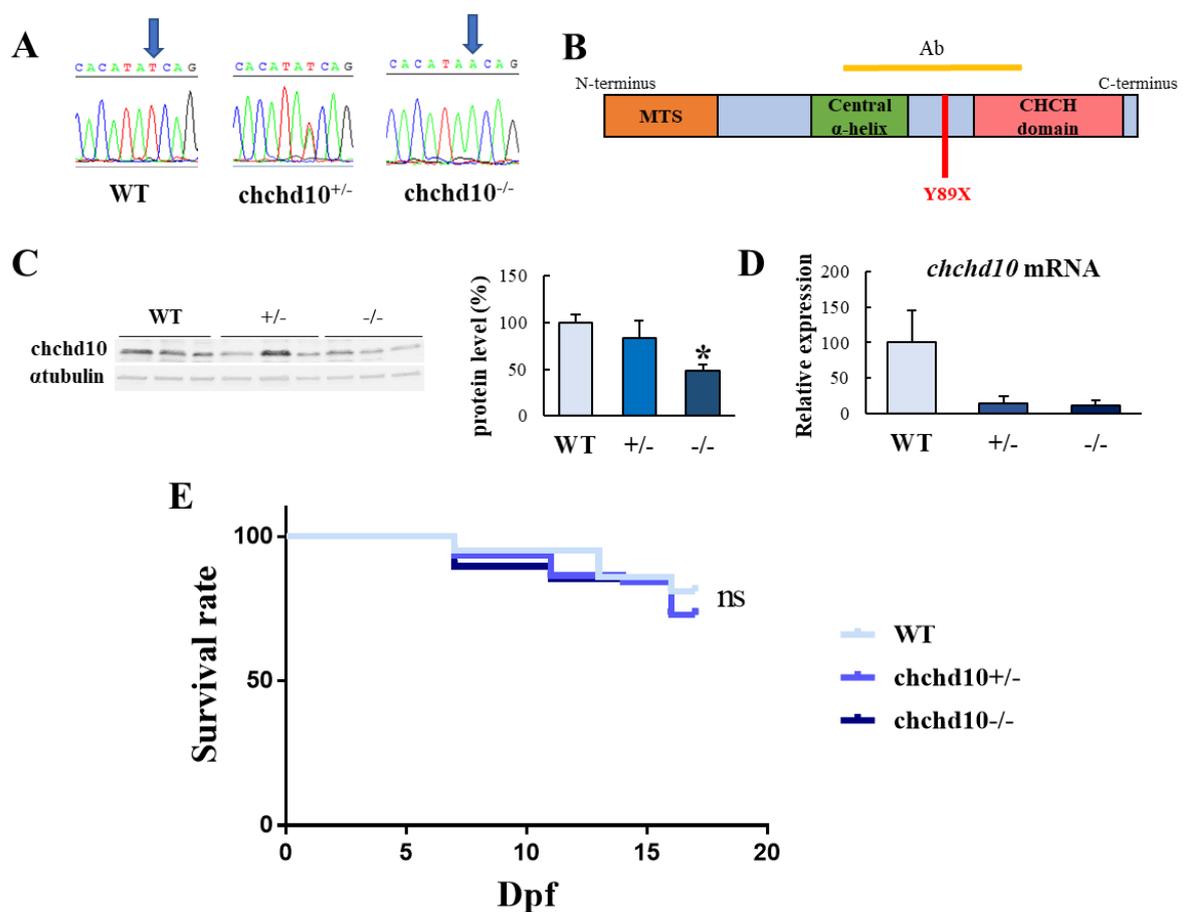


Figure 12: Characterization of the zebrafish *Chchd10* mutant model. **A**, Sanger sequencing results with arrows showing the mutation target site. **B**, schematic representation of zebrafish *Chchd10* protein showing the site of mutation before the CHCH domain that gives its function. The antigen sequence recognized by the antibody is also displayed. **C**, *Left*, immunoblot analysis of *Chchd10* protein in total extract from WT, hetero and homozygous embryos at 48hpf. The band detected is around 15 KDa. *Right*, densitometry quantification of *Fus* protein level normalized to α tubulin. (One-way ANOVA with Bonferroni post-hoc test. * $p < 0, 05$). **D**, RT-PCR analysis of *chchd10* messenger RNA. **E**, Kaplan-Meier survival curves for WT, *chchd10*^{+/-} and *chchd10*^{-/-}. Time is shown in days post fertilization (dpf). An *n* of 20 embryos was used for WT, while 43 have been used for heterozygous mutants and 47 for homozygous mutants.

3.2. Locomotion assessment in *chchd10* zebrafish models

To further the characterization of the model, we assessed its swimming behavior at 2 dpf through TEER assay. Similar to the *fus* mutant model, by measuring the distance, time and velocity of each animal, we observed that only *chchd10*^{-/-} embryos (dark blue) presented an altered locomotion and paralysis in some cases (**Figure 13B**), whereas the *chchd10*^{+/-} and the WT had a comparable swimming behavior. This suggests that *chchd10* may impact motor function in the zebrafish.

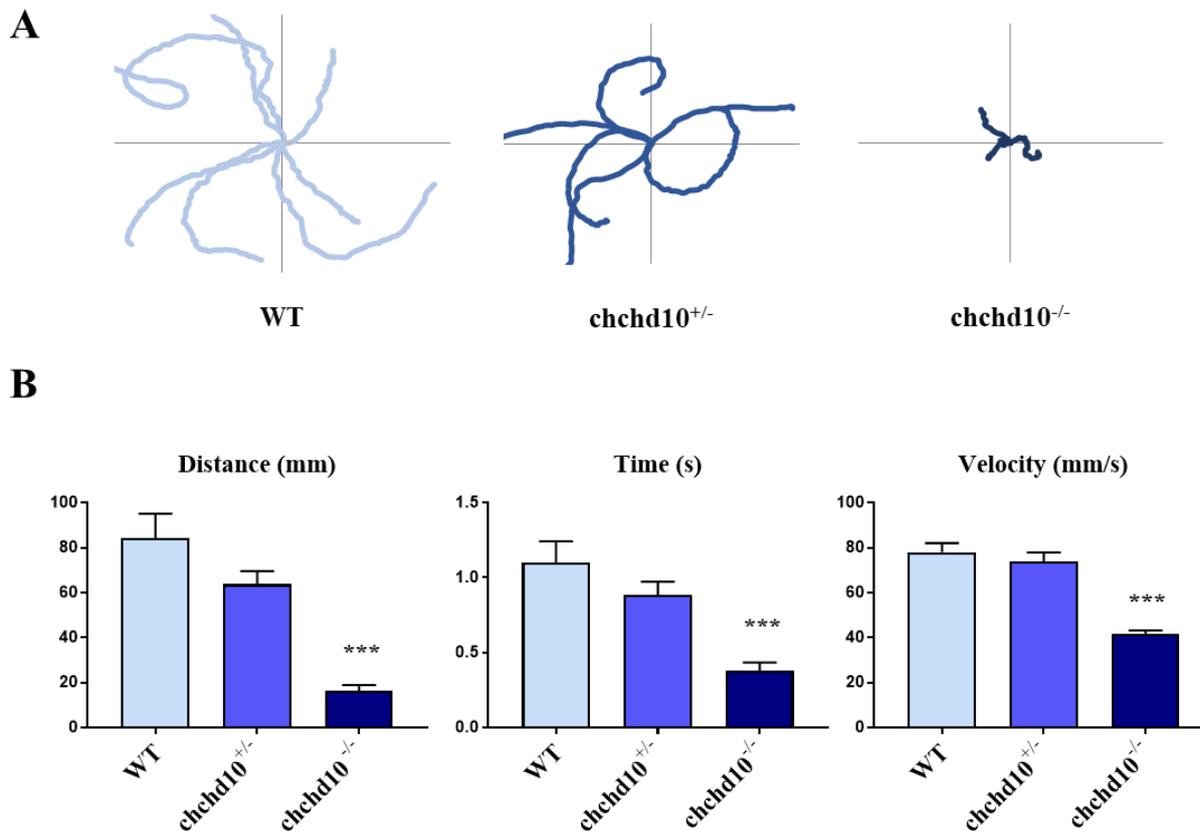


Figure 13: *chchd10*^{-/-} 2 dpf embryos show locomotion defects at TEER. **A**, traces representing the swim response of heterozygous and homozygous embryos compared to WT controls during the touch-evoked escape response assays (TEER). Briefly, 2 dpf zebrafish embryos are gently touched on the tail with a glass pipette and their escape behavior is assessed and recorded. **B**, Quantitative analyses of TEER. *Chchd10*^{-/-} (dark blue) showed a significant decrease in the distance travelled, time spent and velocity of swimming (One-way ANOVA with Bonferroni post-hoc test. **p*<0, 05; ***p*<0, 01; *** *p*<0, 001).

We also tried to rescue the phenotype through the use of decylubiquinone (DU), a synthesized analogue of Coenzyme Q-10 (CoQ10, ubiquinone) that acts as an electron acceptor for complexes I and III of the transport chain in the mitochondria and as an antioxidant (Ernster & Dallner, 1995). It has been shown that DU prevented ROS production and the activation of the mitochondrial permeability transition, which is a key event in apoptotic and necrotic cell death (Armstrong, Whiteman, Rose, & Jones, 2003). After incubating the embryos at 24 hpf in 1.5 μ M of DU, a TEER test was realized the next day at 48 hpf. However, no significant improvement in the motor phenotype has been observed (**Supplementary Material, Figure S7**).

We also wanted to determine if the motor impairments observed at 2 dpf would persist at further stages (3, 4 and 5 dpf). For this, we used the previously described light stimulation protocol with the ZebraLab platform and recorded the behavior of the embryos (**Supplementary Material, Figure S8**). However, at every stage, there was no significant change in the global activity of the embryos between *chchd10*^{-/-}, *chchd10*^{+/-} and the WT controls (**Supplementary Material, Figure S8B, C, D**). Although not significant, there is a noticeable tendency in global activity decrease of *chchd10*^{-/-} embryos compared to the other conditions at 4 dpf (**Supplementary Material, Figure S8C**).

Overall, we saw an impaired locomotion at 2 dpf in the *chchd10*^{-/-} embryos that did not persist over time. This could be explained by a developmental delay caused by the mutation at early stage that is possibly compensated afterwards by other pathways.

3.3. *Chchd10* mutation does not impact NMJ formation in zebrafish

Similar to the *fus* mutant model, we performed an immunolabeling analysis for NMJ components both at the pre-synaptic (SV2) and post-synaptic terminals (α BTX) using the same procedure (**Supplementary Material, Figure S3**).

Here, at 2 dpf, no difference was observed between the *chchd10* mutants and the WT condition, with no aberrantly non-colocalized pre- and post-synaptic elements (**Figure 14B, C**), even though the motor axons seem less branched in the mutant conditions. This suggests that *chchd10* loss of function does not impact the formation of NMJ in zebrafish and that other hallmarks of ALS should be investigated through this mutation.

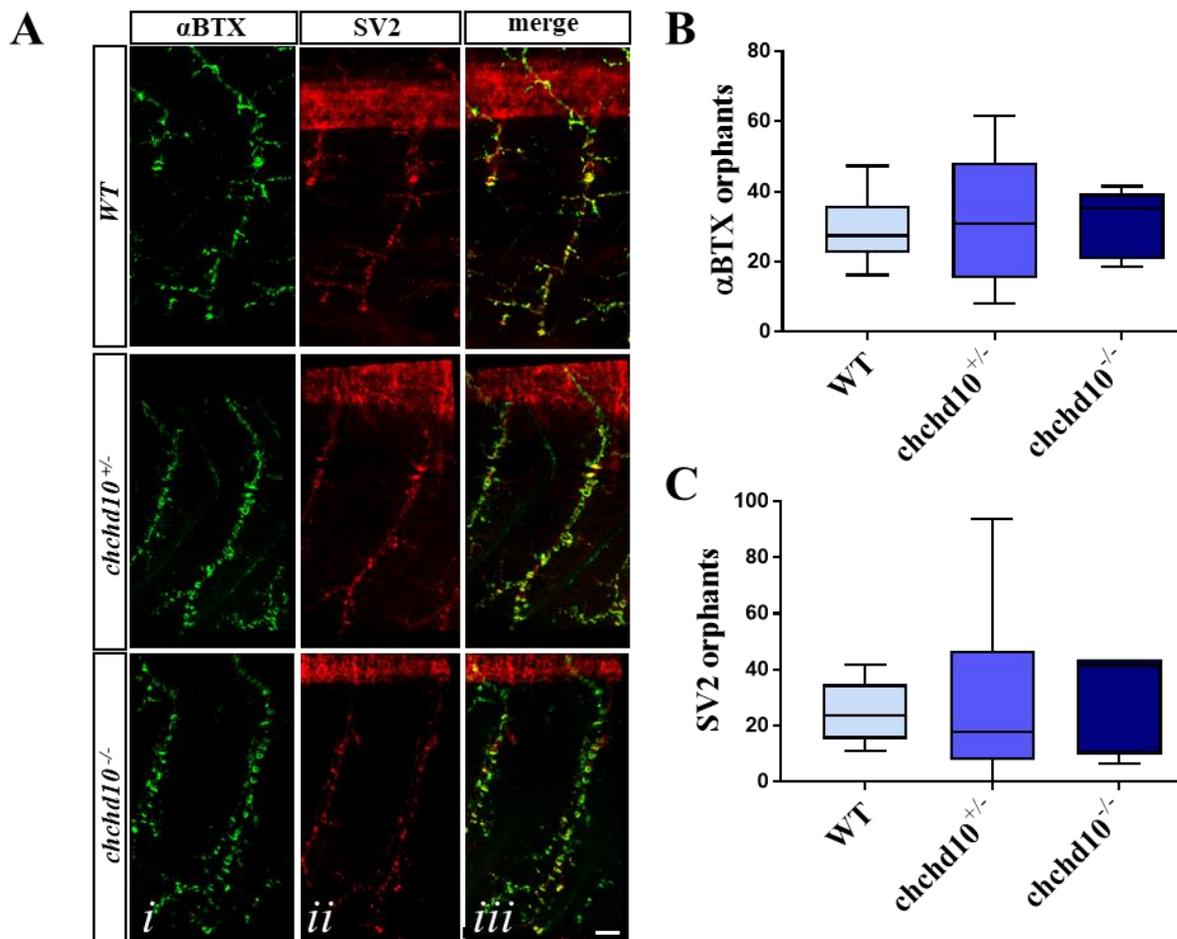


Figure 14: NMJ formation in *chchd10* mutant zebrafish models. **A**, Primary motor axon (Cap) of WT and mutant *chchd10* embryos stained immunofluorescently with the antibody against SV2 (red) and AChRs (green). At 48 hpf, all conditions presented grown motor axons along the myosepta and well colocalized pre- and post-synaptic terminals. *chchd10*^{+/-} and *chchd10*^{-/-} seem to show motor axons with less branching. **B**, spots quantification with Imaris software of uninervated AChR. **C**, quantification of orphaned SV2 pre-synaptic markers.

3.4. Muscle morphology of *chchd10* zebrafish mutants

Following the investigation on the NMJ, we assessed the muscle structure in this mutant model by immunolabeling analysis using specific antibodies for fast (F310; **Figure 15A**) and slow muscles (F59; **Figure 15B**). In all the conditions, the fast muscle fibers seemed unaltered and well organized in straight parallel lines (**Figure 15Ai, ii, iii**). The same goes for slow muscle fibers in all the conditions (**Figure 15Bi, ii, iii**). *chchd10*^{-/-} embryos seemed to show some curvy slow muscle fibers but not in the same proportions as in the *fus*^{-/-} mutant models, suggesting no significant impact of *chchd10* loss of function on muscle structure in zebrafish.

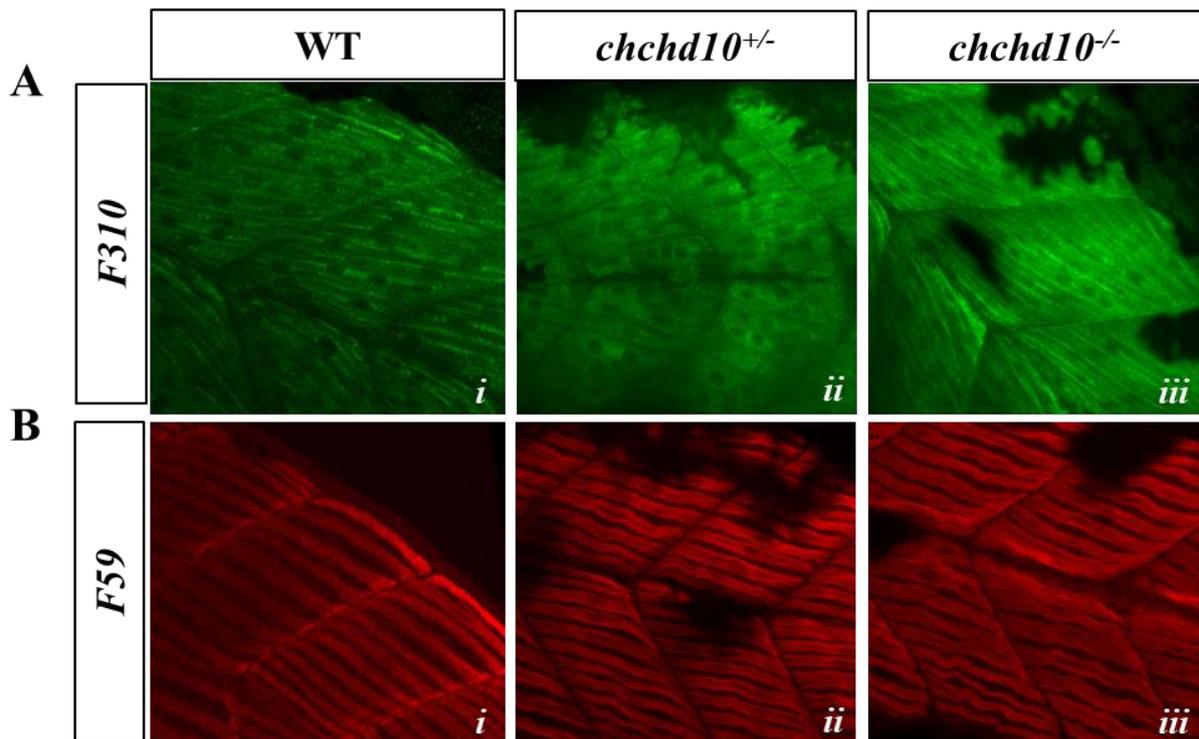


Figure 15: Muscle morphology in *chchd10* mutants at 2 dpf. **A**, immunolabelling of fast muscle fibers with F310 antibody in WT (i) and *chchd10* mutant embryos (ii, iii). No significant difference was observed between WT and *chchd10* mutants. **B**, immunolabelling of slow muscle fibers with F59 antibody in WT (i) and *chchd10* mutant embryos (ii, iii). No significant difference was observed between WT and *chchd10* mutants. Homozygous mutants presented some curvy slow muscle fibers but only in rare areas of the fish, contrarily to *fus*^{-/-} embryos.

3.5. Mitochondrial network and membrane potential permeability in *chchd10* mutants

With *chchd10* proteins located in the intermembrane space of the mitochondria and involved in their stability, we assessed the mitochondrial network morphology in the *chchd10* mutant zebrafish embryos. We performed the same immunolabelling technique used for *fus* models, using the TOMM20 antibody that targets the outer mitochondrial membrane.

Here, *chchd10*^{-/-} embryos seem to show lower mitochondrial density and fragmentation in some locations compared to WT and *chchd10*^{+/-} (**Figure 16C**). However, this approach remains qualitative and incite us to deepen our investigation on mitochondria dysfunction in *chchd10* mutants.

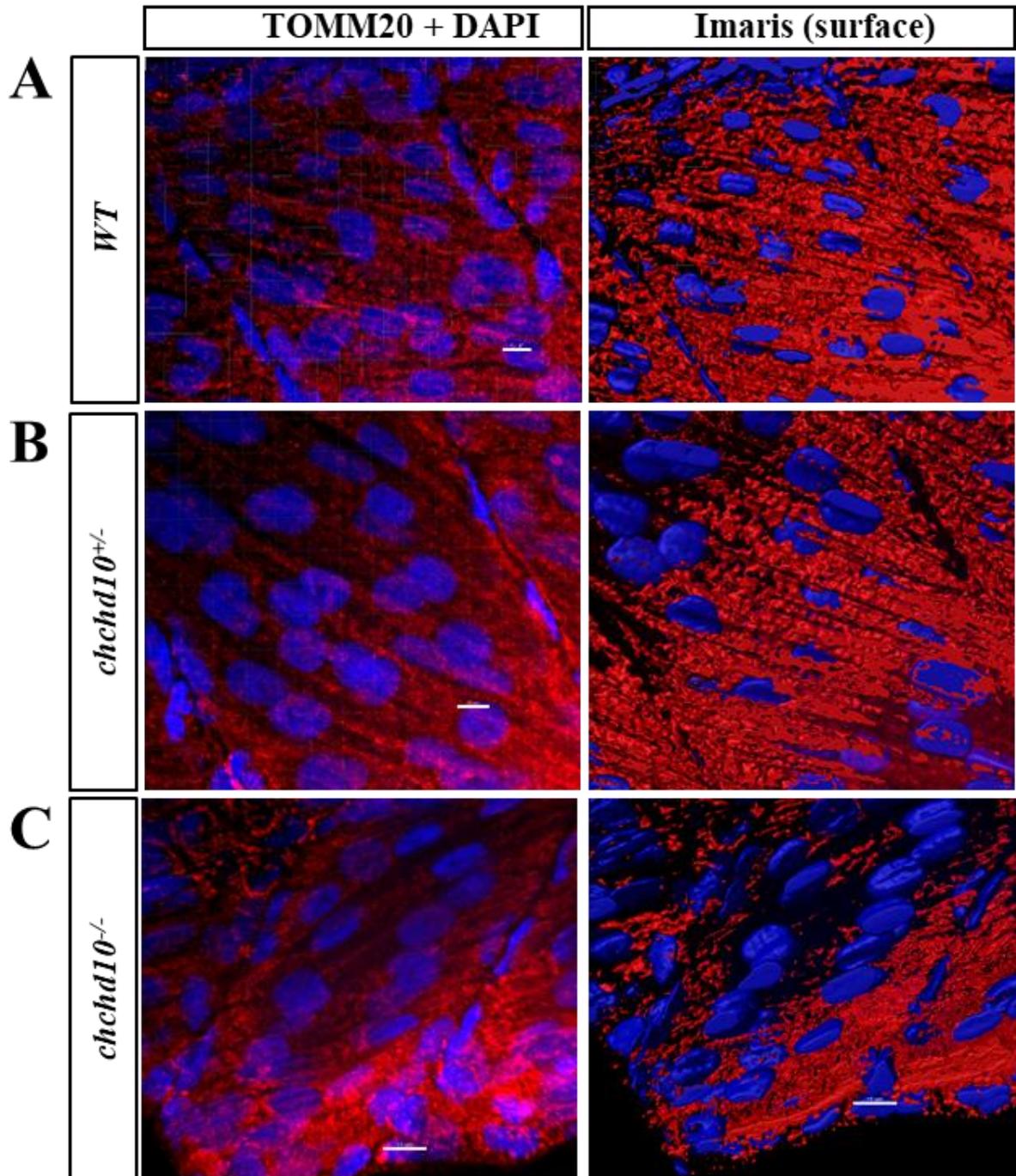


Figure 16: Mitochondrial network in *chchd10* mutants. Immunostaining (TOMM20) of WT (A), *chchd10^{+/-}* (B) and *chchd10^{-/-}* (C) conditions. The nuclei were also marked with DAPI to better visualize the network in the muscle fibers. Confocal z-stacks of 0,15 μm were used to build the mitochondrial network 3D structure with Imaris. The “surface” option was used to create a 3D structure from the confocal microscopy data. This allowed us to identify relevant entities within the z-stacks to build the mitochondrial network in 3D. Scale bar, 100 μm .

Next, we investigated whether *chchd10* non-sense mutation in zebrafish could impact the mitochondrial membrane permeability (MMP). Mitochondria generate a potential across their membrane due to the enzyme activity of the respiratory chain, named mitochondrial membrane potential ($\Delta\Psi_m$), and it serves as an intermediate form of energy storage which is used by ATP synthase to make ATP (Zorova et al., 2018). $\Delta\Psi_m$ is also considered to be a factor determining the viability of mitochondria (Zorova et al., 2018). Indeed, one apoptotic pathway involves an increase of outer MMP causing the release of various proteins from the intermembrane space into the cytoplasm, leading to a loss of $\Delta\Psi_m$, as well as mitochondrial swelling and rupture of the outer mitochondrial membrane (Tsujimoto & Shimizu, 2007).

In order to assess the MMP in our model, we used a kit optimized for its analysis by flow cytometry with a mitochondrial potential dye on 2 dpf embryos. This dye accumulates in normal mitochondria thanks to the MMP, resulting in an increase in fluorescence. In apoptotic conditions, MMP is supposed to be collapsing, which is manifested by a decrease in fluorescence.

Here, the MMP analysis showed a notable tendency in fluorescence decrease in *chchd10* mutants compared to WT conditions (**Figure 17**), however without reaching significance. The decrease observed in *chchd10* heterozygotes is peculiar as they do not show locomotion defects and any alteration in the mitochondrial network at 2 dpf. These data suggest a possible effect of *chchd10* on mitochondrial membrane permeability that could facilitate apoptotic conditions, but further investigation needs to be performed to ascertain this observation.

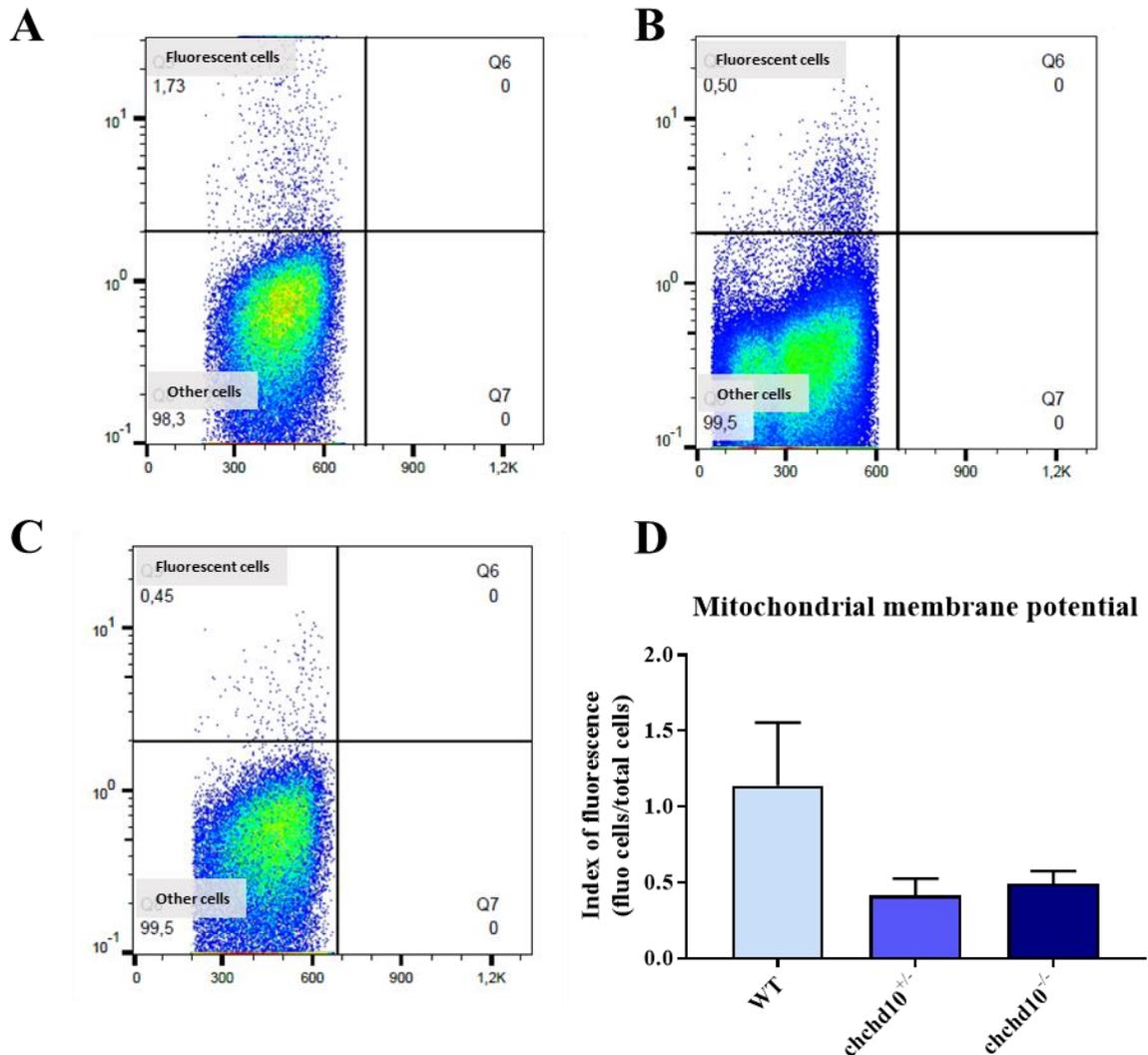


Figure 17: Mitochondrial membrane potential assessment in *chchd10* mutants. Flow cytometry results showing percentage of fluorescent cells in WT (A), *chchd10*^{+/-} (B) and *chchd10*^{-/-} (C) conditions. D, Quantification of the index of fluorescence. *chchd10* mutants showed a decrease in fluorescence, indicating a possible collapse of the mitochondrial membrane potential, and thus aberrant apoptotic conditions in the mutants.

3.6. Mitochondrial gene expression changes in *chchd10* mutants

Like for *fus*, we decided to assess the transcript expression of *polg1*, *twinkle*, *tk2*, *cox5ab* and *atp5a1*, which are involved in mtDNA maintenance and respiratory chain complexes biogenesis.

At 2 dpf, *twinkle*, *tk2* and *polg1* transcripts showed a tendency in increased expression in *chchd10*^{-/-} embryos (**Figure 18A**). In parallel, the genes involved in the biogenesis of mitochondrial complexes also displayed a tendency to increase in both *chchd10* mutants (**Figure 18B**). However, due to high variability, there was no statistical difference between WT and the mutants. Altogether, these data hint towards an altered expression of key genes in mitochondrial functions caused by *chchd10* loss of function, but assessments need to be repeated and refined for confirmation.

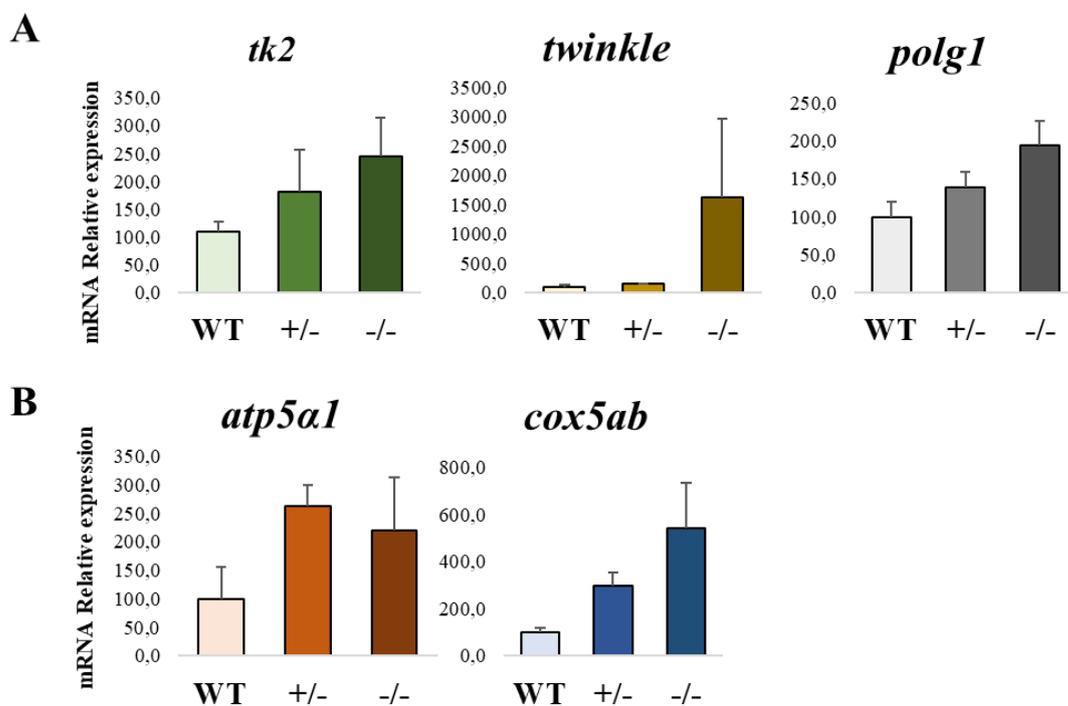


Figure 18: Mitochondrial gene expression changes in *chchd10* mutants. **A**, qPCR analysis of *twinkle*, *tk2* and *polg1*, genes involved in mtDNA maintenance and replication. **B**, qPCR analysis of *atp5a1* and *cox5ab*, genes involved in the biogenesis of mitochondrial complexes.

3.7. Expression of *tardbp* and *fus* in *chchd10* mutant zebrafish

A recent study reported a pathological link between CHCHD10 mutation and cytoplasmic TDP-43 inclusions that are associated with mitochondrial dysfunctions (Woo et al., 2017). With the use of different model organisms, they could show that CHCHD10 plays a protective role in

mitochondrial and synaptic integrity and that CHCHD10 mutations induces cytoplasmic TDP-43 accumulation, resulting in mitochondrial and synaptic damage (Woo et al., 2017). For this reason, we wanted to check the expression of *tardbp* and tdp-43 in our *chchd10* mutant model. In parallel, tdp-43 shares multiple roles with *fus* (Ling, Polymenidou, & Cleveland, 2013), and they both colocalize with mitochondrial markers in different ALS models (Magrané, Cortez, Gan, & Manfredi, 2013; Wang et al., 2013). Therefore, since a major part of the thesis is focused on *fus* characterization, we decided to investigate possible expression changes of *fus* in the *chchd10* mutants.

To do so, we performed Western Blot analyses to determine the protein levels of tdp-43 at 2 dpf embryos. No significant change was found between the conditions (**Figure 19A**). We also checked for levels of *tardbp* transcripts and observed a slight increase but also not significant (**Figure 19B**). We also did the measurements to determine *fus* expression. At the protein level, there seems to be a decrease in *chchd10*^{-/-} embryos compared to the other conditions, although not significant (**Figure 19C**). At the mRNA level, no change in expression was observed (**Figure 19D**). Therefore, these results suggest that *chchd10* loss-of-function does not influence the expression patterns of tdp-43 and *fus*.

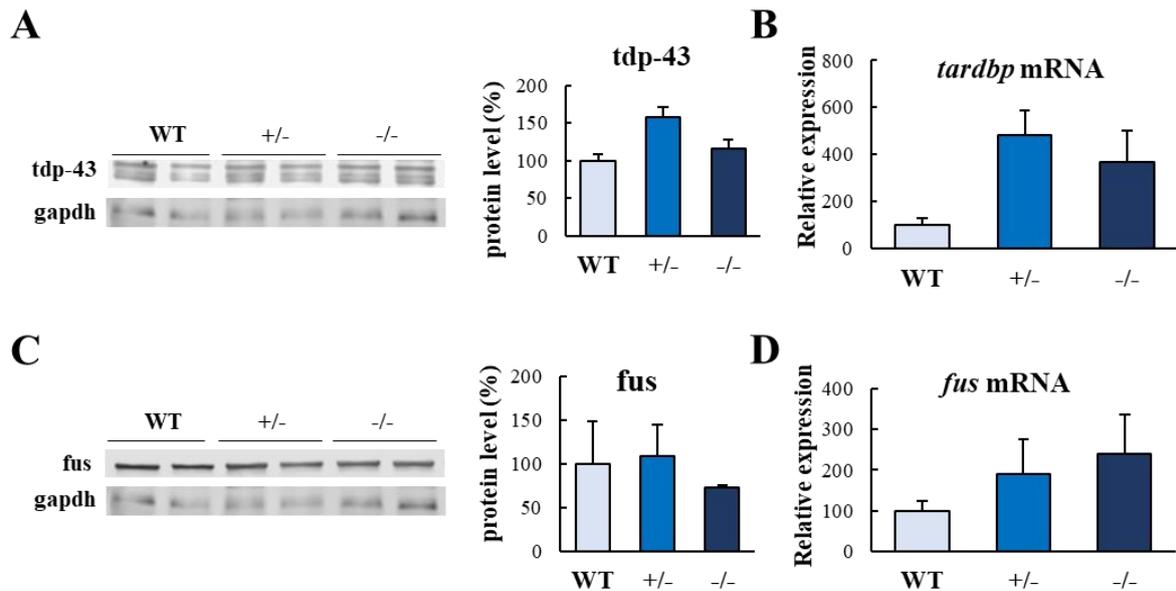


Figure 19: Expression profiles of *tdp-43* and *fus* in *chchd10* mutants. **A**, *Left*, immunoblot analysis of *tdp-43* protein in total extract from WT, hetero and homozygous embryos at 48hpf. The band detected is around 43 KDa. *Right*, densitometry quantification of *tdp-43* protein level normalized to *gapdh*. **B**, RT-PCR analysis of *tadbpb* mRNA. **C**, *Left*, immunoblot analysis of *fus* protein in total extract from WT, hetero and homozygous embryos at 48hpf. The band detected is around 77 KDa. *Right*, densitometry quantification of *fus* protein level normalized to *gapdh*. **D**, RT-PCR analysis of *fus* mRNA.

3.8. Expression of *chchd10* in the *fus* mutant zebrafish model

Following the results highlighting mitochondrial network alteration, we decided to look at the expression of *chchd10* by western blot and qPCR analysis in the *fus* mutant models. Interestingly, both *chchd10* proteins and mRNA were decreased in *fus*^{-/-} embryos compared to WT and *fus*^{+/-} conditions (**Figure 20A and B**), suggesting the important role that *fus* may have on *chchd10* expression in zebrafish.

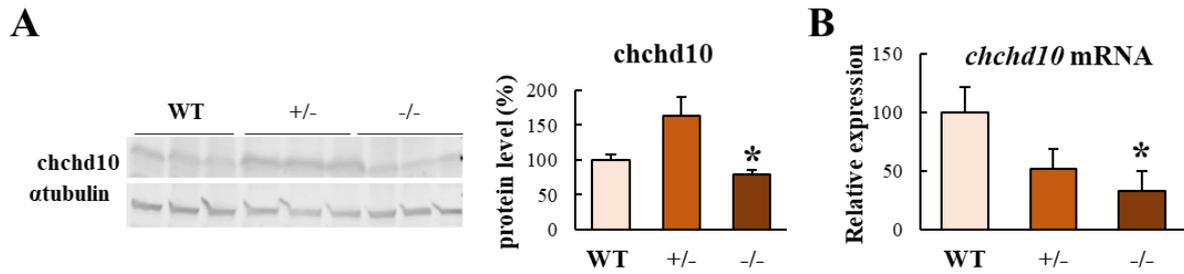


Figure 20: *chchd10* expression changes in *fus* mutants. **A**, *Left*, immunoblot analysis of *chchd10* protein in total extract from WT, hetero and homozygous embryos at 48hpf. The band detected is around 15 KDa. *Right*, densitometry quantification of *chchd10* protein level normalized to α tubulin. (One-way ANOVA with Bonferroni post-hoc test. * $p < 0, 05$). **B**, RT-PCR analysis of *chchd10* mRNA (One-way ANOVA with Bonferroni post-hoc test. * $p < 0, 05$).

3.9. Discussion

The investigation of ALS hallmarks in these new models led us to explore the muscle and mitochondrial features of the disease, which are less explored and present little available information. We demonstrated here that loss of *fus* impacts the organization of slow muscle fibers during development in zebrafish. This muscle structure alteration was associated with changes in the morphology of mitochondrial network, which is more fragmented and less elongated. In addition, we described expression changes in key mitochondrial genes induced by *fus* non-sense mutation, further strengthening the relevance of mitochondrial dysfunction in ALS-associated FUS.

The disruption of slow muscle fibers structure found in *fus* models is consistent with previous studies highlighting muscle pathology in ALS. Several studies on SOD1 mutant mice reported a reduction in muscle volume (Marcuzzo et al., 2011) and strength associated with mitochondrial dysfunction (Dobrowolny, Aucello, Molinaro, & Musarò, 2008). Other studies found that the overexpression of mutant SOD1 in muscle displayed muscle weakness associated

with altered NMJs and MN degeneration (Wong & Martin, 2010). Recently, a direct link was established between mutant FUS and post-synaptic defects in a knock-in *Fus*-ALS mouse model (Picchiarelli et al., 2019). The authors showed that ALS mutant FUS displayed intrinsic toxicity in both MN and induced pluripotent stem cell-derived myotubes of FUS-ALS patients, as well as myogenic pathological activity in muscle biopsy samples from patients (Picchiarelli et al., 2019). In addition, they showed that FUS regulates the expression of *Chrn* genes, thus impacting NMJ formation and stability. These data reinforce the findings described in this thesis. In fact, both NMJ and muscle structure disruption are identified in zebrafish lacking *fus*. However, we need to perform further studies on muscle to confirm the alterations we found. Electrical stimulations on muscle fibers have been performed on zebrafish embryos and could provide information on muscle contraction efficiency and muscle strength (Dou, Andersson-Lendahl, & Arner, 2008).

Mitochondrial pathology plays an important role in pathogenesis of ALS as has been reported in numerous studies. SOD1 mutants displayed increased ROS levels and altered levels of PGC-1 α would disrupt mitochondrial biogenesis and function in SOD1 models (Da Cruz et al., 2012; Halter et al., 2010). TDP-43 and FUS have also been linked with mitochondrial dysfunctions, such as decreased mitochondrial membrane potential and elevation of ROS levels (Wang et al., 2019), or disruption of the ATP synthase complex assembly, suppressing mitochondrial ATP synthase and causing mitochondrial stress (Deng et al., 2018). In our work, we revealed disrupted mitochondrial networks in absence of *fus*, hinting towards *fus* loss of function in mitochondrial dysfunction. However, the visualization techniques we used present limitations that compel us to be cautious in our conclusions. Indeed, to the best of our knowledge, visualizing the mitochondrial network has been mostly done on cell lines and primary cultures, providing a simpler environment for the penetration of these fluorescent markers and thus a better representation of the network at the cellular level. Here, we wanted

to analyze this network in a full organism, providing a context closer to reality, with the cells behaving according to their specific disposition in the organs. But this approach made the labelling more complex and, in some cases, inconsistent, as the markers would sometimes not be homogeneously diffused. This limitation might interfere with the 3D reconstruction analysis and thus making the assessment less accurate.

Furthermore, the quantification of ROS levels in our models presented high variability and we could not see any significant increase that has been previously reported in other ALS studies (Capitano et al., 2012; Halter et al., 2010). Therefore, more precise labelling techniques for zebrafish and other experiments evaluating different metabolic features need to be developed and performed. Further investigation on this direction would provide solid clues on mitochondrial implication in ALS pathogenesis.

We also put our interest in the characterization of a stable mutant zebrafish lacking CHCHD10, which protein is strictly implicated in mitochondrial activity. The model displayed locomotor deficits at 2 dpf that were not persistent in later stages. It presented also some mitochondrial dysfunctions such as less dense and fragmented mitochondrial network, as well as a collapse of the mitochondrial membrane potential, suggesting the enhancement of apoptotic conditions. Expression changes in key mitochondrial genes were also hinted. However, these alterations were not significant and only provide interesting trends that need to be further developed.

Several reasons could explain the lack of clear pathological features in this model. First, a number of partners of CHCHD10 have been identified and could compensate for its loss of function. Indeed, CHCHD10 is part of the mitochondrial contact site and cristae organizing system (MICOS) complex that also includes other CHCH proteins including CHCHD3 and 6, as well as mitofilin and mitofusin (Genin et al., 2016). These proteins share similar roles with CHCHD10 and have been shown to all interact with the same proteins to contribute to crista

stability (Xie, Marusich, Souda, Whitelegge, & Capaldi, 2007; Zhou, Saw, & Tan, 2017). Another major partner is CHCHD2, which is structurally almost identical to CHCHD10 (Imai, Meng, Shiba-Fukushima, & Hattori, 2019) and has been reported to form a multimodal complex with CHCHD10, associating these twin proteins to various neurodegenerative diseases (Imai et al., 2019; Klemann et al., 2017). CHCHD2 and CHCHD10 form both homodimers and heterodimers to maintain mitochondrial activity, and it has been hypothesized that the disruption of these complexes might be involved in neurodegeneration (Huang et al., 2018; Meng et al., 2017). In our case, the mutation on *chchd10* might be insufficient to disturb the binding between *chchd2* and the truncated *chchd10* proteins, thus keeping the homo and heterodimers intact and possibly temper the effects of *chchd10* loss of function.

Our data are also in line with recent studies on mutant CHCHD10 mouse models (Anderson et al., 2019b; Burstein et al., 2017). In one report, they demonstrated that knock-in mice harboring a mutant form of CHCHD10 displayed ALS-like clinical features, including motor deficits, myopathy, early lethality and diverse mitochondrial alterations, whereas CHCHD10 KO mice did not induce disease pathology or mitochondrial alterations, suggesting that CHCHD10-dependent disease pathology is not particularly caused by loss-of-function (Anderson et al., 2019b). Burstein and colleagues demonstrated similar results with CHCHD10 KO mice being viable, having no significant phenotype and no mitochondrial abnormalities, suggesting the occurrence of functional redundancy or compensatory mechanisms for CHCHD10 loss (Burstein et al., 2017).

CHCHD10 being only recently identified as an ALS-associated gene, there is still a solid need for new models and further studies to clarify this gain versus loss-of-function matter.

Pathological links between CHCHD10 and other ALS-associated genes are yet to be defined. Only one study showed in different models that CHCHD10 ALS/FTD mutations caused mitochondrial and synaptic damage associated with TDP-43 accumulation (Woo et al.,

2017), providing a link between these two genes. Here, we propose a possible connection between *fus* and *chchd10*, since *fus* loss of function significantly change *chchd10* mRNA and protein expression. Therefore, further analyses have to be done in this direction and may provide interesting information on the potential link that these two genes have in ALS etiology.

3.10. Material & Methods

Animal Care: Adult and larval zebrafish (*Danio rerio*) were maintained at the ICM (Institut du Cerveau et de la Moelle épinière, Paris) zebrafish facility and bred according to the National and European Guidelines for Animal Welfare. All procedures were approved by the Institutional Ethics Committees at the Research Centers of ICM and Imagine.

Screening of mutant lines: The *chchd10* mutant zebrafish line was obtained from the European Zebrafish Resource Center (EZRC) (reference: sa18461) and originates from the mutagenesis screen performed at the Wellcome Trust Sanger Institute (Stemple Laboratory). The mutation (c.266T>A; p.Y89X) in the *chchd10* genetic line is a *nonsense* point mutation at the exon 3 of *chchd10*. Heterozygous parents and homozygous offspring were screened through high-precision sequencing (GATC Biotech, Eurofins Genomics). DNA was extracted from by cutting a part of the fin from adult zebrafish. Prior to fin-clipping, the adult zebrafish were anesthetized in tricaine (160 µg/mL). A part of their caudal fin was cut and put in 75 µL of extraction solution (25mM NaOH, 0,2 mM EDTA). The samples were then incubated at 98°C for 1 hour and 75 µL of a Tris-HCl (40 mM, pH 5.5) solution was added to the tubes before centrifugation at 4000 rpm for 3 minutes. The samples were amplified by PCR using Taq polymerase Master Mix (Thermo Scientific K1071) and the following primers: CCTAAGCAGCCAGGTCTCAT (forward primer) and

TTGACTCAATGCCACTTAAAGAG (reverse primer). The PCR products were then sent to GATC Biotech for sequencing.

Survival assay: Embryos were maintained according to the procedures of the ICM fish facility. Larvae were checked daily and maintained in petri dish in a 28.5°C incubator for the first 5 days post-fertilization (dpf). After 5 dpf, the fish were transferred in tanks with continuous water flux. They were then fed regularly and the number of dead larvae was scored each day for 20 days by a blinded observer.

Western Blot: 30 ug of proteins from zebrafish lysates (equal amount of protein in each lane) were separated by sodium dodecylpolyacrylamide gel electrophoresis. Samples were denatured at 98°C for 7 minutes. The separated proteins were transferred to nitrocellulose membranes (0.45µm, Life Sciences) and probed with the following primary antibodies: c-terminal FUS antibody (sc-47711, Santacruz), C-terminal TDP-43 antibody (12892-1-AP, Proteintech), CHCHD10 antibody (HPA003440, Sigma-Aldrich). A α -tubulin antibody (T5168, Sigma-Aldrich) and a GAPDH antibody (ab8245, abcam) were used as loading controls. The blots were incubated with the corresponding fluorescent secondary antibody and the signal was detected using the ODYSSEY® CLx. The intensity of bands in each of the lanes from the Western blots were measured by ImageJ.

RNA isolation and analysis of transcripts by q-PCR: Total RNA was isolated from fish using TRIzol Reagent (Sigma) according to the manufacturer's protocol. First-strand cDNAs were obtained by reverse transcription of 1 µg of total RNA using the High Capacity cDNA Reverse Transcription Kit (Roche), according to the manufacturer's instructions. Quantitative PCR amplification was performed with SyBer2X Gene Expression Assays using the primers listed in **Table S2**. Data were analyzed transforming raw Cq values into relative quantification data using the delta Cq method.

Locomotion assessment: *Touch-evoked escape response (TEER):* 2 days post-fertilization (dpf) zebrafish embryos were tested for behavior under a stereomicroscope (Zeiss, Germany) by inducing a light touch-stimulus at the level of the tail with a tip and were scored for the distance travelled, the time spent swimming and speed in a 15-cm diameter petri dish. Their responses were recorded with a Grasshopper digital camera (Point Grey) at a rate of 30 frame/s and quantified using ImageJ. *Spontaneous locomotion:* 3 dpf-zebrafish larvae were maintained in a 96-well plate and assayed for total locomotor activity in ZebraBox (Viewpoint Life Sciences, Lyon, France). The embryos were tested for 10 min in a 5 min dark, 2 min light (100%), 3 min dark paradigm, repeated 3 times. The experiment was done on the same fish at 4 and 5 dpf. Total swim activity was analyzed using ZebraLab V3 software (Viewpoint Life Sciences, Lyon, France).

Immunohistochemistry: Animals were fixed in 4% paraformaldehyde for 2 hours at RT. After fixation the larvae were rinsed several times with PBS and then incubated in PBS containing 1 mg/ml collagenase (20 min) to remove skin. The collagenase was washed off with PBS Triton X-100 (PBST; 1 h) and heads were cut away. For the NMJ study, after an incubation of 30 min in blocking solution (1% BSA, 1% triton, PBS, 2% Goat serum), the embryos were incubated overnight at 4°C in synaptic vesicle 2 (SV2, 1:200; DSHB) antibody diluted in blocking solution. The embryos were then washed and incubated for 30 min in PBST containing α -bungarotoxin conjugated to Alexa 488 (α BTX, 1:1000; Abcam). The larvae were then rinsed several times with PBST and then incubated in freshly prepared block solution containing a secondary antibody (Alexa Fluor 568, 1:1000; Life Technologies) for 3h at RT before mounting on glass slide in 50% glycerol. The NMJs were visualized using a Spinning disk system (Intelligent Imaging Innovations, USA), an Examiner.Z1 upright stand (Carl Zeiss, Germany), a CSU-W1 head (Yokogawa, Japan), and an ORCA-Flash 4.0 camera (Hamamatsu, Japan). For the muscle, the same procedures were followed using the corresponding antibodies: anti-F59

(Developmental Studies Hybridoma Bank) for slow muscles, anti-F310 (Developmental Studies Hybridoma Bank) for fast muscles, anti-TOMM20 (HPA011562, Sigma-Aldrich) for mitochondria. For mitochondria, embryos were incubated for 5 minutes in DAPI (4',6-diamidino-2-phénylindole) to mark the nuclei just before mounting procedure. All images were captured at 40x and stained embryos were processed using Imaris Image Analysis software and ImageJ.

Reactive Oxygen Species measurements: 15 embryos were washed with cold Phosphate Buffer solution (PBS; pH 7.4) twice and then homogenized in cold buffer (0.32 mM of sucrose, 20 mM of HEPES, 1 mM of MgCl₂, and 0.5 mM of phenylmethyl sulfonylfluoride (PMSF), pH 7.4). The homogenate was centrifuged at 15,000g at 4°C for 20 minutes, and the supernatant was transferred to new tubes for further experimentation. 20µl of the homogenate was added to a 96-well plate and incubated at room temperature for 5 min, after which 100 µl of PBS (pH 7.4) and 8.3 µl of DFH stock solution (10 mg/ml) were added to each well. The plate was incubated at 37°C for 30 minutes. The fluorescence intensity was measured using a microplate reader with excitation and emission at 485 and 530 nm, respectively. The reactive oxygen species concentration was expressed as arbitrary emission units per mg protein.

Mitochondrial membrane permeability assessment: zebrafish embryos were first dissociated to work on cell lysates. Embryos were transferred into a 6-well plate with 2 mL EDTA-trypsin (0.25%) and incubated at 28°C for 90 minutes. Samples were triturated regularly with pipettes. The digestion is then stopped with 10% fetal calf serum. Cells are transferred to 50ml tubes and 15mL of HBSS (Sigma-Aldrich). The mix is then centrifuged at 4°C, 3000rpm for 5 minutes. HBSS is then removed to add fresh HBSS to wash again with the same parameters. Cells are then resuspended in 3ml Leibovitz medium (Sigma-Aldrich) with 10%FPBS, 1% L-glutamine, 2% Pen/Strep. The mix is then centrifuged at 4°C, 3000rpm for 5 minutes. Supernatant is discarded and cells are put in 1mL of the same medium. 2uL of 500x

Mitochondrial Potential Dye (MAK146, Sigma-Aldrich) is added per sample. Cells are then incubated in a 5%, CO₂, 37°C incubator for 30 minutes. Cells are then centrifuged at 1000rpm for 4 minutes and resuspended in 1mL of buffer of choice. The fluorescence is then monitored using the flow cytometer MACSQuant[®] Analyzer 10 (Miltenyi Biotec) (emission at 535 nm). The results are then analyzed using FlowJo[®] software.

Statistical analysis: All data values for the zebrafish experiments are represented as average standard error of mean (SEM) with significance determined using one-way ANOVAs. Differences between groups were identified via post hoc comparisons, specified in the legend of each figure. All analyses were performed using Prism 5.0 (Graph Pad, CA). Significance level was set at $p < 0.05$.

3.11. Supplementary Material

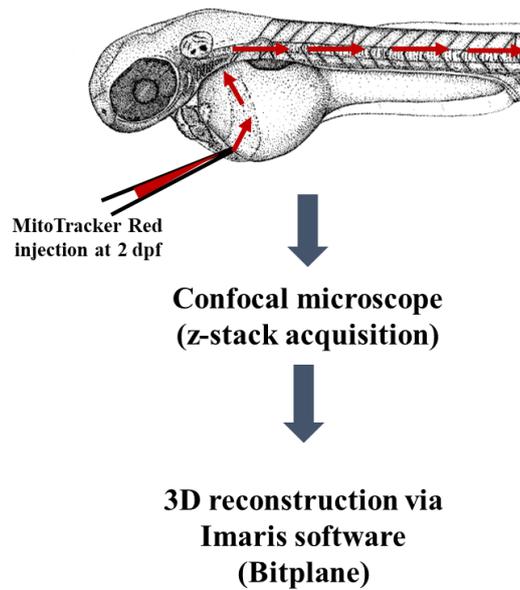


Figure S6: Mitotracker injection and acquisition protocol

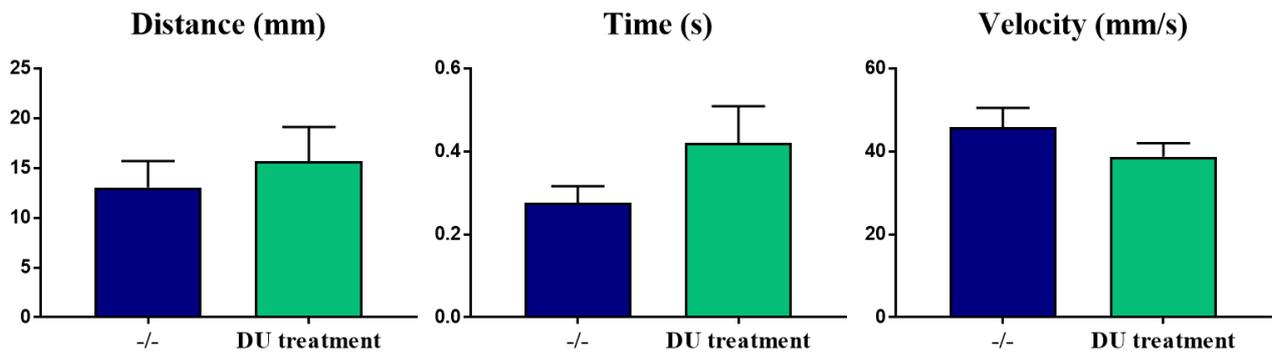


Figure S7: TEER analysis on $chchd10^{-/-}$ mutants after decylubiquinone (DU) treatment. No particular rescue of the locomotion was observed.

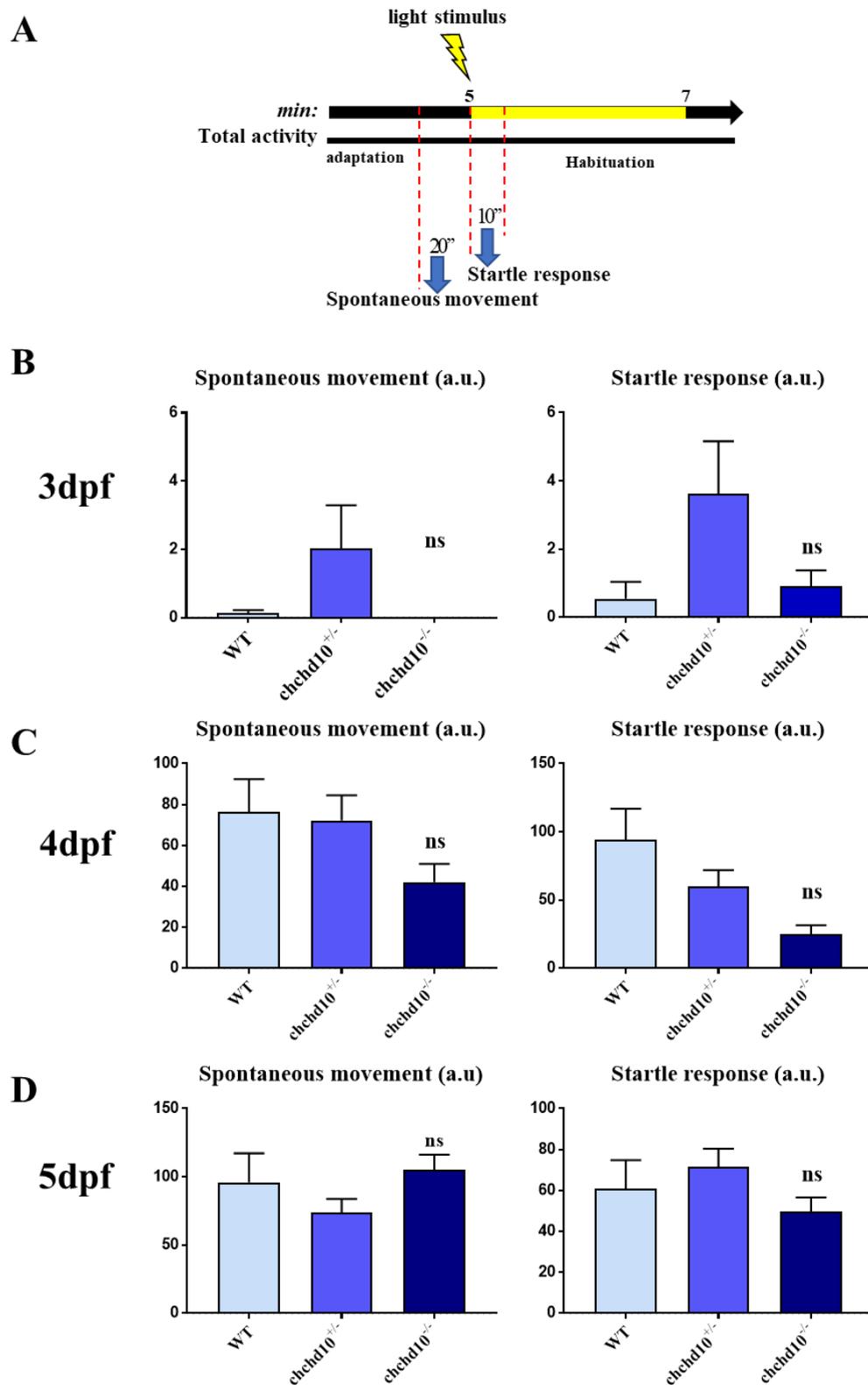


Figure S8: Behavioral activity in light/dark stimulation. **A**, automatized protocol used at the Viewpoint Zebrazox system. Light stimulation is preceded by a period of five minutes of darkness, called adaptation period. This period represents the favorite environment for zebrafish, probably mimicking a protective area from predators, in which we can measure an

increased locomotion activity that we call Spontaneous movements. An abrupt stimulation by light, the startle response, is then applied and the fish responds with a brief period of elevated activity, where anxiety increases. The total experiment duration is of 7 minutes and it is repeated over time until day 5. **B, C, D**, quantification for activity during darkness (spontaneous movement) and light (startle response) at 3, 4 and 5 dpf.

Fus F	CGACTGTACGTATAACACCT
Fus R	CCATAATCATTGACGCCAT
Tdp-43 F	GGAAACCTAGTCTATGTGGT
Tdp-43 R	CATCGATCTCATCCATCTTC
chchd10 F	CCTAAGCAGCCAGGTCTCAT
chchd10 R	TTGACTCAATGCCACTTAAAGAG
polg1 F	GGTGACCAGTGAAGACCGATA
polg1 R	GTCCACTGCGCTAAAGAAGG
tk2 F	CCTGTATGAGGACTGGCTGA
tk2 R	TCTGTTCTCCTCAAAGTATGC
twinkle F	TGTGGGCTGACAAGTTTGAGG
twinkle R	TGTCCACAGACAGATTTTCTTG
cox5ab F	GGTCACCGGAGCTTCAGGAT
cox5ab R	TCGAGCCGAGAGGTAGAAAAACC
atp5a1 F	TTCTTGAGCCGACACTGGA
atp5a1 R	CGAACACCACAACCAACG

Table S2: Primers list for qPCR analyses

CHAPTER 3 – CONCLUSION AND PERSPECTIVES

Conclusion and Perspectives

Neurodegenerative diseases represent a major issue to human health and, due to increased population ageing, their prevalence tends to increase (Heemels, 2016). These diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis or frontotemporal dementia, are diverse in their pathophysiology, ranging from memory and cognitive impairments to severe locomotion abilities (Gitler, Dhillon, & Shorter, 2017). One of the common features that characterize them as neurodegenerative is the accumulation and aggregation of disease-specific proteins, and advances in genome sequencing technology have made it possible to identify in patients the genetic causes of these complex diseases (Pihlstrøm, Wiethoff, & Houlden, 2018). However, the underlying mechanisms that define these diseases still need to be fully unraveled in order to provide efficient treatments.

One way to reinforce our understanding is to develop model systems that can recapitulate the typical hallmarks of these diseases (Gitler et al., 2017). In the case of ALS, efficient experimental model organisms, like mouse, *Drosophila*, zebrafish, nematode, cell cultures or more recently human patient-derived stem cell models, have been generated and provided the scientific community with useful insights into genetic and molecular bases of the disease (reviewed in Van Damme, Robberecht, & Van Den Bosch, 2017). However, all these models have limitations and the translation to patients remains unclear, thus inciting for developing new models as well as undertake cross-model approaches to validate disease mechanisms found in less-complex systems in more-complex models and human samples (Van Damme et al., 2017).

ALS is a devastating disease characterized by the selective loss of upper motor neurons in the motor cortex and lower motor neurons in the brainstem and the spinal cord. Over the years, multiple pathogenic mechanisms have been identified to be potential causes for ALS, including

excitotoxicity, oxidative stress and mitochondrial dysfunction, RNA processing defects, axonal transport defects, metabolic alterations and accumulation of protein aggregates (reviewed in Hardiman et al., 2017), thus highlighting the complexity of the disease. Another fact to consider is that, even though ALS has been usually described as a disease of the motor neuron cell body, several studies have demonstrated that other cells are affected (Brites & Vaz, 2014; Philips & Robberecht, 2011; Ramírez-Jarquín, Lazo-Gómez, Tovar-y-Romo, & Tapia, 2014).

Also, with reports showing that NMJ dysfunction is one of the first events occurring in pathological conditions prior to MN degeneration (Moloney, de Winter, & Verhaagen, 2014), ALS has been redefined as a distal axonopathy, where molecular changes causing MN degeneration occur at nerve terminals and post-synaptic sites prior to symptom onset (Moloney et al., 2014). Particularly, this “dying-back” hypothesis stipulates that alterations first occur distally at the NMJ and progress proximally toward the cell body. Multiple studies are in line with this hypothesis by demonstrating denervation and innervation changes at the muscle associated with ALS hallmarks independently of MN pathological changes (Fischer et al., 2004; Loeffler, Picchiarelli, Dupuis, & Gonzalez De Aguilar, 2016; Pansarasa, Rossi, Berardinelli, & Cereda, 2014).

In this thesis, we provide multiple lines of evidence that ascertain the dying-back hypothesis. Indeed, we show, through the characterization of a stable mutant KO zebrafish model for the gene *fus*, specific ALS hallmarks such as accelerated lethality, persisting motor deficits, as well as disorganized NMJ morphology associated with dysregulation of several AChR subunits and *hdac4* level, hinting towards denervation and reinnervation processes. This model also displayed altered muscle structure and aberrant mitochondrial network morphology. In addition, transcriptomic and protein expression changes of tau and kinases acting on tau phosphorylation have been found in the model, providing potential clues towards a link between ALS and FTD pathologies.

From this work, we formulate the following hypothesis, represented in **Figure 21**. Briefly, loss of *fus* expression is responsible for the occurrence of distal pathological signs at the NMJ. Specifically, the tight juxtaposition of pre- and post- elements is lost, resulting in several AChR clusters being denervated and diffuse on the muscular membrane. As already reported in human, a phenomenon of reinnervation occurs. Expression of genes for skeletal muscle differentiation decrease, such as *hdac4*, and others link to juvenile stage increase, as it is the case for AChR γ subunit. Also, at the MNs, there is an attempt of reinnervation by enhancing cytoskeletal plasticity. This plasticity depends on microtubule dynamics which are in turn dependent on tau function. This scenario could possibly explain the changed expression of tau towards the smaller isoforms and the increase of its related kinases, conditions that are particularly abundant during development.

These findings are corroborated by a recent study demonstrating the critical involvement of ALS-FUS in muscle (Picchiarelli et al., 2019). However, our respective *fus* models differ from theirs since we identified deficits in a loss-of-function approach, whereas they attribute their findings to a toxic *FUS* gain-of-function (Picchiarelli et al., 2019). Therefore, other models need to be generated and compared to elucidate these discrepancies.

Also, the use of other genome editing techniques could also reinforce these findings in zebrafish. With the development of these tools, including transcription activator-like effector nucleases (TALEN) and the clustered regularly interspaced short palindromic repeats/CRISPR-associated 9 (CRISPR/Cas9) system, more stable knock-in and knock-out models can be and are now generated for a better study of late-onset diseases like ALS.

Furthermore, zebrafish is an easily accessible model for screening potential therapeutic molecules that must be validated later in mammals (Babin, Goizet, & Raldúa, 2014).

Therefore, as stated above, there is a need for more cross-model approaches to validate the pathogenic mechanisms identified in these different models, to provide additional information on the complex consequences and possible co-occurrences of gain and loss of function phenomenon in ALS pathology, and to be able to translate these findings into human pathology in order to generate efficient therapeutic strategies. In this context, the transcriptomic and proteomic sequencing are particularly important, since they are permitting the identifications of numerous biomarker candidates and therapeutic targets in FUS ALS patients (De Santis et al., 2017; Scekcic-Zahirovic et al., 2016).

The data gathered by our *fus* model incite us to explore more deeply its whole-transcriptome profile. The first steps have already been initiated with the generation of a specific transgenic line harboring the *fus* non sense mutation on a *hb9:GFP* background. In this way, MNs express the GFP (green fluorescent protein) construct, as the *hb9* gene is involved in MN differentiation (Broihier & Skeath, 2002). We designed this line to sort through FACS (Fluorescence activated cell sorting) the MN and perform specifically the transcriptomic analysis in this cell type. Preliminary work indicate that this approach is feasible for proteomics and transcriptomics analysis that will certainly advance our knowledge of the pathogenic mechanisms due to loss of function of FUS in ALS.

In this thesis, we also characterized a stable zebrafish mutant of the *chchd10* gene. The model displayed only tendencies that resemble some ALS features, including enhanced mitochondrial membrane permeability, but overall no clear phenotype has been highlighted. Our model tends to be in line with a lack of loss of function effects and it would be interesting to generate a knock-in model to validate the reported data.

CHCHD10 being only recently associated with ALS, more studies need to be performed on this gene and particularly on its possible partners that may contribute to ALS pathology or compensate the effects of CHCHD10 mutations. One of its partners, CHCHD2, has been

reported to accumulate in pathological conditions (Huang et al., 2018). Since it is not duplicated in zebrafish, CHCHD2 gene could represent an interesting candidate to target for genetic analysis. A vertebrate model would certainly extend our understanding on CHCHD10-associated ALS pathology.

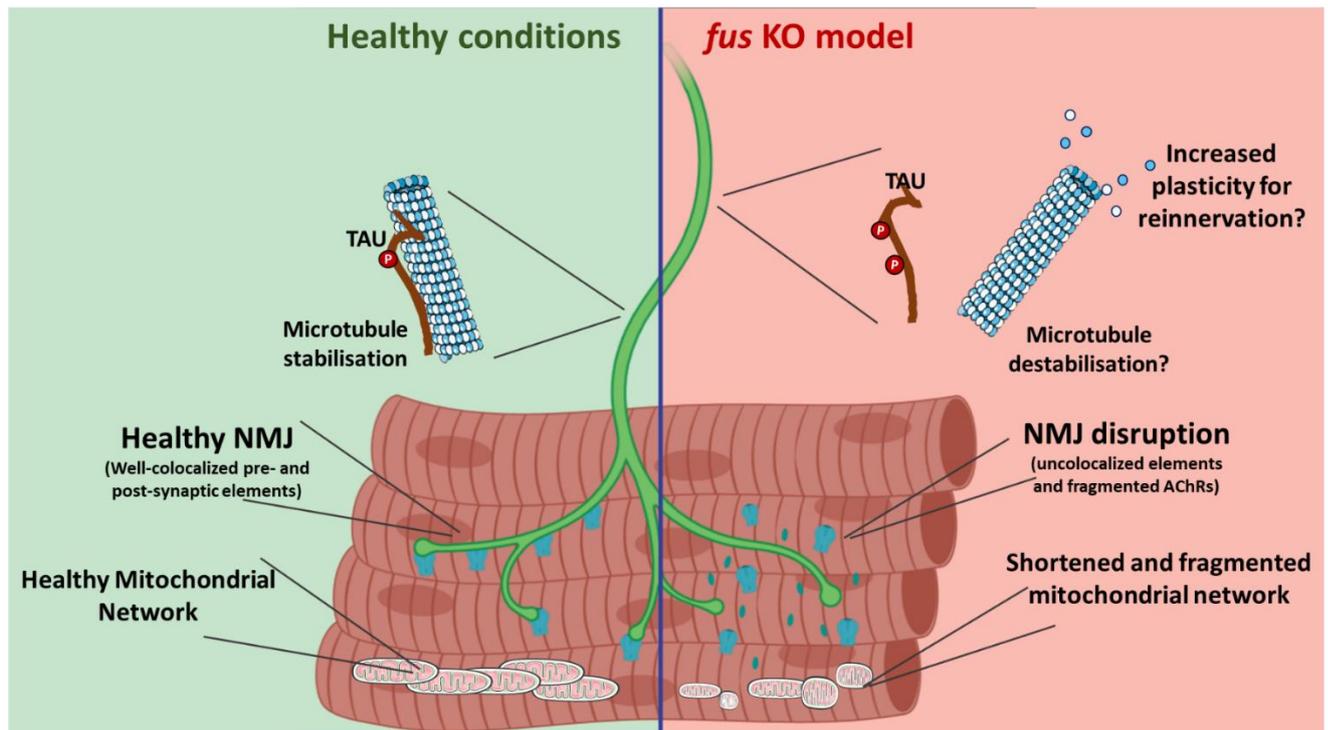


Figure 21: Summary of the proposed hypothesis of ALS features occurrence in our *fus KO* zebrafish model. In healthy conditions, pre- and post-synaptic elements are well-colocalized, constituting functional NMJs and proper innervation from the MN to the muscle. When *fus* is knocked-out in zebrafish, pre- and post-synaptic elements fail to colocalize properly to form functional synapses, with AChRs presenting pathological fragmentations. Thus, denervation occurs which triggers compensatory reinnervation processes that go through the adaptive plasticity of the axon to form new junctions with the skeletal muscle. This plasticity depends on enhanced cytoskeletal dynamics involving the microtubules and thus, modulating tau function as well as tau-related kinases to ensure tau phosphorylation.

Bibliography

- Abe, K., Aoki, M., Tsuji, S., Itoyama, Y., Sobue, G., Togo, M., ... Yoshino, H. (2017). Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*, *16*(7), 505–512. [https://doi.org/10.1016/S1474-4422\(17\)30115-1](https://doi.org/10.1016/S1474-4422(17)30115-1)
- Akiyama, S., Endo, H., Inohara, N., Ohta, S., & Kagawa, Y. (1994). Gene structure and cell type-specific expression of the human ATP synthase alpha subunit. *Biochimica et Biophysica Acta*, *1219*(1), 129–140. [https://doi.org/10.1016/0167-4781\(94\)90255-0](https://doi.org/10.1016/0167-4781(94)90255-0)
- Al-Chalabi, A., & Hardiman, O. (2013). The epidemiology of ALS: a conspiracy of genes, environment and time. *Nature Reviews Neurology*, *9*, 617.
- Alvarez, S., Calin, A., Graffmo, K. S., Moldovan, M., & Krarup, C. (2013). Peripheral motor axons of SOD1G127X mutant mice are susceptible to activity-dependent degeneration. *Neuroscience*, *241*, 239–249. <https://doi.org/https://doi.org/10.1016/j.neuroscience.2013.03.017>
- Åman, P., Panagopoulos, I., Lassen, C., Fioretos, T., Mencinger, M., Toresson, H., ... Mitelman, F. (1996a). Expression patterns of the human sarcoma-associated genes FUS and EWS and the genomic structure of FUS. *Genomics*, *37*(1), 1–8. <https://doi.org/10.1006/geno.1996.0513>
- Åman, P., Panagopoulos, I., Lassen, C., Fioretos, T., Mencinger, M., Toresson, H., ... Mitelman, F. (1996b). Expression Patterns of the Human Sarcoma-Associated Genes FUS and EWS and the Genomic Structure of FUS. *Genomics*, *37*(1), 1–8. <https://doi.org/https://doi.org/10.1006/geno.1996.0513>
- Ambros, V. (2004). The functions of animal microRNAs. *Nature*, *431*(7006), 350–355. <https://doi.org/10.1038/nature02871>
- Amlie-Wolf, A., Ryvkin, P., Tong, R., Dragomir, I., Suh, E., Xu, Y., ... Lee, E. B. (2015). Transcriptomic changes due to cytoplasmic TDP-43 expression reveal dysregulation of histone transcripts and nuclear chromatin. *PLoS ONE*, *10*(10), 1–20. <https://doi.org/10.1371/journal.pone.0141836>
- An, H., Skelt, L., Notaro, A., Highley, J. R., Fox, A. H., La Bella, V., ... Shelkovernikova, T. A. (2019). ALS-linked FUS mutations confer loss and gain of function in the nucleus by promoting excessive formation of dysfunctional paraspeckles. *Acta Neuropathologica Communications*, *7*(1), 7. <https://doi.org/10.1186/s40478-019-0658-x>
- An, J., Shi, J., He, Q., Lui, K., Liu, Y., Huang, Y., & Sheikh, M. S. (2012). CHCM1/CHCHD6, novel mitochondrial protein linked to regulation of mitofilin and mitochondrial cristae morphology. *Journal of Biological Chemistry*, *287*(10), 7411–7426. <https://doi.org/10.1074/jbc.M111.277103>
- Anagnostou, G., Akbar, M. T., Paul, P., Angelinetta, C., Steiner, T. J., & de Bellerocche, J. (2010). Vesicle associated membrane protein B (VAPB) is decreased in ALS spinal cord. *Neurobiology of Aging*, *31*(6), 969–985. <https://doi.org/10.1016/j.neurobiolaging.2008.07.005>
- Anderson, C. J., Bredvik, K., Burstein, S. R., Davis, C., Meadows, S. M., Dash, J., ... Manfredi, G. (2019a). ALS/FTD mutant CHCHD10 mice reveal a tissue-specific toxic gain-of-function and mitochondrial stress response. *Acta Neuropathologica*. <https://doi.org/10.1007/s00401-019-01989-y>
- Anderson, C. J., Bredvik, K., Burstein, S. R., Davis, C., Meadows, S. M., Dash, J., ... Manfredi, G. (2019b). ALS/FTD mutant CHCHD10 mice reveal a tissue-specific toxic gain-of-function and mitochondrial stress response. *Acta Neuropathologica*, *138*(1), 103–121. <https://doi.org/10.1007/s00401-019-01989-y>

- Andersson, M. K., Ståhlberg, A., Arvidsson, Y., Olofsson, A., Semb, H., Stenman, G., ... Åman, P. (2008a). The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biology*, 9, 1–17. <https://doi.org/10.1186/1471-2121-9-37>
- Andersson, M. K., Ståhlberg, A., Arvidsson, Y., Olofsson, A., Semb, H., Stenman, G., ... Åman, P. (2008b). The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biology*, 9(1), 37. <https://doi.org/10.1186/1471-2121-9-37>
- Antar, L. N., Li, C., Zhang, H., Carroll, R. C., & Bassell, G. J. (2006). Local functions for FMRP in axon growth cone motility and activity-dependent regulation of filopodia and spine synapses. *Molecular and Cellular Neurosciences*, 32(1–2), 37–48. <https://doi.org/10.1016/j.mcn.2006.02.001>
- Aoki, Y., Manzano, R., Lee, Y., Dafinca, R., Aoki, M., Douglas, A. G. L., ... Wood, M. J. A. (2017). C9orf72 and RAB7L1 regulate vesicle trafficking in amyotrophic lateral sclerosis and frontotemporal dementia. *Brain*, 140(4), 887–897. <https://doi.org/10.1093/brain/awx024>
- Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., ... Oda, T. (2006). TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochemical and Biophysical Research Communications*, 351(3), 602–611. <https://doi.org/10.1016/j.bbrc.2006.10.093>
- Araki, M., Ito, G., & Tomita, T. (2018). Physiological and pathological functions of LRRK2: implications from substrate proteins. *Neuronal Signaling*, 2(4), NS20180005. <https://doi.org/10.1042/ns20180005>
- Argyriou, A. A., Polychronopoulos, P., Talelli, P., & Chroni, E. (2006). F wave study in amyotrophic lateral sclerosis: assessment of balance between upper and lower motor neuron involvement. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, 117(6), 1260–1265. <https://doi.org/10.1016/j.clinph.2006.03.002>
- Armstrong, G. A. B., & Drapeau, P. (2013). Loss and gain of FUS function impair neuromuscular synaptic transmission in a genetic model of ALS. *Human Molecular Genetics*, 22(21), 4282–4292. <https://doi.org/10.1093/hmg/ddt278>
- Armstrong, J. S., Whiteman, M., Rose, P., & Jones, D. P. (2003). The Coenzyme Q10 analog decylubiquinone inhibits the redox-activated mitochondrial permeability transition: role of mitochondrial [correction mitochondrial] complex III. *The Journal of Biological Chemistry*, 278(49), 49079–49084. <https://doi.org/10.1074/jbc.M307841200>
- Arnold, E. S., Ling, S.-C., Huelga, S. C., Lagier-Tourenne, C., Polymenidou, M., Ditsworth, D., ... Cleveland, D. W. (2013). ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. *Proceedings of the National Academy of Sciences*, 110(8), E736–E745. <https://doi.org/10.1073/pnas.1222809110>
- Artero, R., Prokop, A., Paricio, N., Begemann, G., Pueyo, I., Mlodzik, M., ... Baylies, M. K. (1998). The muscleblind gene participates in the organization of Z-bands and epidermal attachments of *Drosophila* muscles and is regulated by Dmef2. *Developmental Biology*, 195(2), 131–143. <https://doi.org/10.1006/dbio.1997.8833>
- Artuso, L., Romano, A., Verri, T., Domenichini, A., Argenton, F., Santorelli, F. M., & Petruzzella, V. (2012). Mitochondrial DNA metabolism in early development of zebrafish (*Danio rerio*). *Biochimica et Biophysica Acta - Bioenergetics*, 1817(7), 1002–1011. <https://doi.org/10.1016/j.bbabi.2012.03.019>
- Ash, P. E. A., Zhang, Y. J., Roberts, C. M., Saldi, T., Hutter, H., Buratti, E., ... Link, C. D. (2010). Neurotoxic effects of TDP-43 overexpression in *C. elegans*. *Human Molecular Genetics*, 19(16),

3206–3218. <https://doi.org/10.1093/hmg/ddq230>

- Atkin, J. D., Farg, M. A., Soo, K. Y., Walker, A. K., Halloran, M., Turner, B. J., ... Horne, M. K. (2014). Mutant SOD1 inhibits ER-Golgi transport in amyotrophic lateral sclerosis. *Journal of Neurochemistry*, *129*(1), 190–204. <https://doi.org/10.1111/jnc.12493>
- Atkin, J. D., Farg, M. A., Walker, A. K., McLean, C., Tomas, D., & Horne, M. K. (2008). Endoplasmic reticulum stress and induction of the unfolded protein response in human sporadic amyotrophic lateral sclerosis. *Neurobiology of Disease*, *30*(3), 400–407. <https://doi.org/10.1016/j.nbd.2008.02.009>
- Atsumi, T. (1981). The ultrastructure of intramuscular nerves in amyotrophic lateral sclerosis. *Acta Neuropathologica*, *55*(3), 193–198. <https://doi.org/10.1007/BF00691318>
- Aulas, A., Stabile, S., & Vande Velde, C. (2012). Endogenous TDP-43, but not FUS, contributes to stress granule assembly via G3BP. *Molecular Neurodegeneration*, *7*(1). <https://doi.org/10.1186/1750-1326-7-54>
- Ayala, Y. M., Zago, P., D'Ambrogio, A., Xu, Y.-F., Petrucelli, L., Buratti, E., & Baralle, F. E. (2008). Structural determinants of the cellular localization and shuttling of TDP-43. *Journal of Cell Science*, *121*(22), 3778–3785. <https://doi.org/10.1242/jcs.038950>
- Babin, P. J., Goizet, C., & Raldúa, D. (2014). Zebrafish models of human motor neuron diseases: Advantages and limitations. *Progress in Neurobiology*, *118*, 36–58. <https://doi.org/10.1016/j.pneurobio.2014.03.001>
- Baechtold, H., Kuroda, M., Sok, J., Ron, D., Lopez, B. S., & Akhmedov, A. T. (1999). Human 75-kDa DNA-pairing protein is identical to the pro-oncoprotein TLS/FUS and is able to promote D-loop formation. *The Journal of Biological Chemistry*, *274*(48), 34337–34342. <https://doi.org/10.1074/jbc.274.48.34337>
- Bailey, R. M., Covy, J. P., Melrose, H. L., Rousseau, L., Watkinson, R., Knight, J., ... Lewis, J. (2013). LRRK2 phosphorylates novel tau epitopes and promotes tauopathy. *Acta Neuropathologica*, *126*(6), 809–827. <https://doi.org/10.1007/s00401-013-1188-4>
- Ballatore, C., Lee, V. M.-Y., & Trojanowski, J. Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nature Reviews. Neuroscience*, *8*(9), 663–672. <https://doi.org/10.1038/nrn2194>
- Bannwarth, S., Ait-El-Mkadem, S., Chaussonot, A., Genin, E. C., Lacas-Gervais, S., Fragaki, K., ... Paquis-Flucklinger, V. (2014a). A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. *Brain*, *137*(8), 2329–2345. <https://doi.org/10.1093/brain/awu138>
- Bannwarth, S., Ait-El-Mkadem, S., Chaussonot, A., Genin, E. C., Lacas-Gervais, S., Fragaki, K., ... Paquis-Flucklinger, V. (2014b). A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. *Brain*, *137*(8), 2329–2345. <https://doi.org/10.1093/brain/awu138>
- Barber, S. C., & Shaw, P. J. (2010a). Oxidative stress in ALS: Key role in motor neuron injury and therapeutic target. *Free Radical Biology and Medicine*, *48*(5), 629–641. <https://doi.org/https://doi.org/10.1016/j.freeradbiomed.2009.11.018>
- Barber, S. C., & Shaw, P. J. (2010b, March 1). Oxidative stress in ALS: Key role in motor neuron injury and therapeutic target. *Free Radical Biology and Medicine*, Vol. 48, pp. 629–641. <https://doi.org/10.1016/j.freeradbiomed.2009.11.018>
- Barmada, S. J., Skibinski, G., Korb, E., Rao, E. J., Wu, J. Y., & Finkbeiner, S. (2010). Cytoplasmic Mislocalization of TDP-43 Is Toxic to Neurons and Enhanced by a Mutation Associated with Familial Amyotrophic Lateral Sclerosis. *Journal of Neuroscience*, *30*(2), 639–649.

<https://doi.org/10.1523/JNEUROSCI.4988-09.2010>

- Baron, D. M., Kaushansky, L. J., Ward, C. L., Sama, R. R. K., Chian, R. J., Boggio, K. J., ... Bosco, D. A. (2013). Amyotrophic lateral sclerosis-linked FUS/TLS alters stress granule assembly and dynamics. *Molecular Neurodegeneration*, 8(1), 1–18. <https://doi.org/10.1186/1750-1326-8-30>
- Barschke, P., Oeckl, P., Steinacker, P., Ludolph, A., & Otto, M. (2017). Proteomic studies in the discovery of cerebrospinal fluid biomarkers for amyotrophic lateral sclerosis. *Expert Review of Proteomics*, 14(9), 769–777. <https://doi.org/10.1080/14789450.2017.1365602>
- Batista, P. J., & Chang, H. Y. (2013). Long noncoding RNAs: cellular address codes in development and disease. *Cell*, 152(6), 1298–1307. <https://doi.org/10.1016/j.cell.2013.02.012>
- Bäumer, D., Hilton, D., Paine, S. M. L., Turner, M. R., Lowe, J., Talbot, K., & Ansorge, O. (2010). Juvenile ALS with basophilic inclusions is a FUS proteinopathy with FUS mutations. *Neurology*, 75(7), 611–618. <https://doi.org/10.1212/WNL.0b013e3181ed9cde>
- Bayer, H., Lang, K., Buck, E., Higelin, J., Barteczko, L., Pasquarelli, N., ... Witting, A. (2017). ALS-causing mutations differentially affect PGC-1 α expression and function in the brain vs. peripheral tissues. *Neurobiology of Disease*, 97, 36–45. <https://doi.org/10.1016/j.nbd.2016.11.001>
- Beck, J., Poulter, M., Hensman, D., Rohrer, J. D., Mahoney, C. J., Adamson, G., ... Mead, S. (2013). Large C9orf72 Hexanucleotide Repeat Expansions Are Seen in Multiple Neurodegenerative Syndromes and Are More Frequent Than Expected in the UK Population. *The American Journal of Human Genetics*, 92(3), 345–353. <https://doi.org/https://doi.org/10.1016/j.ajhg.2013.01.011>
- Beddoe, T., & Lithgow, T. (2002, September 2). Delivery of nascent polypeptides to the mitochondrial surface. *Biochimica et Biophysica Acta - Molecular Cell Research*, Vol. 1592, pp. 35–39. [https://doi.org/10.1016/S0167-4889\(02\)00262-8](https://doi.org/10.1016/S0167-4889(02)00262-8)
- Beers, D. R., Henkel, J. S., Xiao, Q., Zhao, W., Wang, J., Yen, A. A., ... Appel, S. H. (2006). Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, 103(43), 16021–16026. <https://doi.org/10.1073/pnas.0607423103>
- Behra, M., Cousin, X., Bertrand, C., Vonesch, J.-L., Biellmann, D., Chatonnet, A., & Strähle, U. (2002). Acetylcholinesterase is required for neuronal and muscular development in the zebrafish embryo. *Nature Neuroscience*, 5(2), 111–118. <https://doi.org/10.1038/nn788>
- Behzadnia, N., Golas, M. M., Hartmuth, K., Sander, B., Kastner, B., Deckert, J., ... Lührmann, R. (2007). Composition and three-dimensional EM structure of double affinity-purified, human prespliceosomal A complexes. *EMBO Journal*, 26(6), 1737–1748. <https://doi.org/10.1038/sj.emboj.7601631>
- Belly, A., Moreau-Gachelin, F., Sadoul, R., & Goldberg, Y. (2005). Delocalization of the multifunctional RNA splicing factor TLS/FUS in hippocampal neurones: Exclusion from the nucleus and accumulation in dendritic granules and spine heads. *Neuroscience Letters*, 379(3), 152–157. <https://doi.org/10.1016/j.neulet.2004.12.071>
- Bertolotti, A., Lutz, Y., Heard, D. J., Chambon, P., & Tora, L. (1996a). hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. *The EMBO Journal*, 15(18), 5022–5031. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8890175>
- Bertolotti, A., Lutz, Y., Heard, D. J., Chambon, P., & Tora, L. (1996b). hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. *Embo Journal*, 15(18), 5022–5031. <https://doi.org/10.1002/j.1460-2075.1996.tb00882.x>

- Bertram, L., & Tanzi, R. E. (2005). The genetic epidemiology of neurodegenerative disease. *The Journal of Clinical Investigation*, *115*(6), 1449–1457. <https://doi.org/10.1172/JCI24761>
- Bill, B. R., Petzold, A. M., Clark, K. J., Schimmenti, L. A., & Ekker, S. C. (2009). A Primer for Morpholino Use in Zebrafish. *Zebrafish*, *6*(1), 69–77. <https://doi.org/10.1089/zeb.2008.0555>
- Bilsland, L. G., Sahai, E., Kelly, G., Golding, M., Greensmith, L., & Schiavo, G. (2010). Deficits in axonal transport precede ALS symptoms in vivo. *Proceedings of the National Academy of Sciences*, *107*(47), 20523 LP – 20528. <https://doi.org/10.1073/pnas.1006869107>
- Blechingberg, J., Luo, Y., Bolund, L., Damgaard, C. K., & Nielsen, A. L. (2012). Gene Expression Responses to FUS, EWS, and TAF15 Reduction and Stress Granule Sequestration Analyses Identifies FET-Protein Non-Redundant Functions. *PLoS ONE*, *7*(9). <https://doi.org/10.1371/journal.pone.0046251>
- Blokhuis, A. M., Koppers, M., Groen, E. J. N., van den Heuvel, D. M. A., Dini Modigliani, S., Anink, J. J., ... Pasterkamp, R. J. (2016). Comparative interactomics analysis of different ALS-associated proteins identifies converging molecular pathways. *Acta Neuropathologica*, *132*(2), 175–196. <https://doi.org/10.1007/s00401-016-1575-8>
- Bodea, L. G., Eckert, A., Ittner, L. M., Piguët, O., & Götz, J. (2016). Tau physiology and pathomechanisms in frontotemporal lobar degeneration. *Journal of Neurochemistry*, *138*, 71–94. <https://doi.org/10.1111/jnc.13600>
- Bogdanov, M., Brown, R. H., Matson, W., Smart, R., Hayden, D., O'Donnell, H., ... Cudkovicz, M. (2000). Increased oxidative damage to DNA in ALS patients. *Free Radical Biology and Medicine*, *29*(7), 652–658. [https://doi.org/10.1016/S0891-5849\(00\)00349-X](https://doi.org/10.1016/S0891-5849(00)00349-X)
- Boillée, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., ... Cleveland, D. W. (2006). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science*, *312*(5778), 1389–1392. <https://doi.org/10.1126/science.1123511>
- Bond, C. S., & Fox, A. H. (2009, September 7). Paraspeckles: Nuclear bodies built on long noncoding RNA. *Journal of Cell Biology*, Vol. 186, pp. 637–644. <https://doi.org/10.1083/jcb.200906113>
- Boore, J. L. (1999). Animal mitochondrial genomes. *Nucleic Acids Research*, *27*(8), 1767–1780. <https://doi.org/10.1093/nar/27.8.1767>
- Bosco, D. A., Lemay, N., Ko, H. K., Zhou, H., Burke, C., Kwiatkowski, T. J., ... Hayward, L. J. (2010). Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. *Human Molecular Genetics*, *19*(21), 4160–4175. <https://doi.org/10.1093/hmg/ddq335>
- Bosco, D. A., Morfini, G., Karabacak, N. M., Song, Y., Gros-Louis, F., Pasinelli, P., ... Brown, R. H. (2010). Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. *Nature Neuroscience*, *13*(11), 1396–1403. <https://doi.org/10.1038/nn.2660>
- Bose, J. K., Huang, C.-C., & Shen, C.-K. J. (2011). Regulation of autophagy by neuropathological protein TDP-43. *The Journal of Biological Chemistry*, *286*(52), 44441–44448. <https://doi.org/10.1074/jbc.M111.237115>
- Bostock, H., Cikurel, K., & Burke, D. (1998). Threshold tracking techniques in the study of human peripheral nerve. *Muscle & Nerve*, *21*(2), 137–158. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9466589>
- Bostock, H., Sharief, M. K., Reid, G., & Murray, N. M. F. (1995). Axonal ion channel dysfunction in amyotrophic lateral sclerosis. *Brain*, *118*(1), 217–225. <https://doi.org/10.1093/brain/118.1.217>
- Bozzo, F., Mirra, A., & Carrì, M. T. (2017). Oxidative stress and mitochondrial damage in the pathogenesis of ALS: New perspectives. *Neuroscience Letters*, *636*, 3–8. <https://doi.org/10.1016/j.neulet.2016.04.065>

- Braak, H., Brettschneider, J., Ludolph, A. C., Lee, V. M., Trojanowski, J. Q., & Tredici, K. Del. (2013). Amyotrophic lateral sclerosis - A model of corticofugal axonal spread. *Nature Reviews Neurology*, 9(12), 708–714. <https://doi.org/10.1038/nrneurol.2013.221>
- Braakman, I., Hebert, D. N., Spang, A., Mandon, E. C., Trueman, S. F., Schwartz, T., ... Miller, E. A. (2014). *Protein Folding in the Endoplasmic Reticulum Protein Folding in the Endoplasmic Reticulum*. <https://doi.org/10.1101/cshperspect.a013201>
- Bramblett, G. T., Goedert, M., Jakes, R., Merrick, S. E., Trojanowski, J. Q., & Lee, V. M. Y. (1993). Abnormal tau phosphorylation at Ser396 in alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron*, 10(6), 1089–1099. [https://doi.org/10.1016/0896-6273\(93\)90057-X](https://doi.org/10.1016/0896-6273(93)90057-X)
- Brandt, R. (1996, August). The tau proteins in neuronal growth and development. *Frontiers in Bioscience : A Journal and Virtual Library*, Vol. 1, pp. d118-30. <https://doi.org/10.2741/A120>
- Brandt, Roland, Lee, G., Teplow, D. B., Shalloway, D., & Abdel-Ghany, M. (1994). Differential effect of phosphorylation and substrate modulation on tau's ability to promote microtubule growth and nucleation. *Journal of Biological Chemistry*, 269(16), 11776–11782.
- Braun, K. (2000). FMRP Involvement in Formation of Synapses among Cultured Hippocampal Neurons. *Cerebral Cortex*, 10(10), 1045–1052. <https://doi.org/10.1093/cercor/10.10.1045>
- Brenner, D., Müller, K., Wieland, T., Weydt, P., Böhm, S., Lule, D., ... Weishaupt, J. H. (2016). NEK1 mutations in familial amyotrophic lateral sclerosis. *Brain*, 139(5), e28. <https://doi.org/10.1093/brain/aww033>
- Brenner, D., Yilmaz, R., Müller, K., Grehl, T., Petri, S., Meyer, T., ... Kassubek, J. (2018). Hot-spot KIF5A mutations cause familial ALS. *Brain*, 141(3), 688–697. <https://doi.org/10.1093/brain/awx370>
- Brites, D., & Vaz, A. R. (2014a). Microglia centered pathogenesis in ALS: insights in cell interconnectivity. *Frontiers in Cellular Neuroscience*, 8, 117. <https://doi.org/10.3389/fncel.2014.00117>
- Brites, D., & Vaz, A. R. (2014b, May 22). Microglia centered pathogenesis in ALS: Insights in cell interconnectivity. *Frontiers in Cellular Neuroscience*, Vol. 8. <https://doi.org/10.3389/fncel.2014.00117>
- Brockmann, S. J., Freischmidt, A., Oeckl, P., Müller, K., Srinivas, K., Helferich, A. M., ... Andersen, P. M. (2018). *CHCHD10* mutations p . R15L and p . G66V cause motoneuron disease by haploinsufficiency. 0(January), 1–10. <https://doi.org/10.1093/hmg/ddx436/4772769>
- Broihier, H. T., & Skeath, J. B. (2002). Drosophila homeodomain protein dHb9 directs neuronal fate via crossrepressive and cell-nonautonomous mechanisms. *Neuron*, 35(1), 39–50. [https://doi.org/10.1016/S0896-6273\(02\)00743-2](https://doi.org/10.1016/S0896-6273(02)00743-2)
- Brooks, B. R., Miller, R. G., Swash, M., & Munsat, T. L. (2000). El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis*, 1(5), 293–299. <https://doi.org/10.1080/146608200300079536>
- Brown, R. H., & Al-Chalabi, A. (2017). Amyotrophic Lateral Sclerosis. *New England Journal of Medicine*, 377(2), 162–172. <https://doi.org/10.1056/NEJMra1603471>
- Brown, T. A., Waring, R. B., Scazzocchio, C., & Davies, R. W. (1985). The *Aspergillus nidulans* mitochondrial genome. *Current Genetics*, 9(2), 113–117. <https://doi.org/10.1007/BF00436957>
- Brujin, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., ... Cleveland, D. W. (1997). ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron*, 18(2), 327–338.

Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9052802>

- Bruneteau, G., Simonet, T., Bauché, S., Mandjee, N., Malfatti, E., Girard, E., ... Hantai, D. (2013). Muscle histone deacetylase 4 upregulation in amyotrophic lateral sclerosis: Potential role in reinnervation ability and disease progression. *Brain*, *136*(8), 2359–2368. <https://doi.org/10.1093/brain/awt164>
- Buée, L., Bussièrè, T., Buée-Scherrer, V., Delacourte, A., & Hof, P. R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Research. Brain Research Reviews*, *33*(1), 95–130. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10967355>
- Burd, C., & Cullen, P. J. (2014). Retromer: A master conductor of endosome sorting. *Cold Spring Harbor Perspectives in Biology*, *6*(2). <https://doi.org/10.1101/cshperspect.a016774>
- Burk, K., & Pasterkamp, R. J. (2019). Disrupted neuronal trafficking in amyotrophic lateral sclerosis. *Acta Neuropathologica*, *137*(6), 859–877. <https://doi.org/10.1007/s00401-019-01964-7>
- Burrell, J. R., Kiernan, M. C., Vucic, S., & Hodges, J. R. (2011). Motor Neuron dysfunction in frontotemporal dementia. *Brain*, *134*(9), 2582–2594. <https://doi.org/10.1093/brain/awr195>
- Burstein, S., Valsecchi, F., Kawamata, H., Bourens, M., Zeng, R., Zuberi, A., ... Manfredi, G. (2017). In vitro and in vivo studies of the ALS-FTLD protein CHCHD10 reveal novel mitochondrial topology and protein interactions. *Human Molecular Genetics*, *0*(0), 1–18. <https://doi.org/10.1093/hmg/ddx397>
- Butovsky, O., Siddiqui, S., Gabriely, G., Lanser, A. J., Dake, B., Murugaiyan, G., ... Weiner, H. L. (2012). Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. *Journal of Clinical Investigation*, *122*(9), 3063–3087. <https://doi.org/10.1172/JCI62636>
- C Kiernan DSc, A. M., Cheah MBIostat, B. C., Burrell MBBS, J., Zoing BNurs, M. C., eld, N., to, C., ... Zoing, M. C. (2011). Amyotrophic lateral sclerosis. *The Lancet*, *377*(377), 942–955. [https://doi.org/10.1016/S0140-6736\(10\)61156-7](https://doi.org/10.1016/S0140-6736(10)61156-7)
- Calvio, C., Neubauer, G., Mann, M., & Lamond, A. I. (1995). Identification of hnRNP P2 as TLS/FUS using electrospray mass spectrometry. *RNA (New York, N.Y.)*, *1*(7), 724–733. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7585257>
- Campanari, M.-L., García-Ayllón, M.-S., Ciura, S., Sáez-Valero, J., & Kabashi, E. (2016). Neuromuscular Junction Impairment in Amyotrophic Lateral Sclerosis: Reassessing the Role of Acetylcholinesterase. *Frontiers in Molecular Neuroscience*, *9*(DEC2016). <https://doi.org/10.3389/fnmol.2016.00160>
- Capitano, D., Vasso, M., Ratti, A., Grignaschi, G., Volta, M., Moriggi, M., ... Gelfi, C. (2012). Molecular signatures of amyotrophic lateral sclerosis disease progression in hind and forelimb muscles of an SOD1(G93A) mouse model. *Antioxidants & Redox Signaling*, *17*(10), 1333–1350. <https://doi.org/10.1089/ars.2012.4524>
- Cappello, V., & Francolini, M. (2017). Neuromuscular junction dismantling in amyotrophic lateral sclerosis. *International Journal of Molecular Sciences*, *18*(10). <https://doi.org/10.3390/ijms18102092>
- Cappello, V., Vezzoli, E., Righi, M., Fossati, M., Mariotti, R., Crespi, A., ... Francolini, M. (2012). Analysis of neuromuscular junctions and effects of anabolic steroid administration in the SOD1G93A mouse model of ALS. *Molecular and Cellular Neurosciences*, *51*(1–2), 12–21. <https://doi.org/10.1016/j.mcn.2012.07.003>
- Carrì, M. T., Ferri, A., Battistoni, A., Famhy, L., Gabbianelli, R., Poccia, F., & Rotilio, G. (1997). Expression of a Cu,Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis induces mitochondrial alteration and increase of cytosolic Ca²⁺ concentration in transfected

- neuroblastoma SH-SY5Y cells. *FEBS Letters*, 414(2), 365–368. [https://doi.org/10.1016/S0014-5793\(97\)01051-X](https://doi.org/10.1016/S0014-5793(97)01051-X)
- Carrì, M. T., Valle, C., Bozzo, F., & Cozzolino, M. (2015). Oxidative stress and mitochondrial damage: Importance in non-SOD1 ALS. *Frontiers in Cellular Neuroscience*, 9(FEB), 1–6. <https://doi.org/10.3389/fncel.2015.00041>
- Cavallaro, G. (2010). Genome-wide analysis of eukaryotic twin CX9C proteins. *Molecular BioSystems*, 6(12), 2459–2470. <https://doi.org/10.1039/c0mb00058b>
- Chacinska, A., Guiard, B., Müller, J. M., Schulze-Specking, A., Gabriel, K., Kutik, S., & Pfanner, N. (2008). Mitochondrial biogenesis, switching the sorting pathway of the intermembrane space receptor Mia40. *Journal of Biological Chemistry*, 283(44), 29723–29729. <https://doi.org/10.1074/jbc.M805356200>
- Champagne, D. L., Hoefnagels, C. C. M., De Kloet, R. E., & Richardson, M. K. (2010). Translating rodent behavioral repertoire to zebrafish (*Danio rerio*): Relevance for stress research. *Behavioural Brain Research*, 214, 332–342. <https://doi.org/10.1016/j.bbr.2010.06.001>
- Chan, Y. A., Hieter, P., & Stirling, P. C. (2014). Mechanisms of genome instability induced by RNA-processing defects. *Trends in Genetics*, 30(6), 245–253. <https://doi.org/10.1016/j.tig.2014.03.005>
- Chang, L., & Monteiro, M. J. (2015). Defective proteasome delivery of polyubiquitinated proteins by ubiquilin-2 proteins containing ALS mutations. *PLoS ONE*, 10(6), 1–15. <https://doi.org/10.1371/journal.pone.0130162>
- Chang, Y., Kong, Q., Shan, X., Tian, G., Ilieva, H., Cleveland, D. W., ... Lin, C. L. G. (2008). Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. *PLoS ONE*, 3(8). <https://doi.org/10.1371/journal.pone.0002849>
- Chaudhury, A., Chander, P., & Howe, P. H. (2010). hnRNP function and KH domain bind to ssRNA (stabilize mRNA). *RNA (New York, N.Y.)*, 16(8), 1449–1462. <https://doi.org/10.1261/rna.2254110>
- Chen-Plotkin, A. S., Lee, V. M.-Y., & Trojanowski, J. Q. (2010). TAR DNA-binding protein 43 in neurodegenerative disease. *Nature Reviews Neurology*, 6, 211. Retrieved from <https://doi.org/10.1038/nrneurol.2010.18>
- Chen, C., Ding, X., Akram, N., Xue, S., & Luo, S.-Z. (2019). Fused in Sarcoma: Properties, Self-Assembly and Correlation with Neurodegenerative Diseases. *Molecules*, 24(8), 1622. <https://doi.org/10.3390/molecules24081622>
- Chen, M., Martins, R. N., & Lardelli, M. (2009). Complex splicing and neural expression of duplicated tau genes in zebrafish embryos. *Journal of Alzheimer's Disease*, 18(2), 305–317. <https://doi.org/10.3233/JAD-2009-1145>
- Chiò, A., Calvo, A., Bovio, G., Canosa, A., Bertuzzo, D., Galmozzi, F., ... Piemonte and Valle d'Aosta Register for Amyotrophic Lateral Sclerosis. (2014). Amyotrophic Lateral Sclerosis Outcome Measures and the Role of Albumin and Creatinine. *JAMA Neurology*, 71(9), 1134. <https://doi.org/10.1001/jamaneurol.2014.1129>
- Chiò, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., & Traynor, B. G. (2009). Prognostic factors in ALS: A critical review. *Amyotrophic Lateral Sclerosis*, 10(5–6), 310–323. <https://doi.org/10.3109/17482960802566824>
- Cho, J.-H., & Johnson, G. V. W. (2004). Primed phosphorylation of tau at Thr231 by glycogen synthase kinase 3beta (GSK3beta) plays a critical role in regulating tau's ability to bind and stabilize microtubules. *Journal of Neurochemistry*, 88(2), 349–358. <https://doi.org/10.1111/j.1471-4159.2004.02155.x>

- Cho, J. H., & Johnson, G. V. W. (2003). Glycogen synthase kinase 3 β phosphorylates tau at both primed and unprimed sites: Differential impact on microtubule binding. *Journal of Biological Chemistry*, 278(1), 187–193. <https://doi.org/10.1074/jbc.M206236200>
- Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., ... Goldstein, D. B. (2015). Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science*, 347(6229), 1436 LP – 1441. <https://doi.org/10.1126/science.aaa3650>
- Ciura, S., Sellier, C., Campanari, M.-L., Charlet-Berguerand, N., & Kabashi, E. (2016). The most prevalent genetic cause of ALS-FTD, C9orf72 synergizes the toxicity of ATXN2 intermediate polyglutamine repeats through the autophagy pathway. *Autophagy*, 12(8). <https://doi.org/10.1080/15548627.2016.1189070>
- Ciura, Sorana, Lattante, S., Le Ber, I., Latouche, M., Tostivint, H., Brice, A., & Kabashi, E. (2013). Loss of function of C9orf72 causes motor deficits in a zebrafish model of amyotrophic lateral sclerosis. *Annals of Neurology*, 74(2), 180–187. <https://doi.org/10.1002/ana.23946>
- Clark, J. A., Southam, K. A., Blizzard, C. A., King, A. E., & Dickson, T. C. (2016). Axonal degeneration, distal collateral branching and neuromuscular junction architecture alterations occur prior to symptom onset in the SOD1G93A mouse model of amyotrophic lateral sclerosis. *Journal of Chemical Neuroanatomy*, 76, 35–47. <https://doi.org/10.1016/j.jchemneu.2016.03.003>
- Codina, M., Li, J., Gutiérrez, J., Kao, J. P. Y., & Du, S. J. (2010). Loss of Smyhc1 or Hsp90 α 1 Function Results in Different Effects on Myofibril Organization in Skeletal Muscles of Zebrafish Embryos. *PLoS ONE*, 5(1), e8416. <https://doi.org/10.1371/journal.pone.0008416>
- Cogliati, S., Frezza, C., Soriano, M. E., Varanita, T., Quintana-Cabrera, R., Corrado, M., ... Scorrano, L. (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell*, 155(1), 160–171. <https://doi.org/10.1016/j.cell.2013.08.032>
- Cohen, T. J., Barrientos, T., Hartman, Z. C., Garvey, S. M., Cox, G. A., & Yao, T.-P. (2009). The deacetylase HDAC4 controls myocyte enhancing factor-2-dependent structural gene expression in response to neural activity. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 23(1), 99–106. <https://doi.org/10.1096/fj.08-115931>
- Cohen, T. J., Hwang, A. W., Restrepo, C. R., Yuan, C. X., Trojanowski, J. Q., & Lee, V. M. Y. (2015). An acetylation switch controls TDP-43 function and aggregation propensity. *Nature Communications*, 6. <https://doi.org/10.1038/ncomms6845>
- Cohen, T. J., Hwang, A. W., Unger, T., Trojanowski, J. Q., & Lee, V. M. Y. (2012). Redox signalling directly regulates TDP-43 via cysteine oxidation and disulphide cross-linking. *EMBO Journal*, 31(5), 1241–1252. <https://doi.org/10.1038/emboj.2011.471>
- Cohen, T. J., Waddell, D. S., Barrientos, T., Lu, Z., Feng, G., Cox, G. A., ... Yao, T.-P. (2007). The histone deacetylase HDAC4 connects neural activity to muscle transcriptional reprogramming. *The Journal of Biological Chemistry*, 282(46), 33752–33759. <https://doi.org/10.1074/jbc.M706268200>
- Colombrita, C., Onesto, E., Buratti, E., de la Grange, P., Gumina, V., Baralle, F. E., ... Ratti, A. (2015). From transcriptomic to protein level changes in TDP-43 and FUS loss-of-function cell models. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 1849(12), 1398–1410. <https://doi.org/10.1016/j.bbagr.2015.10.015>
- Colombrita, C., Onesto, E., Megiorni, F., Pizzuti, A., Baralle, F. E., Buratti, E., ... Ratti, A. (2012). TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells. *Journal of Biological Chemistry*, 287(19), 15635–15647. <https://doi.org/10.1074/jbc.M111.333450>
- Conlon, E. G., & Manley, J. L. (2017). RNA-binding proteins in neurodegeneration: mechanisms in

- aggregate. *Genes & Development*, 31(15), 1509–1528. <https://doi.org/10.1101/gad.304055.117>
- Copeland, W. C., & Longley, M. J. (2003). DNA polymerase gamma in mitochondrial DNA replication and repair. *TheScientificWorldJournal*, Vol. 3, pp. 34–44. <https://doi.org/10.1100/tsw.2003.09>
- Corcia, P., Danel, V., Lacour, A., Beltran, S., Andres, C., Couratier, P., ... Vourc'h, P. (2017). A novel mutation of the C-terminal amino acid of FUS (Y526C) strengthens FUS gene as the most frequent genetic factor in aggressive juvenile ALS. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 18(3–4), 298–301. <https://doi.org/10.1080/21678421.2016.1265564>
- Corcia, P., Tauber, C., Vercoullie, J., Arlicot, N., Prunier, C., Praline, J., ... Ribeiro, M.-J. (2012). Molecular imaging of microglial activation in amyotrophic lateral sclerosis. *PLoS One*, 7(12), e52941. <https://doi.org/10.1371/journal.pone.0052941>
- Couthouis, J., Hart, M. P., Erion, R., King, O. D., Diaz, Z., Nakaya, T., ... Gitler, A. D. (2012). Evaluating the role of the FUS/TLS-related gene EWSR1 in amyotrophic lateral sclerosis. *Human Molecular Genetics*, 21(13), 2899–2911. <https://doi.org/10.1093/hmg/dds116>
- Cozzolino, M., Rossi, S., Mirra, A., & Carrì, M. T. (2015). Mitochondrial dynamism and the pathogenesis of amyotrophic lateral sclerosis. *Frontiers in Cellular Neuroscience*, 9(February), 1–5. <https://doi.org/10.3389/fncel.2015.00031>
- Crozat, A., Åman, P., Mandahl, N., & Ron, D. (1993). Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. *Nature*, 363, 640. Retrieved from <http://dx.doi.org/10.1038/363640a0>
- Cruz, J. C., Tseng, H.-C., Goldman, J. A., Shih, H., & Tsai, L.-H. (2003). Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. *Neuron*, 40(3), 471–483. [https://doi.org/10.1016/s0896-6273\(03\)00627-5](https://doi.org/10.1016/s0896-6273(03)00627-5)
- Da Cruz, S., Parone, P. A., Lopes, V. S., Lillo, C., McAlonis-Downes, M., Lee, S. K., ... Cleveland, D. W. (2012). Elevated PGC-1 α activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS. *Cell Metabolism*, 15(5), 778–786. <https://doi.org/10.1016/j.cmet.2012.03.019>
- Dafinca, R., Scaber, J., Ababneh, N., Lalic, T., Weir, G., Christian, H., ... Talbot, K. (2016). C9orf72 Hexanucleotide Expansions Are Associated with Altered Endoplasmic Reticulum Calcium Homeostasis and Stress Granule Formation in Induced Pluripotent Stem Cell-Derived Neurons from Patients with Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Stem Cells*, 34(8), 2063–2078. <https://doi.org/10.1002/stem.2388>
- Daikoku, E., Saito, M., & Ono, F. (2015). Zebrafish mutants of the neuromuscular junction: swimming in the gene pool. *Journal of Physiological Sciences*, 65(3), 217–221. <https://doi.org/10.1007/s12576-015-0372-9>
- Dal Canto, M. C., & Gurney, M. E. (1994). Development of central nervous system pathology in a murine transgenic model of human amyotrophic lateral sclerosis. *The American Journal of Pathology*, 145(6), 1271–1279. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/7992831>
- Damiano, M., Starkov, A. A., Petri, S., Kipiani, K., Kiaei, M., Mattiazzi, M., ... Manfredi, G. (2006). Neural mitochondrial Ca²⁺ capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. *Journal of Neurochemistry*, 96(5), 1349–1361. <https://doi.org/10.1111/j.1471-4159.2006.03619.x>
- Darshi, M., Mendiola, V. L., Mackey, M. R., Murphy, A. N., Koller, A., Perkins, G. A., ... Taylor, S. S. (2011). ChChd3, an inner mitochondrial membrane protein, is essential for maintaining Crista integrity and mitochondrial function. *Journal of Biological Chemistry*, 286(4), 2918–2932. <https://doi.org/10.1074/jbc.M110.171975>

- Daube, J. R. (1985). Electrophysiologic studies in the diagnosis and prognosis of motor neuron diseases. *Neurologic Clinics*, 3(3), 473–493.
- Davenport, R. J., Swingler, R. J., Chancellor, A. M., & Warlow, C. P. (1996). Avoiding false positive diagnoses of motor neuron disease: lessons from the Scottish Motor Neuron Disease Register. In *Neurol Neurosurg Psychiatry* (Vol. 60).
- de Carvalho, M., & Swash, M. (2000). Nerve conduction studies in amyotrophic lateral sclerosis. *Muscle & Nerve*, 23(3), 344–352.
- De Carvalho, Mamede, & Swash, M. (2009). Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. *Amyotrophic Lateral Sclerosis*, 10(1), 53–57. <https://doi.org/10.1080/17482960802521126>
- De Santis, R., Santini, L., Colantoni, A., Peruzzi, G., de Turreis, V., Alfano, V., ... Rosa, A. (2017). FUS Mutant Human Motoneurons Display Altered Transcriptome and microRNA Pathways with Implications for ALS Pathogenesis. *Stem Cell Reports*, 9(5), 1450–1462. <https://doi.org/10.1016/j.stemcr.2017.09.004>
- De vos, K. J., Chapman, A. L., Tennant, M. E., Manser, C., Tudor, E. L., Lau, K. F., ... Grierson, A. J. (2007). Familial amyotrophic lateral sclerosis-linked SOD1 mutants perturb fast axonal transport to reduce axonal mitochondria content. *Human Molecular Genetics*, 16(22), 2720–2728. <https://doi.org/10.1093/hmg/ddm226>
- De vos, K. J., Mórotz, G. M., Stoica, R., Tudor, E. L., Lau, K. F., Ackerley, S., ... Miller, C. C. J. (2012). VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. *Human Molecular Genetics*, 21(6), 1299–1311. <https://doi.org/10.1093/hmg/ddr559>
- DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., ... Rademakers, R. (2011). Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron*, 72(2), 245–256. <https://doi.org/10.1016/j.neuron.2011.09.011>
- DeJesus-Hernandez, M., Rayaprolu, S., Soto-Ortolaza, A. I., Rutherford, N. J., Heckman, M. G., Traynor, S., ... Ross, O. A. (2013). Analysis of the C9orf72 repeat in Parkinson's disease, essential tremor and restless legs syndrome. *Parkinsonism & Related Disorders*, 19(2), 198–201. <https://doi.org/10.1016/j.parkreldis.2012.09.013>
- Demestre, M., Parkin-Smith, G., Petzold, A., & Pullen, A. H. (2005). The pro and the active form of matrix metalloproteinase-9 is increased in serum of patients with amyotrophic lateral sclerosis. *Journal of Neuroimmunology*, 159(1–2), 146–154. <https://doi.org/10.1016/j.jneuroim.2004.09.015>
- Deng, H., Gao, K., & Jankovic, J. (2014). The role of FUS gene variants in neurodegenerative diseases. *Nature Reviews Neurology*, 10(6), 337–348. <https://doi.org/10.1038/nrneurol.2014.78>
- Deng, H. X., Chen, W., Hong, S. T., Boycott, K. M., Gorrie, G. H., Siddique, N., ... Siddique, T. (2011). Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature*, 477(7363), 211–215. <https://doi.org/10.1038/nature10353>
- Deng, J., Yang, M., Chen, Y., Chen, X., Liu, J., Sun, S., ... Wu, J. Y. (2015). FUS Interacts with HSP60 to Promote Mitochondrial Damage. *PLoS Genetics*, 11(9), 1–30. <https://doi.org/10.1371/journal.pgen.1005357>
- Detrich, H. W., Westerfield, M., & Zon, L. I. (1999). Overview of the Zebrafish system. *Methods in Cell Biology*, 59, 3–10. [https://doi.org/10.1016/s0091-679x\(08\)61816-6](https://doi.org/10.1016/s0091-679x(08)61816-6)
- Devlin, A. C., Burr, K., Borooh, S., Foster, J. D., Cleary, E. M., Geti, I., ... Miles, G. B. (2015). Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. *Nature Communications*, 6.

<https://doi.org/10.1038/ncomms6999>

- Dewey, C. M., Cenik, B., Sephton, C. F., Dries, D. R., Mayer, P., Good, S. K., ... Yu, G. (2011). TDP-43 Is Directed to Stress Granules by Sorbitol, a Novel Physiological Osmotic and Oxidative Stressor. *Molecular and Cellular Biology*, *31*(5), 1098–1108. <https://doi.org/10.1128/mcb.01279-10>
- Di Giorgio, F. P., Boulting, G. L., Bobrowicz, S., & Eggan, K. C. (2008). Human Embryonic Stem Cell-Derived Motor Neurons Are Sensitive to the Toxic Effect of Glial Cells Carrying an ALS-Causing Mutation. *Cell Stem Cell*, *3*(6), 637–648. <https://doi.org/10.1016/j.stem.2008.09.017>
- Diaper, D. C., Adachi, Y., Sutcliffe, B., Humphrey, D. M., Elliott, C. J. H., Stepto, A., ... Hirth, F. (2013). Loss and gain of Drosophila TDP-43 impair synaptic efficacy and motor control leading to age-related neurodegeneration by loss-of-function phenotypes. *Human Molecular Genetics*, *22*(8), 1539–1557. <https://doi.org/10.1093/hmg/ddt005>
- Dichmann, D. S., & Harland, R. M. (2012). Fus/TLS orchestrates splicing of developmental regulators during gastrulation. *Genes and Development*, *26*(12), 1351–1363. <https://doi.org/10.1101/gad.187278.112>
- Dini Modigliani, S., Morlando, M., Errichelli, L., Sabatelli, M., & Bozzoni, I. (2014). An ALS-associated mutation in the FUS 3'-UTR disrupts a microRNA-FUS regulatory circuitry. *Nature Communications*, *5*, 4335. <https://doi.org/10.1038/ncomms5335>
- Dobbin, M. M., Madabhushi, R., Pan, L., Chen, Y., Kim, D., Gao, J., ... Tsai, L. H. (2013). SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. *Nature Neuroscience*, *16*(8), 1008–1015. <https://doi.org/10.1038/nn.3460>
- Dobrowolny, G., Aucello, M., Molinaro, M., & Musarò, A. (2008). Local expression of mIgf-1 modulates ubiquitin, caspase and CDK5 expression in skeletal muscle of an ALS mouse model. *Neurological Research*, *30*(2), 131–136. <https://doi.org/10.1179/174313208X281235>
- Dormann, D., & Haass, C. (2011, July). TDP-43 and FUS: A nuclear affair. *Trends in Neurosciences*, Vol. 34, pp. 339–348. <https://doi.org/10.1016/j.tins.2011.05.002>
- Dormann, D., Madl, T., Valori, C. F., Bentmann, E., Tahirovic, S., Abou-Ajram, C., ... Haass, C. (2012). Arginine methylation next to the PY-NLS modulates Transportin binding and nuclear import of FUS. *EMBO Journal*, *31*(22), 4258–4275. <https://doi.org/10.1038/emboj.2012.261>
- Dormann, D., Rodde, R., Edbauer, D., Bentmann, E., Fischer, I., Hruscha, A., ... Haass, C. (2010). ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import. *The EMBO Journal*, *29*(16), 2841–2857. <https://doi.org/10.1038/emboj.2010.143>
- Dorst, J., Kühnlein, P., Hendrich, C., Kassubek, J., Sperfeld, A. D., & Ludolph, A. C. (2011). Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. *Journal of Neurology*, *258*(4), 613–617. <https://doi.org/10.1007/s00415-010-5805-z>
- Dou, Y., Andersson-Lendahl, M., & Arner, A. (2008). Structure and function of skeletal muscle in zebrafish early larvae. *Journal of General Physiology*, *131*(5), 445–453. <https://doi.org/10.1085/jgp.200809982>
- DP, B. (2004). MicroRNAs : Genomics, Biogenesis, Mechanism, and Function.pdf. *Cell*, *116*(2), 281–297. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/14744438>
- Drapeau, P., Saint-Amant, L., Buss, R. R., Chong, M., McDearmid, J. R., & Brustein, E. (2002). Development of the locomotor network in zebrafish. *Progress in Neurobiology*, *68*(2), 85–111. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12450489>
- Dreyfuss, G., Kim, V. N., & Kataoka, N. (2002). Messenger-RNA-binding proteins and the messages

- they carry. *Nature Reviews Molecular Cell Biology*, 3(3), 195–205.
<https://doi.org/10.1038/nrm760>
- Droppelmann, C. A., Campos-Melo, D., Ishtiaq, M., Volkening, K., & Strong, M. J. (2014). RNA metabolism in ALS: When normal processes become pathological. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 15(5–6), 321–336.
<https://doi.org/10.3109/21678421.2014.881377>
- Drubin, D. G., Caput, D., & Kirschner, M. W. (1984). Studies on the expression of the microtubule-associated protein, tau, during mouse brain development, with newly isolated complementary DNA probes. *Journal of Cell Biology*, 98(3), 1090–1097. <https://doi.org/10.1083/jcb.98.3.1090>
- Duleep, A., & Shefner, J. (2013). *Electrodiagnosis of Motor Neuron Disease*.
<https://doi.org/10.1016/j.pmr.2012.08.022>
- Dunlop, J., Beal McIlvain, H., She, Y., & Howland, D. S. (2003). Impaired spinal cord glutamate transport capacity and reduced sensitivity to riluzole in a transgenic superoxide dismutase mutant rat model of amyotrophic lateral sclerosis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 23(5), 1688–1696. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/12629173>
- Dupuis, L., Corcia, P., Fergani, A., Gonzalez De Aguilar, J.-L., Bonnefont-Rousselot, D., Bittar, R., ... Meininger, V. (2008). Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. *Neurology*, 70(13), 1004–1009. <https://doi.org/10.1212/01.wnl.0000285080.70324.27>
- Dupuis, Luc, & Loeffler, J.-P. (2009). Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models. *Current Opinion in Pharmacology*, 9, 341–346.
<https://doi.org/10.1016/j.coph.2009.03.007>
- Echaniz-Laguna, A., Degos, B., Mohr, M., Kessler, R., Urban-Kraemer, E., & Tranchant, C. (2006). A study of three patients with amyotrophic lateral sclerosis and a polyneuropathy resembling CIDP. *Muscle & Nerve*, 33(3), 356–362. <https://doi.org/10.1002/mus.20475>
- Ederle, H., & Dormann, D. (2017). TDP-43 and FUS en route from the nucleus to the cytoplasm. *FEBS Letters*, 591(11), 1489–1507. <https://doi.org/10.1002/1873-3468.12646>
- Ekinci, F. J., & Shea, T. B. (1999). Hyperactivation of mitogen-activated protein kinase increases phospho-tau immunoreactivity within human neuroblastoma: additive and synergistic influence of alteration of additional kinase activities. *Cellular and Molecular Neurobiology*, 19(2), 249–260. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10081608>
- Ellis, D. Z., Rabe, J., & Sweadner, K. J. (2003). Global Loss of Na,K-ATPase and Its Nitric Oxide-Mediated Regulation in a Transgenic Mouse Model of Amyotrophic Lateral Sclerosis. *The Journal of Neuroscience*, 23(1), 43 LP – 51. <https://doi.org/10.1523/JNEUROSCI.23-01-00043.2003>
- Ernster, L., & Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *BBA - Molecular Basis of Disease*, 1271(1), 195–204. [https://doi.org/10.1016/0925-4439\(95\)00028-3](https://doi.org/10.1016/0925-4439(95)00028-3)
- Feiguin, F., Godena, V. K., Romano, G., D'Ambrogio, A., Klima, R., & Baralle, F. E. (2009). Depletion of TDP-43 affects Drosophila motoneurons terminal synapsis and locomotive behavior. *FEBS Letters*, 583(10), 1586–1592. <https://doi.org/10.1016/j.febslet.2009.04.019>
- Fernandez, H. L., & Inestrosa, N. C. (1976). Role of axoplasmic transport in neurotrophic regulation of muscle end plate acetylcholinesterase. *Nature*, 262(5563), 55–56.
<https://doi.org/10.1038/262055a0>
- Ferrari, R., Hernandez, D. G., Nalls, M. A., Rohrer, J. D., Ramasamy, A., Kwok, J. B. J., ... Momeni, P. (2014). Frontotemporal dementia and its subtypes: A genome-wide association study. *The*

Lancet Neurology, 13(7), 686–699. [https://doi.org/10.1016/S1474-4422\(14\)70065-1](https://doi.org/10.1016/S1474-4422(14)70065-1)

- Ferrer, I., Blanco, R., Carmona, M., Ribera, R., Goutan, E., Puig, B., ... Ribalta, T. (2001). Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy an. *Brain Pathology (Zurich, Switzerland)*, 11(2), 144–158.
- Ferrer, I., Gomez-Isla, T., Puig, B., Freixes, M., Ribé, E., Dalfo, E., & Avila, J. (2005). Current advances on different kinases involved in tau phosphorylation, and implications in Alzheimer's disease and tauopathies. *Current Alzheimer Research*, 2(1), 3–18.
- Ferrer, Isidre, Legati, A., García-Monco, J. C., Gomez-Beldarrain, M., Carmona, M., Blanco, R., ... Coppola, G. (2015). Familial Behavioral Variant Frontotemporal Dementia Associated With Astrocyte-Predominant Tauopathy. *Journal of Neuropathology & Experimental Neurology*, 74(4), 370–379. <https://doi.org/10.1097/NEN.0000000000000180>
- Ferri, A., Cozzolino, M., Crosio, C., Nencini, M., Casciati, A., Gralla, E. B., ... Carri, M. T. (2006). Familial ALS-superoxide dismutases associate with mitochondria and shift their redox potentials. *Proceedings of the National Academy of Sciences of the United States of America*, 103(37), 13860–13865. <https://doi.org/10.1073/pnas.0605814103>
- Ferri, A., Fiorenza, P., Nencini, M., Cozzolino, M., Pesaresi, M. G., Valle, C., ... Carri, M. T. (2010). Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. *Human Molecular Genetics*, 19(22), 4529–4542. <https://doi.org/10.1093/hmg/ddq383>
- Festoff, B. W., & Fernandez, H. L. (1981). Plasma and red blood cell acetylcholinesterase in amyotrophic lateral sclerosis. *Muscle and Nerve*, 4(1), 41–47. <https://doi.org/10.1002/mus.880040108>
- Filippi, M., Agosta, F., Abrahams, S., Fazekas, F., Grosskreutz, J., Kalra, S., ... European Federation of Neurological Societies. (2010). EFNS guidelines on the use of neuroimaging in the management of motor neuron diseases. *European Journal of Neurology*, 17(4), 526–e20. <https://doi.org/10.1111/j.1468-1331.2010.02951.x>
- Finelli, M. J., Liu, K. X., Wu, Y., Oliver, P. L., & Davies, K. E. (2015). Oxr1 improves pathogenic cellular features of ALS-associated FUS and TDP-43 mutations. *Human Molecular Genetics*, 24(12), 3529–3544. <https://doi.org/10.1093/hmg/ddv104>
- Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., ... Glass, J. D. (2004a). Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Experimental Neurology*, 185(2), 232–240. <https://doi.org/10.1016/j.expneurol.2003.10.004>
- Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., ... Glass, J. D. (2004b). Amyotrophic lateral sclerosis is a distal axonopathy: Evidence in mice and man. *Experimental Neurology*, 185(2), 232–240. <https://doi.org/10.1016/j.expneurol.2003.10.004>
- Flanagan-Steet, H., Fox, M. A., Meyer, D., & Sanes, J. R. (2005). Neuromuscular synapses can form in vivo by incorporation of initially aneural postsynaptic specializations. *Development*, 132(20), 4471–4481. <https://doi.org/10.1242/dev.02044>
- Forsberg, K., Andersen, P. M., Marklund, S. L., & Brännström, T. (2011). Glial nuclear aggregates of superoxide dismutase-1 are regularly present in patients with amyotrophic lateral sclerosis. *Acta Neuropathologica*, 121(5), 623–634. <https://doi.org/10.1007/s00401-011-0805-3>
- Forsberg, K., Jonsson, P. A., Andersen, P. M., Bergemalm, D., Graffmo, K. S., Hultdin, M., ... Brännström, T. (2010). Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. *PLoS ONE*, 5(7). <https://doi.org/10.1371/journal.pone.0011552>
- Freibaum, B. D., Chitta, R. K., High, A. A., & Taylor, J. P. (2010). Global analysis of TDP-43

- interacting proteins reveals strong association with RNA splicing and translation machinery. *Journal of Proteome Research*, 9(2), 1104–1120. <https://doi.org/10.1021/pr901076y>
- Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K., ... Weishaupt, J. H. (2015). Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nature Neuroscience*, 18(5), 631–636. <https://doi.org/10.1038/nn.4000>
- Frickenhaus, M., Wagner, M., Mallik, M., Catinozzi, M., & Storkebaum, E. (2015). Highly efficient cell-type-specific gene inactivation reveals a key function for the Drosophila FUS homolog cabeza in neurons. *Scientific Reports*, 5, 1–10. <https://doi.org/10.1038/srep09107>
- Friedman, J. R., Mourier, A., Yamada, J., Michael McCaffery, J., & Nunnari, J. (2015). MICOS coordinates with respiratory complexes and lipids to establish mitochondrial inner membrane architecture. *ELife*, 2015(4), 1–61. <https://doi.org/10.7554/eLife.07739>
- Fritz, E., Izaurieta, P., Weiss, A., Mir, F. R., Rojas, P., Gonzalez, D., ... van Zundert, B. (2013). Mutant SOD1-expressing astrocytes release toxic factors that trigger motoneuron death by inducing hyperexcitability. *Journal of Neurophysiology*, 109(11), 2803–2814. <https://doi.org/10.1152/jn.00500.2012>
- Fujii, R., Okabe, S., Urushido, T., Inoue, K., Yoshimura, A., Tachibana, T., ... Takumi, T. (2005). The RNA binding protein TLS is translocated to dendritic spines by mGluR5 activation and regulates spine morphology. *Current Biology : CB*, 15(6), 587–593. <https://doi.org/10.1016/j.cub.2005.01.058>
- Fujii, R., & Takumi, T. (2005). TLS facilitates transport of mRNA encoding an actin-stabilizing protein to dendritic spines. *Journal of Cell Science*, 118(Pt 24), 5755–5765. <https://doi.org/10.1242/jcs.02692>
- Funayama, M., Ohe, K., Amo, T., Furuya, N., Yamaguchi, J., Saiki, S., ... Hattori, N. (2015). CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: A genome-wide linkage and sequencing study. *The Lancet Neurology*, 14(3), 274–282. [https://doi.org/10.1016/S1474-4422\(14\)70266-2](https://doi.org/10.1016/S1474-4422(14)70266-2)
- Gal, J., Zhang, J., Kwinter, D. M., Zhai, J., Jia, H., Jia, J., & Zhu, H. (2011). Nuclear localization sequence of FUS and induction of stress granules by ALS mutants. *Neurobiology of Aging*, 32(12), 2323.e27–2323.e40. <https://doi.org/https://doi.org/10.1016/j.neurobiolaging.2010.06.010>
- Gandhi, S., & Abramov, A. Y. (2012). Mechanism of oxidative stress in neurodegeneration. *Oxidative Medicine and Cellular Longevity*. <https://doi.org/10.1155/2012/428010>
- Gao, J., Wang, L., Huntley, M. L., Perry, G., & Wang, X. (2018). Pathomechanisms of TDP-43 in neurodegeneration. *Journal of Neurochemistry*, 146(1), 7–20. <https://doi.org/10.1111/jnc.14327>
- García-Ayllón, M. S., Millán, C., Serra-Basante, C., Bataller, R., & Sáez-Valero, J. (2012). Readthrough Acetylcholinesterase Is Increased in Human Liver Cirrhosis. *PLoS ONE*, 7(9). <https://doi.org/10.1371/journal.pone.0044598>
- García-Ayllón, M. S., Riba-Llena, I., Serra-Basante, C., Alom, J., Boopathy, R., & Sáez-Valero, J. (2010). Altered levels of acetylcholinesterase in Alzheimer plasma. *PLoS ONE*, 5(1). <https://doi.org/10.1371/journal.pone.0008701>
- Gardiner, M., Toth, R., Vandermoere, F., Morrice, N. A., & Rouse, J. (2008). Identification and characterization of FUS/TLS as a new target of ATM. *Biochemical Journal*, 415(2), 297–307. <https://doi.org/10.1042/BJ20081135>
- Gargiulo, S., Anzilotti, S., Coda, A. R. D., Gramanzini, M., Greco, A., Panico, M., ... Pappatà, S. (2016). Imaging of brain TSPO expression in a mouse model of amyotrophic lateral sclerosis with 18F-DPA-714 and micro-PET/CT. *European Journal of Nuclear Medicine and Molecular Imaging*, 43(7), 1348–1359. <https://doi.org/10.1007/s00259-016-3311-y>

- Garrido, N., Griparic, L., Jokitalo, E., Wartiovaara, J., van der Blik, A. M., & Spelbrink, J. N. (2003). Composition and dynamics of human mitochondrial nucleoids. *Molecular Biology of the Cell*, *14*(4), 1583–1596. <https://doi.org/10.1091/mbc.e02-07-0399>
- Geevasinga, N., Menon, P., Howells, J., Nicholson, G. A., Kiernan, M. C., & Vucic, S. (2015). Axonal Ion Channel Dysfunction in C9orf72 Familial Amyotrophic Lateral Sclerosis Axonal Ion Channel in Amyotrophic Lateral Sclerosis. *JAMA Neurology*, *72*(1), 49–57. <https://doi.org/10.1001/jamaneurol.2014.2940>
- Genga, A., Bianchi, L., & Foury, F. (1986). A nuclear mutant of *Saccharomyces cerevisiae* deficient in mitochondrial DNA replication and polymerase activity. *The Journal of Biological Chemistry*, *261*(20), 9328–9332. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/3522588>
- Genin, E. C., Madji Hounoum, B., Bannwarth, S., Fragaki, K., Lacas-Gervais, S., Mauri-Crouzet, A., ... Paquis-Flucklinger, V. (2019). Mitochondrial defect in muscle precedes neuromuscular junction degeneration and motor neuron death in CHCHD10S59L/+ mouse. *Acta Neuropathologica*, *10*(0123456789), 1–23. <https://doi.org/10.1007/s00401-019-01988-z>
- Genin, E. C., Plutino, M., Bannwarth, S., Villa, E., Cisneros-Barroso, E., Roy, M., ... Paquis-Flucklinger, V. (2016). CHCHD10 mutations promote loss of mitochondrial cristae junctions with impaired mitochondrial genome maintenance and inhibition of apoptosis. *EMBO Molecular Medicine*, *8*(1), 58–72. <https://doi.org/10.15252/emmm.201505496>
- Genin, E. C., Plutino, M., Bannwarth, S., Villa, E., Cisneros-Barroso, E., Roy, M., ... Paquis-Flucklinger, V. (2016). CHCHD 10 mutations promote loss of mitochondrial cristae junctions with impaired mitochondrial genome maintenance and inhibition of apoptosis. *EMBO Molecular Medicine*, *8*(1), 58–72. <https://doi.org/10.15252/emmm.201505496>
- Gerbino, V., Carrì, M. T., Cozzolino, M., & Achsel, T. (2013). Mislocalised FUS mutants stall spliceosomal snRNPs in the cytoplasm. *Neurobiology of Disease*, *55*, 120–128. <https://doi.org/10.1016/j.nbd.2013.03.003>
- Gerlai, R., Lahav, M., Guo, S., & Rosenthal, A. (2000). Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacology Biochemistry and Behavior*, *67*(4), 773–782. [https://doi.org/10.1016/S0091-3057\(00\)00422-6](https://doi.org/10.1016/S0091-3057(00)00422-6)
- Ghasemi, M. (2016). Iranian Journal of Neurology © 2016 Amyotrophic lateral sclerosis mimic syndromes. In *Iran J Neurol* (Vol. 15).
- Gitler, A. D., Dhillon, P., & Shorter, J. (2017). Neurodegenerative disease: Models, mechanisms, and a new hope. *DMM Disease Models and Mechanisms*, *10*(5), 499–502. <https://doi.org/10.1242/dmm.030205>
- Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., & Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(11), 4051–4055. <https://doi.org/10.1073/pnas.85.11.4051>
- Goedert, Michel, & Spillantini, M. G. (2011). Pathogenesis of the tauopathies. *Journal of Molecular Neuroscience*, *45*(3), 425–431. <https://doi.org/10.1007/s12031-011-9593-4>
- Goode, A., Butler, K., Long, J., Cavey, J., Scott, D., Shaw, B., ... Layfield, R. (2016). Defective recognition of LC3B by mutant SQSTM1/p62 implicates impairment of autophagy as a pathogenic mechanism in ALS-FTLD. *Autophagy*, *12*(7), 1094–1104. <https://doi.org/10.1080/15548627.2016.1170257>
- Götz, J., Halliday, G., & Nisbet, R. M. (2019). Molecular Pathogenesis of the Tauopathies. *Annual Review of Pathology: Mechanisms of Disease*, *14*(1), 239–261. <https://doi.org/10.1146/annurev-pathmechdis-012418-012936>

- Gospillou, G., & Hepple, R. T. (2016, October 6). Editorial: Mitochondria in Skeletal muscle health, aging and diseases. *Frontiers in Physiology*, 7(OCT). <https://doi.org/10.3389/fphys.2016.00446>
- Gregory, R. I., Yan, K.-P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., & Shiekhattar, R. (2004). The Microprocessor complex mediates the genesis of microRNAs. *Nature*, 432(7014), 235–240. <https://doi.org/10.1038/nature03120>
- Groen, E. J. N., Fumoto, K., Blokhuis, A. M., Engelen-Lee, J. Y., Zhou, Y., van den Heuvel, D. M. A., ... Pasterkamp, R. J. (2013). ALS-associated mutations in FUS disrupt the axonal distribution and function of SMN. *Human Molecular Genetics*, 22(18), 3690–3704. <https://doi.org/10.1093/hmg/ddt222>
- Gros-Louis, F., Larivière, R., Gowing, G., Laurent, S., Camu, W., Bouchard, J. P., ... Julien, J. P. (2004). A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis. *Journal of Biological Chemistry*, 279(44), 45951–45956. <https://doi.org/10.1074/jbc.M408139200>
- Grundke-iqbal, I., Iqbal, K., Tung, Y., Quinlan, M., Wisniewski, H. M., & Bindert, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (*tau*) in Alzheimer cytoskeletal pathology. 83(July), 4913–4917. <https://doi.org/10.1073/pnas.83.13.4913>
- Gu, Y., & Hall, Z. W. (1988). Characterization of acetylcholine receptor subunits in developing and in denervated mammalian muscle. *Journal of Biological Chemistry*, 263(26), 12878–12885.
- Guerrero, E. N., Wang, H., Mitra, J., Hegde, P. M., Stowell, S. E., Liachko, N. F., ... Hegde, M. L. (2016). TDP-43/FUS in motor neuron disease: Complexity and challenges. *Progress in Neurobiology*. <https://doi.org/10.1016/j.pneurobio.2016.09.004>
- Guise, S., Braguer, D., Carles, G., Delacourte, A., & Briand, C. (2001). Hyperphosphorylation of tau is mediated by ERK activation during anticancer drug-induced apoptosis in neuroblastoma cells. *Journal of Neuroscience Research*, 63(3), 257–267. [https://doi.org/10.1002/1097-4547\(20010201\)63:3<257::AID-JNR1019>3.0.CO;2-T](https://doi.org/10.1002/1097-4547(20010201)63:3<257::AID-JNR1019>3.0.CO;2-T)
- Guo, T., Noble, W., & Hanger, D. P. (2017). Roles of tau protein in health and disease. *Acta Neuropathologica*, 133(5), 665–704. <https://doi.org/10.1007/s00401-017-1707-9>
- Guo, W., Naujock, M., Fumagalli, L., Vandoorne, T., Baatsen, P., Boon, R., ... Van Den Bosch, L. (2017). HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. *Nature Communications*, 8(1), 1–14. <https://doi.org/10.1038/s41467-017-00911-y>
- Gurney, M. E., Liu, R., Althaus, J. S., Hall, E. D., & Decker, D. A. (1998). *MUTANT CU,ZN SUPEROXIDE DISMUTASE IN MOTOR NEURON DISEASE*. 21, 85–89.
- Haidet-Phillips, A. M., Hester, M. E., Miranda, C. J., Meyer, K., Braun, L., Frakes, A., ... Kaspar, B. K. (2011). Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Nature Biotechnology*, 29(9), 824–828. <https://doi.org/10.1038/nbt.1957>
- Halliday, G., Bigio, E. H., Cairns, N. J., Neumann, M., MacKenzie, I. R. A., & Mann, D. M. A. (2012). Mechanisms of disease in frontotemporal lobar degeneration: Gain of function versus loss of function effects. *Acta Neuropathologica*, 124(3), 373–382. <https://doi.org/10.1007/s00401-012-1030-4>
- Halter, B., Gonzalez de Aguilar, J.-L., Rene, F., Petri, S., Fricker, B., Echaniz-Laguna, A., ... Loeffler, J.-P. (2010). Oxidative stress in skeletal muscle stimulates early expression of Rad in a mouse model of amyotrophic lateral sclerosis. *Free Radical Biology & Medicine*, 48(7), 915–923. <https://doi.org/10.1016/j.freeradbiomed.2010.01.014>
- Hamm, M., Bailey, R., Shaw, G., Yen, S. H., Lewis, J., & Giasson, B. I. (2015). Physiologically relevant factors influence tau phosphorylation by leucine-rich repeat kinase 2. *Journal of Neuroscience Research*, 93(10), 1567–1580. <https://doi.org/10.1002/jnr.23614>

- Han, Q., Lu, J., Duan, J., Su, D., Hou, X., Li, F., ... Huang, B. (2008). Gcn5- and Elp3-induced histone H3 acetylation regulates hsp70 gene transcription in yeast. *Biochemical Journal*, 409(3), 779–788. <https://doi.org/10.1042/bj20070578>
- Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E. M., Logroscino, G., Robberecht, W., ... van den Berg, L. H. (2017). Amyotrophic lateral sclerosis. *Nature Reviews Disease Primers*, 3(17071). <https://doi.org/10.1016/bs.pmch.2018.12.001>
- Hardiman, O., Al-Chalabi, A., Chio, A., Corr, E. M., Logroscino, G., Robberecht, W., ... Van Den Berg, L. H. (2017). Amyotrophic lateral sclerosis. *Nature Reviews Disease Primers*, 3. <https://doi.org/10.1038/nrdp.2017.71>
- Hardiman, O., & van den Berg, L. H. (2017). Edaravone: a new treatment for ALS on the horizon? *The Lancet Neurology*, 16(7), 490–491. [https://doi.org/https://doi.org/10.1016/S1474-4422\(17\)30163-1](https://doi.org/https://doi.org/10.1016/S1474-4422(17)30163-1)
- Harms, M., Benitez, B. A., Cairns, N., Cooper, B., Cooper, P., Mayo, K., ... NIA-LOAD/NCRAD Family Study Consortium, for the. (2013). C9orf72 Hexanucleotide Repeat Expansions in Clinical Alzheimer Disease C9orf72 Hexanucleotide Repeat Expansions in AD. *JAMA Neurology*, 70(6), 736–741. <https://doi.org/10.1001/2013.jamaneurol.537>
- Hasegawa, M., Arai, T., Nonaka, T., Kametani, F., Yoshida, M., Hashizume, Y., ... Akiyama, H. (2008). 91/Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Annals of Neurology*, 64(1), 60–70. <https://doi.org/10.1002/ana.21425>
- Haverkamp, L. J., Appel, V., & Appel, S. H. (1995). Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. *Brain : A Journal of Neurology*, 118 (Pt 3, 707–719.
- Heemels, M.-T. (2016). Neurodegenerative diseases. *Nature*, 539, 179. Retrieved from <https://doi.org/10.1038/539179a>
- Hensley, K., Abdel-Moaty, H., Hunter, J., Mhatre, M., Mhou, S., Nguyen, K., ... West, M. (2006). Primary glia expressing the G93A-SOD1 mutation present a neuroinflammatory phenotype and provide a cellular system for studies of glial inflammation. *Journal of Neuroinflammation*, 3. <https://doi.org/10.1186/1742-2094-3-2>
- Hernández, F., Lucas, J. J., Cuadros, R., & Avila, J. (2003). GSK-3 dependent phosphoepitopes recognized by PHF-1 and AT-8 antibodies are present in different tau isoforms. *Neurobiology of Aging*, 24(8), 1087–1094.
- Herrmann, J. M., & Riemer, J. (2014, July 20). Three approaches to one problem: Protein folding in the periplasm, the endoplasmic reticulum, and the intermembrane space. *Antioxidants and Redox Signaling*, Vol. 21, pp. 438–456. <https://doi.org/10.1089/ars.2014.5841>
- Hicks, G G, Singh, N., Nashabi, A., Mai, S., Bozek, G., Klewes, L., ... Ruley, H. E. (2000). Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. *Nature Genetics*, 24(2), 175–179. <https://doi.org/10.1038/72842>
- Hicks, Geoffrey G., Singh, N., Nashabi, A., Mai, S., Bozek, G., Klewes, L., ... Ruley, H. E. (2000). Fus deficiency in mice results in defective B-lymphocyte development and activation, high levels of chromosomal instability and perinatal death. *Nat Genet.*, 24(2), 175–179. [https://doi.org/10.1016/0141-9331\(88\)90141-X](https://doi.org/10.1016/0141-9331(88)90141-X)
- Higgins, C., Jung, C., Ding, H., & Xu, Z. (2002). Mutant Cu, Zn superoxide dismutase that causes motoneuron degeneration is present in mitochondria in the CNS. *Open Access Articles*. Retrieved from <https://escholarship.umassmed.edu/oapubs/1175>
- Higgins, C. M. J., Jung, C., & Xu, Z. (2003). ALS-associated mutant SODIG93A causes

- mitochondrial vacuolation by expansion of the intermembrane space by involvement of SOD1 aggregation and peroxisomes. *BMC Neuroscience*, 4. <https://doi.org/10.1186/1471-2202-4-16>
- Hisano, Y., Ota, S., & Kawahara, A. (2014). Genome editing using artificial site-specific nucleases in zebrafish. *Development, Growth & Differentiation*, 56(1), 26–33. <https://doi.org/10.1111/dgd.12094>
- Hjerpe, R., Bett, J. S., Keuss, M. J., Solovyova, A., McWilliams, T. G., Johnson, C., ... Kurz, T. (2016). UBQLN2 Mediates Autophagy-Independent Protein Aggregate Clearance by the Proteasome. *Cell*, 166(4), 935–949. <https://doi.org/10.1016/j.cell.2016.07.001>
- Hoell, J. I., Larsson, E., Runge, S., Nusbaum, J. D., Duggimpudi, S., Farazi, T. A., ... Tuschl, T. (2011). RNA targets of wild-type and mutant FET family proteins. *Nature Structural and Molecular Biology*, 18(12), 1428–1431. <https://doi.org/10.1038/nsmb.2163>
- Hofmann, S., Rothbauer, U., Mühlenbein, N., Baiker, K., Hell, K., & Bauer, M. F. (2005). Functional and mutational characterization of human MIA40 acting during import into the mitochondrial intermembrane space. *Journal of Molecular Biology*, 353(3), 517–528. <https://doi.org/10.1016/j.jmb.2005.08.064>
- Honda, D., Ishigaki, S., Iguchi, Y., Fujioka, Y., Udagawa, T., Masuda, A., ... Sobue, G. (2014a). The ALS/FTLD-related RNA-binding proteins TDP-43 and FUS have common downstream RNA targets in cortical neurons. *FEBS Open Bio*, 4, 1–10. <https://doi.org/10.1016/j.fob.2013.11.001>
- Honda, D., Ishigaki, S., Iguchi, Y., Fujioka, Y., Udagawa, T., Masuda, A., ... Sobue, G. (2014b). The ALS/FTLD-related RNA-binding proteins TDP-43 and FUS have common downstream RNA targets in cortical neurons. *FEBS Open Bio*, 4, 1–10. <https://doi.org/10.1016/j.fob.2013.11.001>
- Hong, K., Li, Y., Duan, W., Guo, Y., Jiang, H., Li, W., & Li, C. (2012). Full-length TDP-43 and its C-terminal fragments activate mitophagy in NSC34 cell line. *Neuroscience Letters*, 530(2), 144–149. <https://doi.org/10.1016/j.neulet.2012.10.003>
- Hong, M., Zhukareva, V., Vogelsberg-Ragaglia, V., Wszolek, Z., Reed, L., Miller, B. I., ... Lee, V. M. (1998). Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. *Science (New York, N.Y.)*, 282(5395), 1914–1917. <https://doi.org/10.1126/science.282.5395.1914>
- Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., ... Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496(7446), 498–503. <https://doi.org/10.1038/nature12111>
- Howland, D. S., Liu, J., She, Y., Goad, B., Maragakis, N. J., Kim, B., ... Rothstein, J. D. (2002). Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proceedings of the National Academy of Sciences of the United States of America*, 99(3), 1604–1609. <https://doi.org/10.1073/pnas.032539299>
- Huang, B., Johansson, M. J. O., & Byström, A. S. (2005). An early step in wobble uridine tRNA modification requires the Elongator complex. *Rna*, 11(4), 424–436. <https://doi.org/10.1261/rna.7247705>
- Huang, C., Tong, J., Bi, F., Wu, Q., Huang, B., Zhou, H., & Xia, X. G. (2012). Entorhinal cortical neurons are the primary targets of FUS mislocalization and ubiquitin aggregation in FUS transgenic rats. *Human Molecular Genetics*, 21(21), 4602–4614. <https://doi.org/10.1093/hmg/dds299>
- Huang, C., Zhou, H., Tong, J., Chen, H., Liu, Y. J., Wang, D., ... Xia, X. G. (2011). FUS transgenic rats develop the phenotypes of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. *PLoS Genetics*, 7(3). <https://doi.org/10.1371/journal.pgen.1002011>
- Huang, E. J., Zhang, J., Geser, F., Trojanowski, J. Q., Strober, J. B., Dickson, D. W., ... Lomen-Hoerth, C. (2010). Extensive FUS-Immunoreactive Pathology in Juvenile Amyotrophic Lateral

- Sclerosis with Basophilic Inclusions. *Brain Pathology*, 20(6), 1069–1076. <https://doi.org/10.1111/j.1750-3639.2010.00413.x>
- Huang, X., Wu, B. P., Nguyen, D., Liu, Y. T., Marani, M., Hench, J., ... Narendra, D. P. (2018). CHCHD2 accumulates in distressed mitochondria and facilitates oligomerization of CHCHD10. *Human Molecular Genetics*, 27(22), 3881–3900. <https://doi.org/10.1093/hmg/ddy270>
- Hübbers, A., Just, W., Rosenbohm, A., Müller, K., Marroquin, N., Goebel, I., ... Volk, A. E. (2015). De novo FUS mutations are the most frequent genetic cause in early-onset German ALS patients. *Neurobiology of Aging*, 36(11), 3117.e1–3117.e6. <https://doi.org/10.1016/j.neurobiolaging.2015.08.005>
- Hutton, M., Heutink, P., Lendon, C. L., Rizzu, P., Baker, M., Froelich, S., ... Heutink, P. (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*, 393(6686), 702–705. <https://doi.org/10.1038/31508>
- Hwang, W. Y., Fu, Y., Reyon, D., Maeder, M. L., Tsai, S. Q., Sander, J. D., ... Joung, J. K. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology*, 31(3), 227–229. <https://doi.org/10.1038/nbt.2501>
- Ichikawa, H., Shimizu, K., Hayashi, Y., & Ohki, M. (1994). An RNA-binding protein gene, TLS/FUS, is fused to ERG in human myeloid leukemia with t(16;21) chromosomal translocation. *Cancer Research*, 54(11), 2865–2868. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8187069>
- Iguchi, Y., Katsuno, M., Niwa, J. I., Takagi, S., Ishigaki, S., Ikenaka, K., ... Sobue, G. (2013). Loss of TDP-43 causes age-dependent progressive motor neuron degeneration. *Brain*, 136(5), 1371–1382. <https://doi.org/10.1093/brain/awt029>
- Iko, Y., Kodama, T. S., Kasai, N., Oyama, T., Morita, E. H., Muto, T., ... Morikawa, K. (2004). Domain architectures and characterization of an RNA-binding protein, TLS. *Journal of Biological Chemistry*, 279(43), 44834–44840. <https://doi.org/10.1074/jbc.M408552200>
- Ilzecka, J., Stelmasiak, Z., Solski, J., Wawrzycki, S., & Szpetnar, M. (2003). Plasma amino acids concentration in amyotrophic lateral sclerosis patients. *Amino Acids*, 25(1), 69–73. <https://doi.org/10.1007/s00726-002-0352-2>
- Imai, Y., Meng, H., Shiba-Fukushima, K., & Hattori, N. (2019). Twin CHCH Proteins, CHCHD2, and CHCHD10: Key Molecules of Parkinson's Disease, Amyotrophic Lateral Sclerosis, and Frontotemporal Dementia. *International Journal of Molecular Sciences*, 20(4). <https://doi.org/10.3390/ijms20040908>
- Ishigaki, S., Fujioka, Y., Okada, Y., Riku, Y., Udagawa, T., Honda, D., ... Sobue, G. (2017). Altered Tau Isoform Ratio Caused by Loss of FUS and SFPQ Function Leads to FTL-like Phenotypes. *Cell Reports*, 18(5), 1118–1131. <https://doi.org/10.1016/j.celrep.2017.01.013>
- Ishigaki, S., Masuda, A., Fujioka, Y., Iguchi, Y., Katsuno, M., Shibata, A., ... Ohno, K. (2012). Position-dependent FUS-RNA interactions regulate alternative splicing events and transcriptions. *Scientific Reports*, 2, 1–9. <https://doi.org/10.1038/srep00529>
- Ishigaki, S., & Sobue, G. (2018). Importance of Functional Loss of FUS in FTL/ALS. *Frontiers in Molecular Biosciences*, 5(May), 44. <https://doi.org/10.3389/fmolb.2018.00044>
- Ishiguro, A., Kimura, N., Watanabe, Y., Watanabe, S., & Ishihama, A. (2016). TDP-43 binds and transports G-quadruplex-containing mRNAs into neurites for local translation. *Genes to Cells*, 21(5), 466–481. <https://doi.org/10.1111/gtc.12352>
- Iyengar, B., Roote, J., & Campos, A. R. (1999). The *tamas* gene, identified as a mutation that disrupts larval behavior in *Drosophila melanogaster*, codes for the mitochondrial DNA polymerase catalytic subunit (DNApol-gamma125). *Genetics*, 153(4), 1809–1824. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10581287>

- Jackson, H. E., & Ingham, P. W. (2013). Control of muscle fibre-type diversity during embryonic development: The zebrafish paradigm. *Mechanisms of Development*, 130(9–10), 447–457. <https://doi.org/10.1016/j.mod.2013.06.001>
- Jaiswal, M. K. (2019). Riluzole and edaravone: A tale of two amyotrophic lateral sclerosis drugs. *Medicinal Research Reviews*, 39(2), 733–748. <https://doi.org/10.1002/med.21528>
- Jensen, L., Jørgensen, L. H., Bech, R. D., Frandsen, U., & Schrøder, H. D. (2016). Skeletal Muscle Remodelling as a Function of Disease Progression in Amyotrophic Lateral Sclerosis. *BioMed Research International*, 2016, 5930621. <https://doi.org/10.1155/2016/5930621>
- Johansson, M., & Karlsson, A. (1997). Cloning of the cDNA and chromosome localization of the gene for human thymidine kinase 2. *The Journal of Biological Chemistry*, 272(13), 8454–8458. <https://doi.org/10.1074/jbc.272.13.8454>
- Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., ... Traynor, B. J. (2010). Exome Sequencing Reveals VCP Mutations as a Cause of Familial ALS. *Neuron*, 68(5), 857–864. <https://doi.org/10.1016/j.neuron.2010.11.036>
- Jourdain, P., Fukunaga, K., & Muller, D. (2003). *Brief Communication Calcium/Calmodulin-Dependent Protein Kinase II Contributes to Activity-Dependent Filopodia Growth and Spine Formation.*
- Joyce, N. C., & Carter, G. T. (2013). Electrodiagnosis in persons with amyotrophic lateral sclerosis. *PM & R: The Journal of Injury, Function, and Rehabilitation*, 5(5 Suppl), S89-95. <https://doi.org/10.1016/j.pmrj.2013.03.020>
- Ju, J.-S., Fuentealba, R. A., Miller, S. E., Jackson, E., Piwnica-Worms, D., Baloh, R. H., & Weihl, C. C. (2009). Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *The Journal of Cell Biology*, 187(6), 875–888. <https://doi.org/10.1083/jcb.200908115>
- Kabashi, E., Agar, J. N., Taylor, D. M., Minotti, S., & Durham, H. D. (2004). Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *Journal of Neurochemistry*, 89(6), 1325–1335. <https://doi.org/10.1111/j.1471-4159.2004.02453.x>
- Kabashi, E., Bercier, V., Lissouba, A., Liao, M., Brustein, E., Rouleau, G. A., & Drapeau, P. (2011). Fus and tardbp but not sod1 interact in genetic models of amyotrophic lateral sclerosis. *PLoS Genetics*, 7(8), 17–28. <https://doi.org/10.1371/journal.pgen.1002214>
- Kabashi, E., Brustein, E., Champagne, N., & Drapeau, P. (2011). Zebrafish models for the functional genomics of neurogenetic disorders. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1812(3), 335–345. <https://doi.org/10.1016/j.bbadis.2010.09.011>
- Kabashi, E., Champagne, N., Brustein, E., & Drapeau, P. (2010). In the swim of things: Recent insights to neurogenetic disorders from zebrafish. *Trends in Genetics*, 26(8), 373–381. <https://doi.org/10.1016/j.tig.2010.05.004>
- Kabashi, E., Lin, L., Tradewell, M. L., Dion, P. A., Bercier, V., Bourgouin, P., ... Drapeau, P. (2009a). Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo. *Human Molecular Genetics*, 19(4), 671–683. <https://doi.org/10.1093/hmg/ddp534>
- Kabashi, E., Lin, L., Tradewell, M. L., Dion, P. A., Bercier, V., Bourgouin, P., ... Drapeau, P. (2009b). Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo. *Human Molecular Genetics*, 19(4), 671–683. <https://doi.org/10.1093/hmg/ddp534>
- Kabashi, E., Valdmanis, P. N., Dion, P., & Rouleau, G. A. (2007). Oxidized/misfolded superoxide dismutase-1: The cause of all amyotrophic lateral sclerosis? *Annals of Neurology*, 62(6), 553–559. <https://doi.org/10.1002/ana.21319>

- Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., Velde, C. Vande, ... Rouleau, G. A. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nature Genetics*, *40*(5), 572–574. <https://doi.org/10.1038/ng.132>
- Kabuta, T., Suzuki, Y., & Wada, K. (2006). Degradation of amyotrophic lateral sclerosis-linked mutant Cu,Zn-superoxide dismutase proteins by macroautophagy and the proteasome. *Journal of Biological Chemistry*, *281*(41), 30524–30533. <https://doi.org/10.1074/jbc.M603337200>
- Kanadia, R. N., Johnstone, K. A., Mankodi, A., Lungu, C., Thornton, C. A., Esson, D., ... Swanson, M. S. (2003). A Muscleblind Knockout Model for Myotonic Dystrophy. *Science*, *302*(5652), 1978–1980. <https://doi.org/10.1126/science.1088583>
- Kanai, K., Kuwabara, S., Arai, K., Sung, J., Ogawara, K., & Hattori, T. (2003). Muscle cramp in Machado–Joseph disease: Altered motor axonal excitability properties and mexiletine treatment. *Brain*, *126*(4), 965–973. <https://doi.org/10.1093/brain/awg073>
- Kanai, K., Kuwabara, S., Misawa, S., Tamura, N., Ogawara, K., Nakata, M., ... Bostock, H. (2006). Altered axonal excitability properties in amyotrophic lateral sclerosis: impaired potassium channel function related to disease stage. *Brain*, *129*(4), 953–962. <https://doi.org/10.1093/brain/awl024>
- Kapeli, K., Pratt, G. A., Vu, A. Q., Hutt, K. R., Martinez, F. J., Sundararaman, B., ... Yeo, G. W. (2016). Distinct and shared functions of ALS-associated proteins TDP-43, FUS and TAF15 revealed by multisystem analyses. *Nature Communications*, *7*, 1–14. <https://doi.org/10.1038/ncomms12143>
- Katuragi, Y., Ichimura, Y., & Komatsu, M. (2015). p62/SQSTM1 functions as a signaling hub and an autophagy adaptor. *The FEBS Journal*, *282*(24), 4672–4678. <https://doi.org/10.1111/febs.13540>
- Kaur, S. J., McKeown, S. R., & Rashid, S. (2016). Mutant SOD1 mediated pathogenesis of Amyotrophic Lateral Sclerosis. *Gene*, *577*(2), 109–118. <https://doi.org/10.1016/j.gene.2015.11.049>
- Kawakami, F., Shimada, N., Ohta, E., Kagiya, G., Kawashima, R., Maekawa, T., ... Ichikawa, T. (2014, January). Leucine-rich repeat kinase 2 regulates tau phosphorylation through direct activation of glycogen synthase kinase-3 β . *FEBS Journal*, Vol. 281, pp. 3–13. <https://doi.org/10.1111/febs.12579>
- Kawakami, F., Yabata, T., Ohta, E., Maekawa, T., Shimada, N., Suzuki, M., ... Obata, F. (2012). LRRK2 phosphorylates tubulin-associated tau but not the free molecule: LRRK2-mediated regulation of the tau-tubulin association and neurite outgrowth. *PLoS ONE*, *7*(1), 1–9. <https://doi.org/10.1371/journal.pone.0030834>
- Keizman, D., Ish-Shalom, M., Berliner, S., Maimon, N., Vered, Y., Artamonov, I., ... Drory, V. E. (2009). Low uric acid levels in serum of patients with ALS: Further evidence for oxidative stress? *Journal of the Neurological Sciences*, *285*(1–2), 95–99. <https://doi.org/10.1016/j.jns.2009.06.002>
- Kettleborough, R. N. W., Bruijn, E. de, Eeden, F. van, Cuppen, E., & Stemple, D. L. (2011). High-throughput target-selected gene inactivation in zebrafish. In *Methods in Cell Biology* (Third Edit, Vol. 104). <https://doi.org/10.1016/B978-0-12-374814-0.00006-9>
- Kettleborough, R. N. W., Busch-Nentwich, E. M., Harvey, S. A., Dooley, C. M., de Bruijn, E., van Eeden, F., ... Stemple, D. L. (2013). A systematic genome-wide analysis of zebrafish protein-coding gene function. *Nature*, *496*(7446), 494–497. <https://doi.org/10.1038/nature11992>
- Kieran, D., Hafezparast, M., Bohnert, S., Dick, J. R. T., Martin, J., Schiavo, G., ... Greensmith, L. (2005). A mutation in dynein rescues axonal transport defects and extends the life span of ALS mice. *Journal of Cell Biology*, *169*(4), 561–567. <https://doi.org/10.1083/jcb.200501085>

- Kiernan, M. C., & Kaji, R. (2013). Physiology and pathophysiology of myelinated nerve fibers. In *Handbook of Clinical Neurology* (Vol. 115, pp. 43–53). <https://doi.org/10.1016/B978-0-444-52902-2.00004-7>
- Kim, D., Frank, C. L., Dobbin, M. M., Tsunemoto, R. K., Tu, W., Peng, P. L., ... Tsai, L. H. (2008). Deregulation of HDAC1 by p25/Cdk5 in Neurotoxicity. *Neuron*, *60*(5), 803–817. <https://doi.org/10.1016/j.neuron.2008.10.015>
- Kim, N., & Burden, S. J. (2008). MuSK controls where motor axons grow and form synapses. *Nature Neuroscience*, *11*(1), 19–27. <https://doi.org/10.1038/nn2026>
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, *203*(3), 253–310. <https://doi.org/10.1002/aja.1002030302>
- Kimura, T., Ishiguro, K., & Hisanaga, S. (2014). Physiological and pathological phosphorylation of tau by Cdk5. *Frontiers in Molecular Neuroscience*, *7*(July), 1–10. <https://doi.org/10.3389/fnmol.2014.00065>
- Kino, Y., Washizu, C., Kurosawa, M., Yamada, M., Miyazaki, H., Akagi, T., ... Nukina, N. (2015). FUS/TLS deficiency causes behavioral and pathological abnormalities distinct from amyotrophic lateral sclerosis. *Acta Neuropathologica Communications*, *3*, 24. <https://doi.org/10.1186/s40478-015-0202-6>
- Klemann, C. J. H. M., Martens, G. J. M., Sharma, M., Martens, M. B., Isacson, O., Gasser, T., ... Poelmans, G. (2017). Integrated molecular landscape of Parkinson's disease. *Npj Parkinson's Disease*, *3*(1). <https://doi.org/10.1038/s41531-017-0015-3>
- Komine, O., & Yamanaka, K. (2015). Neuroinflammation in motor neuron disease. *Nagoya Journal of Medical Science*, *77*(4), 537–549. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/26663933>
- Koppers, M., Blokhuis, A. M., Westeneng, H.-J., Terpstra, M. L., Zundel, C. A. C., Vieira de Sá, R., ... Pasterkamp, R. J. (2015). C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. *Annals of Neurology*, *78*(3), 426–438. <https://doi.org/10.1002/ana.24453>
- Kosik, K. S., Joachim, C. L., & Selkoe, D. J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(11), 4044–4048. <https://doi.org/10.1073/pnas.83.11.4044>
- Kraemer, B. C., Schuck, T., Wheeler, J. M., Robinson, L. C., Trojanowski, J. Q., Lee, V. M. Y., & Schellenberg, G. D. (2010). Loss of Murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. *Acta Neuropathologica*, *119*(4), 409–419. <https://doi.org/10.1007/s00401-010-0659-0>
- Krarup, C., & Moldovan, M. (2009). Nerve conduction and excitability studies in peripheral nerve disorders. *Current Opinion in Neurology*, *22*(5), 460–466. <https://doi.org/10.1097/WCO.0b013e3283304c9d>
- Krishnan, A. V., Lin, C. S.-Y., Park, S. B., & Kiernan, M. C. (2009). Axonal ion channels from bench to bedside: A translational neuroscience perspective. *Progress in Neurobiology*, *89*(3), 288–313. <https://doi.org/https://doi.org/10.1016/j.pneurobio.2009.08.002>
- Kummer, T. T., Misgeld, T., & Sanes, J. R. (2006). Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. *Current Opinion in Neurobiology*, *16*(1), 74–82. <https://doi.org/10.1016/j.conb.2005.12.003>
- Kuo, J. J., Siddique, T., Fu, R., & Heckman, C. J. (2005). Increased persistent Na⁺ current and its effect on excitability in motoneurons cultured from mutant SOD1 mice. *The Journal of*

- Physiology*, 563(3), 843–854. <https://doi.org/10.1113/jphysiol.2004.074138>
- Kuroda, M., Sok, J., Webb, L., Baechtold, H., Urano, F., Yin, Y., ... Ron, D. (2000). Male sterility and enhanced radiation sensitivity in TLS(-/-) mice. *The EMBO Journal*, 19(3), 453–462. <https://doi.org/10.1093/emboj/19.3.453>
- Kurtishi, A., Rosen, B., Patil, K. S., Alves, G. W., & Møller, S. G. (2019). Cellular Proteostasis in Neurodegeneration. *Molecular Neurobiology*, 56(5), 3676–3689. <https://doi.org/10.1007/s12035-018-1334-z>
- Kwiatkowski, T. J., Bosco D A, Hosler, B. A., Cortelli, P., Jong, P. J. De, Yoshinaga, Y., & Haines, J. L. (2009). Mutations in the FUS/TLS Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis. *Science (New York, N.Y.)*, 547(April), 1205–1209.
- Lacomblez, L., Bensimon, G., Meininger, V., Leigh, P. N., & Guillet, P. (1996). Dose-ranging study of riluzole in amyotrophic lateral sclerosis. *The Lancet*, 347(9013), 1425–1431. [https://doi.org/https://doi.org/10.1016/S0140-6736\(96\)91680-3](https://doi.org/https://doi.org/10.1016/S0140-6736(96)91680-3)
- Lagier-Tourenne, C., Polymenidou, M., Hutt, K. R., Vu, A. Q., Baughn, M., Huelga, S. C., ... Yeo, G. W. (2012a). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nature Neuroscience*, 15(11), 1488–1497. <https://doi.org/10.1038/nn.3230>
- Lagier-Tourenne, C., Polymenidou, M., Hutt, K. R., Vu, A. Q., Baughn, M., Huelga, S. C., ... Yeo, G. W. (2012b). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. *Nature Neuroscience*, 15(11), 1488–1497. <https://doi.org/10.1038/nn.3230>
- Lanson, N. A., Maltare, A., King, H., Smith, R., Kim, J. H., Taylor, J. P., ... Pandey, U. B. (2011). A Drosophila model of FUS-related neurodegeneration reveals genetic interaction between FUS and TDP-43. *Human Molecular Genetics*, 20(13), 2510–2523. <https://doi.org/10.1093/hmg/ddr150>
- Lanson, N. A., & Pandey, U. B. (2012, June 26). FUS-related proteinopathies: Lessons from animal models. *Brain Research*, Vol. 1462, pp. 44–60. <https://doi.org/10.1016/j.brainres.2012.01.039>
- Lattante, S., Rouleau, G. A., & Kabashi, E. (2013). TARDBP and FUS Mutations Associated with Amyotrophic Lateral Sclerosis: Summary and Update. *Human Mutation*, 34(6), 812–826. <https://doi.org/10.1002/humu.22319>
- Lebedeva, S., de Jesus Domingues, A. M., Butter, F., & Ketting, R. F. (2017). Characterization of genetic loss-of-function of Fus in zebrafish. *RNA Biology*, 14(1), 29–35. <https://doi.org/10.1080/15476286.2016.1256532>
- Lee, A., Li, W., Xu, K., Bogert, B. A., Su, K., & Gao, F.-B. (2003). Control of dendritic development by the Drosophila fragile X-related gene involves the small GTPase Rac1. *Development (Cambridge, England)*, 130(22), 5543–5552. <https://doi.org/10.1242/dev.00792>
- Lee, E. B., Lee, V. M.-Y., & Trojanowski, J. Q. (2012). Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. *Nat Rev Neurosci*, 13(1), 38–50. <https://doi.org/10.1038/nrn3121>
- Lee, Y.-B., Baskaran, P., Gomez-Deza, J., Chen, H.-J., Nishimura, A. L., Smith, B. N., ... Shaw, C. E. (2017). C9orf72 poly GA RAN-translated protein plays a key role in amyotrophic lateral sclerosis via aggregation and toxicity. *Human Molecular Genetics*, 26(24), 4765–4777. <https://doi.org/10.1093/hmg/ddx350>
- Lee, Y.-B., Chen, H.-J., Peres, J. N., Gomez-Deza, J., Attig, J., Štálekár, M., ... Shaw, C. E. (2013). Hexanucleotide Repeats in ALS/FTD Form Length-Dependent RNA Foci, Sequester RNA Binding Proteins, and Are Neurotoxic. *Cell Reports*, 5(5), 1178–1186.

<https://doi.org/https://doi.org/10.1016/j.celrep.2013.10.049>

- Lenzken, S. C., Romeo, V., Zolezzi, F., Cordero, F., Lamorte, G., Bonanno, D., ... Barabino, S. M. L. (2011). Mutant SOD1 and mitochondrial damage alter expression and splicing of genes controlling neuritogenesis in models of neurodegeneration. *Human Mutation*, 32(2), 168–182. <https://doi.org/10.1002/humu.21394>
- Lerga, A., Hallier, M., Delva, L., Orvain, C., Gallais, I., Marie, J., & Moreau-Gachelin, F. (2001). Identification of an RNA binding specificity for the potential splicing factor TLS. *The Journal of Biological Chemistry*, 276(9), 6807–6816. <https://doi.org/10.1074/jbc.M008304200>
- Leroy, F., Lamotte d'Incamps, B., Imhoff-Manuel, R. D., & Zytynicki, D. (2014). Early intrinsic hyperexcitability does not contribute to motoneuron degeneration in amyotrophic lateral sclerosis. *eLife*, 3. <https://doi.org/10.7554/eLife.04046>
- Lev, S., Halevy, D. Ben, Peretti, D., & Dahan, N. (2008, June). The VAP protein family: from cellular functions to motor neuron disease. *Trends in Cell Biology*, Vol. 18, pp. 282–290. <https://doi.org/10.1016/j.tcb.2008.03.006>
- Li, L., Xiong, W.-C., & Mei, L. (2018). Neuromuscular Junction Formation, Aging, and Disorders. *Annual Review of Physiology*, 80(1), 159–188. <https://doi.org/10.1146/annurev-physiol-022516-034255>
- Li, Y. R., King, O. D., Shorter, J., & Gitler, A. D. (2013, April). Stress granules as crucibles of ALS pathogenesis. *Journal of Cell Biology*, Vol. 201, pp. 361–372. <https://doi.org/10.1083/jcb.201302044>
- Li, Y., Ray, P., Rao, E. J., Shi, C., Guo, W., Chen, X., ... Wu, J. Y. (2010). A Drosophila model for TDP-43 proteinopathy. *Proceedings of the National Academy of Sciences*, 107(7), 3169–3174. <https://doi.org/10.1073/pnas.0913602107>
- Lim, G. P., Backstrom, J. R., Cullen, M. J., Miller, C. A., Atkinson, R. D., & Tökés, Z. A. (1996). Matrix metalloproteinases in the neocortex and spinal cord of amyotrophic lateral sclerosis patients. *Journal of Neurochemistry*, 67(1), 251–259.
- Ling, J. P., Pletnikova, O., Troncoso, J. C., & Wong, P. C. (2015). TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. *Science*, 349(6248), 650 LP – 655. <https://doi.org/10.1126/science.aab0983>
- Ling, S. C., Polymenidou, M., & Cleveland, D. W. (2013). Converging mechanisms in als and FTD: Disrupted RNA and protein homeostasis. *Neuron*, 79(3), 416–438. <https://doi.org/10.1016/j.neuron.2013.07.033>
- Liu-Yesucevitz, L., Bilgutay, A., Zhang, Y. J., Vanderwyde, T., Citro, A., Mehta, T., ... Wolozin, B. (2010). Tar DNA binding protein-43 (TDP-43) associates with stress granules: Analysis of cultured cells and pathological brain tissue. *PLoS ONE*, 5(10). <https://doi.org/10.1371/journal.pone.0013250>
- Liu, S., & Storrie, B. (2012, December). Are Rab proteins the link between Golgi organization and membrane trafficking? *Cellular and Molecular Life Sciences*, Vol. 69, pp. 4093–4106. <https://doi.org/10.1007/s00018-012-1021-6>
- Liu, Y., Pattamatta, A., Zu, T., Reid, T., Bardhi, O., Borchelt, D. R., ... Ranum, L. P. W. (2016). C9orf72 BAC Mouse Model with Motor Deficits and Neurodegenerative Features of ALS/FTD. *Neuron*, 90(3), 521–534. <https://doi.org/10.1016/j.neuron.2016.04.005>
- Loeffler, J. P., Picchiarelli, G., Dupuis, L., & Gonzalez De Aguilar, J. L. (2016). The role of skeletal muscle in amyotrophic lateral sclerosis. *Brain Pathology*, 26(2), 227–236. <https://doi.org/10.1111/bpa.12350>

- Logroschino, G., Traynor, B. J., Hardiman, O., Chió, A., Mitchell, D., Swingler, R. J., ... Beghi, E. (2010). Incidence of amyotrophic lateral sclerosis in Europe. *Journal of Neurology, Neurosurgery and Psychiatry*, *81*(4), 385–390. <https://doi.org/10.1136/jnnp.2009.183525>
- Lopez-Gonzalez, R., Lu, Y., Gendron, T. F., Karydas, A., Tran, H., Yang, D., ... Gao, F. B. (2016). Poly(GR) in C9ORF72-Related ALS/FTD Compromises Mitochondrial Function and Increases Oxidative Stress and DNA Damage in iPSC-Derived Motor Neurons. *Neuron*, *92*(2), 383–391. <https://doi.org/10.1016/j.neuron.2016.09.015>
- Lorenzl, S., Narr, S., Angele, B., Krell, H. W., Gregorio, J., Kiaei, M., ... Beal, M. F. (2006). The matrix metalloproteinases inhibitor Ro 26-2853 extends survival in transgenic ALS mice. *Experimental Neurology*, *200*(1), 166–171. <https://doi.org/10.1016/j.expneurol.2006.01.026>
- Lourenco, G. F., Janitz, M., Huang, Y., & Halliday, G. M. (2015). Long noncoding RNAs in TDP-43 and FUS/TLS-related frontotemporal lobar degeneration (FTLD). *Neurobiology of Disease*, *82*, 445–454. <https://doi.org/10.1016/j.nbd.2015.07.011>
- Lucas, J. J., Hernández, F., Gómez-Ramos, P., Morán, M. A., Hen, R., & Avila, J. (2001). Decreased nuclear β -catenin, tau hyperphosphorylation and neurodegeneration in GSK-3 β conditional transgenic mice. *EMBO Journal*, *20*(1–2), 27–39. <https://doi.org/10.1093/emboj/20.1.27>
- Luo, G., Yi, J., Ma, C., Xiao, Y., Yi, F., Yu, T., & Zhou, J. (2013). Defective mitochondrial dynamics is an early event in skeletal muscle of an Amyotrophic lateral sclerosis mouse model. *PLoS ONE*, *8*(12). <https://doi.org/10.1371/journal.pone.0082112>
- Ma, J., Huo, X., Jarpe, M. B., Kavelaars, A., & Heijnen, C. J. (2018). Pharmacological inhibition of HDAC6 reverses cognitive impairment and tau pathology as a result of cisplatin treatment. *Acta Neuropathologica Communications*, *6*(1), 103. <https://doi.org/10.1186/s40478-018-0604-3>
- Machamer, J. B., Collins, S. E., & Lloyd, T. E. (2014). The ALS gene FUS regulates synaptic transmission at the *Drosophila* neuromuscular junction. *Human Molecular Genetics*, *23*(14), 3810–3822. <https://doi.org/10.1093/hmg/ddu094>
- Mackenzie, I. R. A., Ansorge, O., Strong, M., Bilbao, J., Zinman, L., Ang, L.-C., ... Neumann, M. (2011). Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. *Acta Neuropathologica*, *122*(1), 87–98. <https://doi.org/10.1007/s00401-011-0838-7>
- Mackenzie, I. R. A., & Neumann, M. (2012). FET proteins in frontotemporal dementia and amyotrophic lateral sclerosis. *Brain Research*, *1462*, 40–43. <https://doi.org/10.1016/J.BRAINRES.2011.12.010>
- Mackenzie, I. R. A., Rademakers, R., & Neumann, M. (2010). TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *The Lancet Neurology*, *9*(10), 995–1007. [https://doi.org/10.1016/S1474-4422\(10\)70195-2](https://doi.org/10.1016/S1474-4422(10)70195-2)
- Mackenzie, I. R., Nicholson, A. M., Sarkar, M., Messing, J., Purice, M. D., Pottier, C., ... Rademakers, R. (2017). TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics. *Neuron*, *95*(4), 808–816.e9. <https://doi.org/10.1016/j.neuron.2017.07.025>
- Magrané, J., Cortez, C., Gan, W.-B., & Manfredi, G. (2013). Abnormal mitochondrial transport and morphology are common pathological denominators in SOD1 and TDP43 ALS mouse models. *Human Molecular Genetics*, *23*(6), 1413–1424. <https://doi.org/10.1093/hmg/ddt528>
- Magrané, J., Cortez, C., Gan, W. B., & Manfredi, G. (2014). Abnormal mitochondrial transport and morphology are common pathological denominators in SOD1 and TDP43 ALS mouse models. *Human Molecular Genetics*, *23*(6), 1413–1424. <https://doi.org/10.1093/hmg/ddt528>
- Majounie, E., Renton, A. E., Mok, K., Dopper, E. G. P., Waite, A., Rollinson, S., ... Traynor, B. J.

- (2012). Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. *The Lancet Neurology*, *11*(4), 323–330. [https://doi.org/10.1016/S1474-4422\(12\)70043-1](https://doi.org/10.1016/S1474-4422(12)70043-1)
- Mallik, M., Catinozzi, M., Hug, C. B., Zhang, L., Wagner, M., Bussmann, J., ... Storkebaum, E. (2018). Xrp1 genetically interacts with the ALS-associated FUS orthologue caz and mediates its toxicity. *The Journal of Cell Biology*, *217*(11), 3947–3964. <https://doi.org/10.1083/jcb.201802151>
- Marchetto, M. C. N., Muotri, A. R., Mu, Y., Smith, A. M., Cezar, G. G., & Gage, F. H. (2008). Non-Cell-Autonomous Effect of Human SOD1G37R Astrocytes on Motor Neurons Derived from Human Embryonic Stem Cells. *Cell Stem Cell*, *3*(6), 649–657. <https://doi.org/10.1016/j.stem.2008.10.001>
- Marcuzzo, S., Zucca, I., Mastropietro, A., de Rosbo, N. K., Cavalcante, P., Tartari, S., ... Bernasconi, P. (2011). Hind limb muscle atrophy precedes cerebral neuronal degeneration in G93A-SOD1 mouse model of amyotrophic lateral sclerosis: A longitudinal MRI study. *Experimental Neurology*, *231*(1), 30–37. <https://doi.org/10.1016/j.expneurol.2011.05.007>
- Marin, B., Boumédiène, F., Logroscino, G., Couratier, P., Babron, M.-C., Leutenegger, A. L., ... Beghi, E. (2017). Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. *International Journal of Epidemiology*, *46*(1), 57–74. <https://doi.org/10.1093/ije/dyw061>
- Marko, M., Vlassis, A., Guialis, A., & Leichter, M. (2012). Domains involved in TAF15 subcellular localisation: Dependence on cell type and ongoing transcription. *Gene*, *506*(2), 331–338. <https://doi.org/10.1016/j.gene.2012.06.088>
- Martin, L., Latypova, X., Wilson, C. M., Magnaudeix, A., Perrin, M. L., Yardin, C., & Terro, F. (2013). Tau protein kinases: Involvement in Alzheimer's disease. *Ageing Research Reviews*, *12*(1), 289–309. <https://doi.org/10.1016/j.arr.2012.06.003>
- Mastrocola, A. S., Kim, S. H., Trinh, A. T., Rodenkirch, L. A., & Tibbetts, R. S. (2013). The RNA-binding protein fused in sarcoma (FUS) functions downstream of poly(ADP-ribose) polymerase (PARP) in response to DNA damage. *The Journal of Biological Chemistry*, *288*(34), 24731–24741. <https://doi.org/10.1074/jbc.M113.497974>
- Masuda, A., Takeda, J. I., Okuno, T., Okamoto, T., Ohkawara, B., Ito, M., ... Ohno, K. (2015). Position-specific binding of FUS to nascent RNA regulates mRNA length. *Genes and Development*, *29*(10), 1045–1057. <https://doi.org/10.1101/gad.255737>
- Matamala, J. M., Howells, J., Dharmadasa, T., Huynh, W., Park, S. B., Burke, D., & Kiernan, M. C. (2018). Excitability of sensory axons in amyotrophic lateral sclerosis. *Clinical Neurophysiology*, *129*(7), 1472–1478. <https://doi.org/10.1016/j.clinph.2018.03.014>
- Matsumoto, G., Shimogori, T., Hattori, N., & Nukina, N. (2015). TBK1 controls autophagosomal engulfment of polyubiquitinated mitochondria through p62/SQSTM1 phosphorylation. *Human Molecular Genetics*, *24*(15), 4429–4442. <https://doi.org/10.1093/hmg/ddv179>
- Mattiazzi, M., D'Aurelio, M., Gajewski, C. D., Martushova, K., Kiaei, M., Flint Beal, M., & Manfredi, G. (2002). Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. *Journal of Biological Chemistry*, *277*(33), 29626–29633. <https://doi.org/10.1074/jbc.M203065200>
- McDonald, K. K., Aulas, A., Destroismaisons, L., Pickles, S., Beleac, E., Camu, W., ... Vande Velde, C. (2011). TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. *Human Molecular Genetics*, *20*(7), 1400–1410. <https://doi.org/10.1093/hmg/ddr021>
- McGown, A., McDearmid, J. R., Panagiotaki, N., Tong, H., Al Mashhadi, S., Redhead, N., ...

- Ramesh, T. M. (2013). Early interneuron dysfunction in ALS: Insights from a mutant sod1 zebrafish model. *Annals of Neurology*, 73(2), 246–258. <https://doi.org/10.1002/ana.23780>
- McGown, A., Shaw, D. P. J., & Ramesh, T. (2016). ZNStress: A high-throughput drug screening protocol for identification of compounds modulating neuronal stress in the transgenic mutant sod1G93R zebrafish model of amyotrophic lateral sclerosis. *Molecular Neurodegeneration*, 11(1), 1–11. <https://doi.org/10.1186/s13024-016-0122-3>
- McMillan, P., Korvatska, E., Poorkaj, P., Evstafjeva, Z., Robinson, L., Greenup, L., ... D'Souza, I. (2008). Tau isoform regulation is region- and cell-specific in mouse brain. *The Journal of Comparative Neurology*, 511(6), 788–803. <https://doi.org/10.1002/cne.21867>
- Medinas, D. B., Valenzuela, V., & Hetz, C. (2017). Proteostasis disturbance in amyotrophic lateral sclerosis. *Human Molecular Genetics*, 26(R2), R91–R104. <https://doi.org/10.1093/hmg/ddx274>
- Meng, H., Yamashita, C., Shiba-Fukushima, K., Inoshita, T., Funayama, M., Sato, S., ... Hattori, N. (2017). Loss of Parkinson's disease-associated protein CHCHD2 affects mitochondrial crista structure and destabilizes cytochrome c. *Nature Communications*, 8. <https://doi.org/10.1038/ncomms15500>
- Menon, K. P., Carrillo, R. A., & Zinn, K. (2013, September 1). Development and plasticity of the Drosophila larval neuromuscular junction. *Wiley Interdisciplinary Reviews. Developmental Biology*, Vol. 2, pp. 647–670. <https://doi.org/10.1002/wdev.108>
- Merlie, J. P., Sebbane, R., Gardner, S., Olson, E., & Lindstrom, J. (1983). The regulation of acetylcholine receptor expression in mammalian muscle. *Cold Spring Harbor Symposia on Quantitative Biology*, 48 Pt 1, 135–146.
- Meyerowitz, J., Parker, S. J., Vella, L. J., Ng, D. C. H., Price, K. A., Liddell, J. R., ... White, A. R. (2011). C-Jun N-terminal kinase controls TDP-43 accumulation in stress granules induced by oxidative stress. *Molecular Neurodegeneration*, 6(1), 57. <https://doi.org/10.1186/1750-1326-6-57>
- Millecamps, S., Boillée, S., Le Ber, I., Seilhean, D., Teyssou, E., Giraudeau, M., ... Salachas, F. (2012). Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. *Journal of Medical Genetics*, 49(4), 258–263. <https://doi.org/10.1136/jmedgenet-2011-100699>
- Miller, R. G., Munsat, T. L., Swash, M., & Brooks, B. R. (1999). Consensus guidelines for the design and implementation of clinical trials in ALS. World Federation of Neurology committee on Research. *Journal of the Neurological Sciences*, 169(1–2), 2–12.
- Mishina, M., Takai, T., Imoto, K., Noda, M., Takahashi, T., Numa, S., ... Sakmann, B. (n.d.). Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature*, 321(6068), 406–411. <https://doi.org/10.1038/321406a0>
- Mitchell, J. C., McGoldrick, P., Vance, C., Hortobagyi, T., Sreedharan, J., Rogelj, B., ... Shaw, C. E. (2013). Overexpression of human wild-type FUS causes progressive motor neuron degeneration in an age- and dose-dependent fashion. *Acta Neuropathologica*, 125(2), 273–288. <https://doi.org/10.1007/s00401-012-1043-z>
- Mitne-Neto, M., Machado-Costa, M., Marchetto, M. C. N., Bengtson, M. H., Joazeiro, C. A., Tsuda, H., ... Zatz, M. (2011). Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. *Human Molecular Genetics*, 20(18), 3642–3652. <https://doi.org/10.1093/hmg/ddr284>
- Mitsumoto, H., Santella, R., Liu, X., Bogdanov, M., Zipprich, J., Wu, H. C., ... Factor-Litvak, P. (2008). Oxidative stress biomarkers in sporadic ALS. *Amyotrophic Lateral Sclerosis*, 9(3), 177–183. <https://doi.org/10.1080/17482960801933942>
- Mizusawa, H., Matsumoto, S., Yen, S. H., Hirano, A., Rojas-Corona, R. R., & Donnenfeld, H. (1989).

- Focal accumulation of phosphorylated neurofilaments within anterior horn cell in familial amyotrophic lateral sclerosis. *Acta Neuropathologica*, 79(1), 37–43.
<https://doi.org/10.1007/BF00308955>
- Mochizuki, Y., Isozaki, E., Takao, M., Hashimoto, T., Shibuya, M., Arai, M., ... Mizutani, T. (2012). Familial ALS with FUS P525L mutation: Two Japanese sisters with multiple systems involvement. *Journal of the Neurological Sciences*, 323(1–2), 85–92.
<https://doi.org/10.1016/j.jns.2012.08.016>
- Mogyoros, I., Kiernan, M. C., Burke, D., & Bostock, H. (1998). Strength-duration properties of sensory and motor axons in amyotrophic lateral sclerosis. *Brain*, 121(5), 851–859.
<https://doi.org/10.1093/brain/121.5.851>
- Moloney, E. B., de Winter, F., & Verhaagen, J. (2014a). ALS as a distal axonopathy: molecular mechanisms affecting neuromuscular junction stability in the presymptomatic stages of the disease. *Frontiers in Neuroscience*, 8, 252. <https://doi.org/10.3389/fnins.2014.00252>
- Moloney, E. B., de Winter, F., & Verhaagen, J. (2014b). ALS as a distal axonopathy: Molecular mechanisms affecting neuromuscular junction stability in the presymptomatic stages of the disease. *Frontiers in Neuroscience*, 8(8 JUL), 1–18. <https://doi.org/10.3389/fnins.2014.00252>
- Mongeon, R., Walogorsky, M., Urban, J., Mandel, G., Ono, F., & Brehm, P. (2011). An acetylcholine receptor lacking both γ and ϵ subunits mediates transmission in zebrafish slow muscle synapses. *The Journal of General Physiology*, 138(3), 353–366. <https://doi.org/10.1085/jgp.201110649>
- Morita, M., Al-Chalabi, A., Andersen, P. M., Hosler, B., Sapp, P., Englund, E., ... Brown, R. H. (2006). A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology*, 66(6), 839 LP – 844. Retrieved from <http://n.neurology.org/content/66/6/839.abstract>
- Morlando, M., Dini Modigliani, S., Torrelli, G., Rosa, A., Di Carlo, V., Caffarelli, E., & Bozzoni, I. (2012). FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. *EMBO Journal*, 31(24), 4502–4510. <https://doi.org/10.1038/emboj.2012.319>
- Morohoshi, F., Ootsuka, Y., Arai, K., Ichikawa, H., Mitani, S., Munakata, N., & Ohki, M. (1998). Genomic structure of the human RBP56/hTAF(II)68 and FUS/TLS genes. *Gene*, 221(2), 191–198. [https://doi.org/10.1016/S0378-1119\(98\)00463-6](https://doi.org/10.1016/S0378-1119(98)00463-6)
- Mórotz, G. M., De Vos, K. J., Vagnoni, A., Ackerley, S., Shaw, C. E., & Miller, C. C. J. (2012). Amyotrophic lateral sclerosis-associated mutant VAPBP56S perturbs calcium homeostasis to disrupt axonal transport of mitochondria. *Human Molecular Genetics*, 21(9), 1979–1988.
<https://doi.org/10.1093/hmg/dds011>
- Morrice, J. R., Gregory-Evans, C. Y., & Shaw, C. A. (2018). Animal models of amyotrophic lateral sclerosis: A comparison of model validity. *Neural Regeneration Research*, 13(12), 2050–2054.
<https://doi.org/10.4103/1673-5374.241445>
- Moussavi Nik, S. H., Newman, M., Ganesan, S., Chen, M., Martins, R., Verdile, G., & Lardelli, M. (2014). Hypoxia alters expression of Zebrafish Microtubule-associated protein Tau (mapta, maptb) gene transcripts. *BMC Research Notes*, 7(1), 767. <https://doi.org/10.1186/1756-0500-7-767>
- Mullins, M. C., Hammerschmidt, M., Haffter, P., & Nüsslein-Volhard, C. (1994). Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Current Biology : CB*, 4(3), 189–202. [https://doi.org/10.1016/s0960-9822\(00\)00048-8](https://doi.org/10.1016/s0960-9822(00)00048-8)
- Münch, C., Meyer, R., Linke, P., Meyer, T., Ludolph, A. C., Haas, J., & Hemmer, B. (2007). The p150 subunit of dynactin (DCTN1) gene in multiple sclerosis. *Acta Neurologica Scandinavica*, 116(4), 231–234. <https://doi.org/10.1111/j.1600-0404.2007.00884.x>
- Nagabhushana, A., Chalasani, M. L., Jain, N., Radha, V., Rangaraj, N., Balasubramanian, D., &

- Swarup, G. (2010). Regulation of endocytic trafficking of transferrin receptor by optineurin and its impairment by a glaucoma-associated mutant. *BMC Cell Biology*, *11*.
<https://doi.org/10.1186/1471-2121-11-4>
- Nagai, M., Re, D. B., Nagata, T., Chalazonitis, A., Jessell, T. M., Wichterle, H., & Przedborski, S. (2007). Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nature Neuroscience*, *10*(5), 615–622. <https://doi.org/10.1038/nn1876>
- Naganawa, Y., & Hirata, H. (2011). Developmental transition of touch response from slow muscle-mediated coilings to fast muscle-mediated burst swimming in zebrafish. *Developmental Biology*, *355*(2), 194–204. <https://doi.org/10.1016/j.ydbio.2011.04.027>
- Nalbandian, A., Llewellyn, K. J., Badadani, M., Yin, H. Z., Nguyen, C., Katheria, V., ... Kimonis, V. E. (2013). A progressive translational mouse model of human valosin-containing protein disease: The VCPR155H/+ mouse. *Muscle and Nerve*, *47*(2), 260–270.
<https://doi.org/10.1002/mus.23522>
- Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., ... Singleton, A. B. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nature Genetics*, *46*(9), 989–993. <https://doi.org/10.1038/ng.3043>
- Neuhauss, S. C. F. (2003). Behavioral genetic approaches to visual system development and function in zebrafish. *Journal of Neurobiology*, *54*(1), 148–160. <https://doi.org/10.1002/neu.10165>
- Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., ... Lee, V. M.-Y. (2006). Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Science*, *314*(5796), 130–133.
<https://doi.org/10.1126/science.1134108>
- Neumann, Manuela, Rademakers, R., Roeber, S., Baker, M., Kretschmar, H. A., & MacKenzie, I. R. A. (2009). A new subtype of frontotemporal lobar degeneration with FUS pathology. *Brain*, *132*(11), 2922–2931. <https://doi.org/10.1093/brain/awp214>
- Newman, J. C., Bailey, A. D., & Weiner, A. M. (2006). Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(25), 9613–9618.
<https://doi.org/10.1073/pnas.0510909103>
- Nicolas, A., Kenna, K., Renton, A. E., Ticozzi, N., Faghri, F., Chia, R., ... Traynor, B. J. (2018). Genome-wide Analyses Identify KIF5A as a Novel ALS Gene. *Neuron*, *97*(6), 1268-1283.e6.
<https://doi.org/10.1016/j.neuron.2018.02.027>
- Niebroj-Dobosz, I., Janik, P., Sokołowska, B., & Kwiecinski, H. (2010). Matrix metalloproteinases and their tissue inhibitors in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *European Journal of Neurology*, *17*(2), 226–231. <https://doi.org/10.1111/j.1468-1331.2009.02775.x>
- Nimchinsky, E. A., Oberlander, A. M., & Svoboda, K. (2001). Abnormal development of dendritic spines in FMR1 knock-out mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *21*(14), 5139–5146.
- Nishimoto, Y., Nakagawa, S., Hirose, T., Okano, H. J., Takao, M., Shibata, S., ... Okano, H. (2013). The long non-coding RNA nuclear-enriched abundant transcript 1-2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. *Molecular Brain*, *6*(1). <https://doi.org/10.1186/1756-6606-6-31>
- Niu, C., Zhang, J., Gao, F., Yang, L., Jia, M., Zhu, H., & Gong, W. (2012). FUS-NLS/Transportin 1 Complex Structure Provides Insights into the Nuclear Targeting Mechanism of FUS and the Implications in ALS. *PLoS ONE*, *7*(10). <https://doi.org/10.1371/journal.pone.0047056>

- Noble, W., Olm, V., Takata, K., Casey, E., Mary, O., Meyerson, J., ... Duff, K. (2003). Cdk5 is a key factor in tau aggregation and tangle formation in vivo. *Neuron*, 38(4), 555–565. [https://doi.org/10.1016/s0896-6273\(03\)00259-9](https://doi.org/10.1016/s0896-6273(03)00259-9)
- Nolan, M., Talbot, K., & Ansorge, O. (2016). Pathogenesis of FUS-associated ALS and FTD: insights from rodent models. *Acta Neuropathologica Communications*, 4(1), 99. <https://doi.org/10.1186/s40478-016-0358-8>
- O'Rourke, J. G., Bogdanik, L., Yáñez, A., Lall, D., Wolf, A. J., Muhammad, A. K. M. G., ... Baloh, R. H. (2016). C9orf72 is required for proper macrophage and microglial function in mice. *Science*, 351(6279), 1324–1329. <https://doi.org/10.1126/science.aaf1064>
- Ohta, E., Nihira, T., Uchino, A., Imaizumi, Y., Okada, Y., Akamatsu, W., ... Okano, H. (2015). I2020T mutant LRRK2 iPSC-derived neurons in the Sagamihara family exhibit increased Tau phosphorylation through the AKT/GSK-3 β signaling pathway. *Human Molecular Genetics*, 24(17), 4879–4900. <https://doi.org/10.1093/hmg/ddv212>
- Onesto, E., Colombrita, C., Gumina, V., Borghi, M. O., Dusi, S., Doretto, A., ... Ratti, A. (2016). Gene-specific mitochondria dysfunctions in human TARDBP and C9ORF72 fibroblasts. *Acta Neuropathologica Communications*, 4(1), 47. <https://doi.org/10.1186/s40478-016-0316-5>
- Ono, S., Hu, J., Shimizu, N., Imai, T., & Nakagawa, H. (2001). Increased interleukin-6 of skin and serum in amyotrophic lateral sclerosis. *Journal of the Neurological Sciences*, 187(1–2), 27–34.
- Orban, P., Devon, R. S., Hayden, M. R., & Leavitt, B. R. (2007). Chapter 15 Juvenile amyotrophic lateral sclerosis. In A. A. Eisen & P. J. B. T.-H. of C. N. Shaw (Eds.), *Motor neuron disorders and related diseases* (Vol. 82, pp. 301–312). [https://doi.org/https://doi.org/10.1016/S0072-9752\(07\)80018-2](https://doi.org/https://doi.org/10.1016/S0072-9752(07)80018-2)
- Orozco, D., Tahirovic, S., Rentzsch, K., Schwenk, B. M., Haass, C., & Edbauer, D. (2012a). Loss of fused in sarcoma (FUS) promotes pathological Tau splicing. *EMBO Reports*, 13(8), 759–764. <https://doi.org/10.1038/embor.2012.90>
- Orozco, D., Tahirovic, S., Rentzsch, K., Schwenk, B. M., Haass, C., & Edbauer, D. (2012b). Loss of fused in sarcoma (FUS) promotes pathological Tau splicing. *EMBO Reports*, 13(8), 759–764. <https://doi.org/10.1038/embor.2012.90>
- Palma, E., Inghilleri, M., Conti, L., Deflorio, C., Frasca, V., Manteca, A., ... Milei, R. (2011). Physiological characterization of human muscle acetylcholine receptors from ALS patients. *Proceedings of the National Academy of Sciences of the United States of America*, 108(50), 20184–20188. <https://doi.org/10.1073/pnas.1117975108>
- Palma, E., Reyes-Ruiz, J. M., Lopercolo, D., Roseti, C., Bertollini, C., Ruffolo, G., ... Inghilleri, M. (2016a). Acetylcholine receptors from human muscle as pharmacological targets for ALS therapy. *Proceedings of the National Academy of Sciences of the United States of America*, 113(11), 3060–3065. <https://doi.org/10.1073/pnas.1600251113>
- Palma, E., Reyes-Ruiz, J. M., Lopercolo, D., Roseti, C., Bertollini, C., Ruffolo, G., ... Inghilleri, M. (2016b). Acetylcholine receptors from human muscle as pharmacological targets for ALS therapy. *Proceedings of the National Academy of Sciences*, 113(11), 3060–3065. <https://doi.org/10.1073/pnas.1600251113>
- Panagopoulos, I., Åman, P., Fioretos, T., Höglund, M., Johansson, B., Mandahl, N., ... Mitelman, F. (1994). Fusion of the FUS gene with ERG in acute myeloid leukemia with t(16;21)(p11;q22). *Genes, Chromosomes and Cancer*, 11(4), 256–262. <https://doi.org/10.1002/gcc.2870110408>
- Pansarasa, O., Rossi, D., Berardinelli, A., & Cereda, C. (2014). Amyotrophic lateral sclerosis and skeletal muscle: an update. *Molecular Neurobiology*, 49(2), 984–990. <https://doi.org/10.1007/s12035-013-8578-4>

- Pansarasa, Orietta, Bordoni, M., Diamanti, L., Sproviero, D., Gagliardi, S., & Cereda, C. (2018). Sod1 in amyotrophic lateral sclerosis: “ambivalent” behavior connected to the disease. *International Journal of Molecular Sciences*, *19*(5), 1–13. <https://doi.org/10.3390/ijms19051345>
- Panzer, J. A., Gibbs, S. M., Dosch, R., Wagner, D., Mullins, M. C., Granato, M., & Balice-Gordon, R. J. (2005a). Neuromuscular synaptogenesis in wild-type and mutant zebrafish. *Developmental Biology*, *285*(2), 340–357. <https://doi.org/10.1016/j.ydbio.2005.06.027>
- Panzer, J. A., Gibbs, S. M., Dosch, R., Wagner, D., Mullins, M. C., Granato, M., & Balice-Gordon, R. J. (2005b). Neuromuscular synaptogenesis in wild-type and mutant zebrafish. *Developmental Biology*, *285*(2), 340–357. <https://doi.org/10.1016/J.YDBIO.2005.06.027>
- Papadeas, S. T., Kraig, S. E., O’Banion, C., Lepore, A. C., & Maragakis, N. J. (2011). Astrocytes carrying the superoxide dismutase 1 (SOD1 G93A) mutation induce wild-type motor neuron degeneration in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(43), 17803–17808. <https://doi.org/10.1073/pnas.1103141108>
- Pardo, A. C., Wong, V., Benson, L. M., Dykes, M., Tanaka, K., Rothstein, J. D., & Maragakis, N. J. (2006). Loss of the astrocyte glutamate transporter GLT1 modifies disease in SOD1G93A mice. *Experimental Neurology*, *201*(1), 120–130. <https://doi.org/10.1016/j.expneurol.2006.03.028>
- Park, J. Y., Mott, M., Williams, T., Ikeda, H., Wen, H., Linhoff, M., & Ono, F. (2014). A single mutation in the acetylcholine receptor δ -subunit causes distinct effects in two types of neuromuscular synapses. *Journal of Neuroscience*, *34*(31), 10211–10218. <https://doi.org/10.1523/JNEUROSCI.0426-14.2014>
- Park, S. B., Kiernan, M. C., & Vucic, S. (2017). Axonal Excitability in Amyotrophic Lateral Sclerosis: Axonal Excitability in ALS. *Neurotherapeutics*, *14*(1), 78–90. <https://doi.org/10.1007/s13311-016-0492-9>
- Park, S. B., Vucic, S., Cheah, B. C., Lin, C. S. Y., Kirby, A., Mann, K. P., ... Kiernan, M. C. (2015). Flecainide in Amyotrophic Lateral Sclerosis as a Neuroprotective Strategy (FANS): A Randomized Placebo-Controlled Trial. *EBioMedicine*, *2*(12), 1916–1922. <https://doi.org/10.1016/j.ebiom.2015.11.022>
- Pascual, M., Vicente, M., Monferrer, L., & Artero, R. (2006). The Muscleblind family of proteins: An emerging class of regulators of developmentally programmed alternative splicing. *Differentiation*, Vol. 74, pp. 65–80. <https://doi.org/10.1111/j.1432-0436.2006.00060.x>
- Pasinelli, P., Belford, M. E., Lennon, N., Bacskai, B. J., Hyman, B. T., Trotti, D., & Brown, R. H. (2004). Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron*, *43*(1), 19–30. <https://doi.org/10.1016/j.neuron.2004.06.021>
- Pasinelli, P., & Brown, R. H. (2006). Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nature Reviews Neuroscience*, *7*(9), 710–723. <https://doi.org/10.1038/nrn1971>
- Patten, S. A., Armstrong, G. A. B., Lissouba, A., Kabashi, E., Parker, J. A., & Drapeau, P. (2014). Fishing for causes and cures of motor neuron disorders. *DMM Disease Models and Mechanisms*, Vol. 7, pp. 799–809. <https://doi.org/10.1242/dmm.015719>
- Pedrini, S., Sau, D., Guareschi, S., Bogush, M., Brown, R. H., Nanciche, N., ... Pasinelli, P. (2010). ALS-linked mutant SOD1 damages mitochondria by promoting conformational changes in Bcl-2. *Human Molecular Genetics*, *19*(15), 2974–2986. <https://doi.org/10.1093/hmg/ddq202>
- Pei, J.-J., Braak, H., An, W.-L., Winblad, B., Cowburn, R. F., Iqbal, K., & Grundke-Iqbal, I. (2002). Up-regulation of mitogen-activated protein kinases ERK1/2 and MEK1/2 is associated with the progression of neurofibrillary degeneration in Alzheimer’s disease. *Brain Research. Molecular Brain Research*, *109*(1–2), 45–55. [https://doi.org/10.1016/s0169-328x\(02\)00488-6](https://doi.org/10.1016/s0169-328x(02)00488-6)

- Peretti, D., Dahan, N., Shimoni, E., Hirschberg, K., & Lev, S. (2008). Coordinated lipid transfer between the endoplasmic reticulum and the golgi complex requires the VAP proteins and is essential for Golgi-mediated transport. *Molecular Biology of the Cell*, *19*(9), 3871–3884. <https://doi.org/10.1091/mbc.E08-05-0498>
- Philips, T., & Robberecht, W. (2011). Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *The Lancet Neurology*, *10*(3), 253–263. [https://doi.org/10.1016/S1474-4422\(11\)70015-1](https://doi.org/10.1016/S1474-4422(11)70015-1)
- Picchiarelli, G., Demestre, M., Zuko, A., Been, M., Higelin, J., Dieterlé, S., ... Storkebaum, E. (2019). FUS-mediated regulation of acetylcholine receptor transcription at neuromuscular junctions is compromised in amyotrophic lateral sclerosis. *Nature Neuroscience*. <https://doi.org/10.1038/s41593-019-0498-9>
- Piedrahita, D., Hernández, I., López-Tobón, A., Fedorov, D., Obara, B., Manjunath, B. S., ... Cardona-Gómez, G. P. (2010). Silencing of CDK5 reduces neurofibrillary tangles in transgenic alzheimer's mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(42), 13966–13976. <https://doi.org/10.1523/JNEUROSCI.3637-10.2010>
- Pieri, M., Carunchio, I., Curcio, L., Mercuri, N. B., & Zona, C. (2009). Increased persistent sodium current determines cortical hyperexcitability in a genetic model of amyotrophic lateral sclerosis. *Experimental Neurology*, *215*(2), 368–379. <https://doi.org/10.1016/j.expneurol.2008.11.002>
- Pigna, E., Renzini, A., Greco, E., Simonazzi, E., Fulle, S., Mancinelli, R., ... Adamo, S. (2018). HDAC4 preserves skeletal muscle structure following long-term denervation by mediating distinct cellular responses. *Skeletal Muscle*, *8*(1), 6. <https://doi.org/10.1186/s13395-018-0153-2>
- Pihlstrøm, L., Wiethoff, S., & Houlden, H. (2018). Genetics of neurodegenerative diseases: an overview. In *Handbook of Clinical Neurology* (Vol. 145, pp. 309–323). <https://doi.org/10.1016/B978-0-12-802395-2.00022-5>
- Pizzo, E., Sarcinelli, C., Sheng, J., Fusco, S., Formiggini, F., Netti, P., ... Hu, G. -f. (2013). Ribonuclease/angiogenin inhibitor 1 regulates stress-induced subcellular localization of angiogenin to control growth and survival. *Journal of Cell Science*, *126*(18), 4308–4319. <https://doi.org/10.1242/jcs.134551>
- Pollari, E., Goldsteins, G., Bart, G., Koistinaho, J., & Giniatullin, R. (2014). The role of oxidative stress in degeneration of the neuromuscular junction in amyotrophic lateral sclerosis. *Frontiers in Cellular Neuroscience*, *8*, 131. <https://doi.org/10.3389/fncel.2014.00131>
- Polymenidou, M., Lagier-Tourenne, C., Hutt, K. R., Huelga, S. C., Moran, J., Liang, T. Y., ... Cleveland, D. W. (2011a). Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nature Neuroscience*, *14*(4), 459–468. <https://doi.org/10.1038/nn.2779>
- Polymenidou, M., Lagier-Tourenne, C., Hutt, K. R., Huelga, S. C., Moran, J., Liang, T. Y., ... Cleveland, D. W. (2011b). Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nature Neuroscience*, *14*(4), 459–468. <https://doi.org/10.1038/nn.2779>
- Poorakaj, P., Bird, T. D., Wijsman, E., Nemens, E., Garruto, R. M., Anderson, L., ... Schellenberg, G. D. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Annals of Neurology*, *43*(6), 815–825. <https://doi.org/10.1002/ana.410430617>
- Pradat, P.-F., Dubourg, O., de Tapia, M., di Scala, F., Dupuis, L., Lenglet, T., ... Gonzalez de Aguilar, J.-L. (2012). Muscle gene expression is a marker of amyotrophic lateral sclerosis severity. *Neuro-Degenerative Diseases*, *9*(1), 38–52. <https://doi.org/10.1159/000329723>
- Pun, S., Santos, A. F., Saxena, S., Xu, L., & Caroni, P. (2006). Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. *Nature Neuroscience*, *9*(3),

408–419. <https://doi.org/10.1038/nn1653>

- Qiu, H., Lee, S., Shang, Y., Wang, W. Y., Au, K. F., Kamiya, S., ... Huang, E. J. (2014). ALS-associated mutation FUS-R521C causes DNA damage and RNA splicing defects. *Journal of Clinical Investigation*, *124*(3), 981–999. <https://doi.org/10.1172/JCI72723>
- Quinlan, K. A. (2011). Links between electrophysiological and molecular pathology of amyotrophic lateral sclerosis. *Integrative and Comparative Biology*, *51*(6), 913–925. <https://doi.org/10.1093/icb/icr116>
- Qureshi, M., Brown, R. H., Rogers, J. T., & Cudkowicz, M. E. (2008). Serum ferritin and metal levels as risk factors for amyotrophic lateral sclerosis. *The Open Neurology Journal*, *2*(1), 51–54. <https://doi.org/10.2174/1874205X00802010051>
- Rabbits, T. H., Forster, A., Larson, R., & Nathan, P. (1993). Fusion of the dominant negative transcription regulator CHOP with a novel gene FUS by translocation t(12;16) in malignant liposarcoma. *Nature Genetics*, *4*(2), 175–180. <https://doi.org/10.1038/ng0693-175>
- Rademakers, R., Stewart, H., DeJesus-Hernandez, M., Krieger, C., Graff-Radford, N., Fabros, M., ... Mackenzie, I. R. A. (2010). Fus gene mutations in familial and sporadic amyotrophic lateral sclerosis. *Muscle & Nerve*, *42*(2), 170–176. <https://doi.org/10.1002/mus.21665>
- Ragagnin, A. M. G., Shadfar, S., Vidal, M., Jamali, M. S., & Atkin, J. D. (2019). Motor Neuron Susceptibility in ALS/FTD. *Frontiers in Neuroscience*, *13*(June). <https://doi.org/10.3389/fnins.2019.00532>
- Ralsler, M., Albrecht, M., Nonhoff, U., Lengauer, T., Lehrach, H., & Krobisch, S. (2005). An Integrative Approach to Gain Insights into the Cellular Function of Human Ataxin-2. *Journal of Molecular Biology*, *346*(1), 203–214. <https://doi.org/https://doi.org/10.1016/j.jmb.2004.11.024>
- Ramesh, N., & Pandey, U. B. (2017). Autophagy Dysregulation in ALS: When Protein Aggregates Get Out of Hand. *Frontiers in Molecular Neuroscience*, *10*(August), 1–18. <https://doi.org/10.3389/fnmol.2017.00263>
- Ramesh, T., Lyon, A. N., Pineda, R. H., Wang, C., Janssen, P. M. L., Canan, B. D., ... Beattie, C. E. (2010). A genetic model of amyotrophic lateral sclerosis in zebrafish displays phenotypic hallmarks of motoneuron disease. *Disease Models & Mechanisms*, *3*(9–10), 652–662. <https://doi.org/10.1242/dmm.005538>
- Ramírez-Jarquín, U. N., Lazo-Gómez, R., Tovar-y-Romo, L. B., & Tapia, R. (2014). Spinal inhibitory circuits and their role in motor neuron degeneration. *Neuropharmacology*, *82*, 101–107. <https://doi.org/https://doi.org/10.1016/j.neuropharm.2013.10.003>
- Rasool, C. G., Chad, D., Bradley, W. G., Connolly, B., & Baruah, J. K. (1983). Acetylcholinesterase and ATPases in motor neuron degenerative diseases. *Muscle & Nerve*, *6*(6), 430–435. <https://doi.org/10.1002/mus.880060606>
- Ratti, A., & Buratti, E. (2016). Physiological functions and pathobiology of TDP-43 and FUS/TLS proteins. *Journal of Neurochemistry*, *138*, 95–111. <https://doi.org/10.1111/jnc.13625>
- Reinhard, S. M., Razak, K., & Ethell, I. M. (2015, July 29). A delicate balance: Role of MMP-9 in brain development and pathophysiology of neurodevelopmental disorders. *Frontiers in Cellular Neuroscience*, Vol. 9. <https://doi.org/10.3389/fncel.2015.00280>
- Renton, A. E., Chiò, A., & Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nature Neuroscience*, *17*(1), 17–23. <https://doi.org/10.1038/nn.3584>
- Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., ... Traynor, B. J. (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*, *72*(2), 257–268. <https://doi.org/10.1016/j.neuron.2011.09.010>

- Ringholz, G. M., Appel, S. H., Bradshaw, M., Cooke, N. A., Mosnik, D. M., & Schulz, P. E. (2005). Prevalence and patterns of cognitive impairment in sporadic ALS. *Neurology*, *65*(4), 586 LP – 590. <https://doi.org/10.1212/01.wnl.0000172911.39167.b6>
- Rizzuto, R., Nakase, H., Zeviani, M., DiMauro, S., & Schon, E. A. (1988). Subunit Va of human and bovine cytochrome c oxidase is highly conserved. *Gene*, *69*(2), 245–256. [https://doi.org/10.1016/0378-1119\(88\)90435-0](https://doi.org/10.1016/0378-1119(88)90435-0)
- Robberecht, W., & Philips, T. (2013). The changing scene of amyotrophic lateral sclerosis. *Nature Reviews Neuroscience*, *14*(4), 248–264. <https://doi.org/10.1038/nrn3430>
- Robinson, H. K., Deykin, A. V., Bronovitsky, E. V., Ovchinnikov, R. K., Ustyugov, A. A., Shelkownikova, T. A., ... Ninkina, N. N. (2015). Early lethality and neuronal proteinopathy in mice expressing cytoplasm-targeted FUS that lacks the RNA recognition motif. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, *16*(5–6), 402–409. <https://doi.org/10.3109/21678421.2015.1040994>
- Robles, E., & Baier, H. (2012, October). Assembly of synaptic laminae by axon guidance molecules. *Current Opinion in Neurobiology*, Vol. 22, pp. 799–804. <https://doi.org/10.1016/j.conb.2012.04.014>
- Rocha, M. C., Pousinha, P. A., Correia, A. M., Sebastião, A. M., & Ribeiro, J. A. (2013). Early changes of neuromuscular transmission in the SOD1(G93A) mice model of ALS start long before motor symptoms onset. *PloS One*, *8*(9), e73846. <https://doi.org/10.1371/journal.pone.0073846>
- Rogelj, B., Easton, L. E., Bogu, G. K., Stanton, L. W., Rot, G., Curk, T., ... Ule, J. (2012). Widespread binding of FUS along nascent RNA regulates alternative splicing in the brain. *Scientific Reports*, *2*, 1–10. <https://doi.org/10.1038/srep00603>
- Roggenbuck, J., Quick, A., & Kolb, S. J. (2017). Genetic testing and genetic counseling for amyotrophic lateral sclerosis: an update for clinicians. *Genetics in Medicine*, *19*(3), 267–274. <https://doi.org/10.1038/gim.2016.107>
- Rooney, J. P. K., Brayne, C., Tobin, K., Logroscino, G., Glymour, M. M., & Hardiman, O. (2017). Benefits, pitfalls, and future design of population-based registers in neurodegenerative disease. *Neurology*, *88*(24), 2321–2329. <https://doi.org/10.1212/WNL.0000000000004038>
- Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., ... Deng, H. X. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*, *362*(6415), 59–62. <https://doi.org/10.1038/362059a0>
- Rossi, A., Kontarakis, Z., Gerri, C., Nolte, H., Hölper, S., Krüger, M., & Stainier, D. Y. R. (2015). Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature*, *524*(7564), 230–233. <https://doi.org/10.1038/nature14580>
- Rozas, P., Bargsted, L., Martínez, F., Hetz, C., & Medinas, D. B. (2017, January 1). The ER proteostasis network in ALS: Determining the differential motoneuron vulnerability. *Neuroscience Letters*, Vol. 636, pp. 9–15. <https://doi.org/10.1016/j.neulet.2016.04.066>
- Ruegsegger, C., Maharjan, N., Goswami, A., Filézac de L'Etang, A., Weis, J., Troost, D., ... Saxena, S. (2016). Aberrant association of misfolded SOD1 with Na⁺/K⁺ATPase- α 3 impairs its activity and contributes to motor neuron vulnerability in ALS. *Acta Neuropathologica*, *131*(3), 427–451. <https://doi.org/10.1007/s00401-015-1510-4>
- Ruegsegger, C., & Saxena, S. (2016). Proteostasis impairment in ALS. *Brain Research*, *1648*, 571–579. <https://doi.org/10.1016/j.brainres.2016.03.032>
- S.L., R., A., R., R.L., G., G.J., G., D.A.Q., M., F., G.-H. G.-H., ... K.W., C. (2014). PARP-1 dependent recruitment of the amyotrophic lateral sclerosis-associated protein FUS/TLS to sites

- of oxidative DNA damage. *Nucleic Acids Research*, 42(1), 307–314.
<https://doi.org/10.1093/nar/gkt835> LK -
<http://sfxprd.tau.ac.il:3410/sfxIc141?sid=EMBASE&issn=13624962&id=doi:10.1093%2Fnar%2Fgkt835&atitle=PARP-1+dependent+recruitment+of+the+amyotrophic+lateral+sclerosis-associated+protein+FUS%2FTLS+to+sites+of+oxidative+DNA+damage&stitle=Nucleic+Acids+Res.&title=Nucleic+Acids+Research&volume=42&issue=1&spage=307&epage=314&aulast=Rulten&aufirst=Stuart+L.&aunit=S.L.&aull=Rulten+S.L.&coden=NARHA&isbn=&pages=307-314&date=2014&aunit1=S&aunitm=L>
- Sabatelli, M., Madia, F., Conte, A., Luigetti, M., Zollino, M., Mancuso, I., ... Tonali, P. (2008). Natural history of young-adult amyotrophic lateral sclerosis. *Neurology*, 71(12), 876 LP – 881.
<https://doi.org/10.1212/01.wnl.0000312378.94737.45>
- Saccon, R. A., Bunton-Stasyshyn, R. K. A., Fisher, E. M. C., & Fratta, P. (2013). Is SOD1 loss of function involved in amyotrophic lateral sclerosis? *Brain*, 136(8), 2342–2358.
<https://doi.org/10.1093/brain/awt097>
- Said Ahmed, M., Hung, W. Y., Zu, J. S., Hockberger, P., & Siddique, T. (2000). Increased reactive oxygen species in familial amyotrophic lateral sclerosis with mutations in SOD1. *Journal of the Neurological Sciences*, 176(2), 88–94. [https://doi.org/10.1016/S0022-510X\(00\)00317-8](https://doi.org/10.1016/S0022-510X(00)00317-8)
- Saint-Amant, L., & Drapeau, P. (1998). Time course of the development of motor behaviors in the zebrafish embryo. *Journal of Neurobiology*, 37(4), 622–632.
- Sakowski, S. A., Lunn, J. S., Busta, A. S., Oh, S. S., Zamora-Berridi, G., Palmer, M., ... Feldman, E. L. (2012). Neuromuscular effects of G93A-SOD1 expression in zebrafish. *Molecular Neurodegeneration*, 7(1). <https://doi.org/10.1186/1750-1326-7-44>
- Sama, R. R. K., Ward, C. L., Kaushansky, L. J., Lemay, N., Ishigaki, S., Urano, F., & Bosco, D. A. (2013). FUS/TLS assembles into stress granules and is a prosurvival factor during hyperosmolar stress. *Journal of Cellular Physiology*, 228(11), 2222–2231. <https://doi.org/10.1002/jcp.24395>
- Sanes, J. R., & Yamagata, M. (2009). Many Paths to Synaptic Specificity. *Annual Review of Cell and Developmental Biology*, 25(1), 161–195.
<https://doi.org/10.1146/annurev.cellbio.24.110707.175402>
- Sasaki, S., & Iwata, M. (2007). Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. *Journal of Neuropathology and Experimental Neurology*, 66(1), 10–16. <https://doi.org/10.1097/nen.0b013e31802c396b>
- Sasayama, H., Shimamura, M., Tokuda, T., Azuma, Y., Yoshida, T., Mizuno, T., ... Yamaguchi, M. (2012). Knockdown of the Drosophila fused in sarcoma (FUS) homologue causes deficient locomotive behavior and shortening of motoneuron terminal branches. *PLoS ONE*, 7(6).
<https://doi.org/10.1371/journal.pone.0039483>
- Saxena, S. K., Rybak, S. M., Davey, R. T., Youle, R. J., & Ackerman, E. J. (1992). Angiogenin is a cytotoxic, tRNA-specific ribonuclease in the RNase A superfamily. *Journal of Biological Chemistry*, 267(30), 21982–21986.
- Saxena, S., Roselli, F., Singh, K., Leptien, K., Julien, J. P., Gros-Louis, F., & Caroni, P. (2013). Neuroprotection through Excitability and mTOR Required in ALS Motoneurons to Delay Disease and Extend Survival. *Neuron*, 80(1), 80–96.
<https://doi.org/10.1016/j.neuron.2013.07.027>
- Scekic-Zahirovic, J., Sendscheid, O., El Oussini, H., Jambeau, M., Sun, Y., Mersmann, S., ... Dupuis, L. (2016). Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss. *The EMBO Journal*, 35(10), e201592559.
<https://doi.org/10.15252/emj.201592559>
- Schiaffino, S., & Reggiani, C. (2011). Fiber types in Mammalian skeletal muscles. *Physiological*

Reviews, 91(4), 1447–1531. <https://doi.org/10.1152/physrev.00031.2010>

- Schmid, B., & Haass, C. (2013). Genomic editing opens new avenues for zebrafish as a model for neurodegeneration. *Journal of Neurochemistry*, 127(4), 461–470. <https://doi.org/10.1111/jnc.12460>
- Schoen, M., Reichel, J. M., Demestre, M., Putz, S., Deshpande, D., Proepper, C., ... Boeckers, T. M. (2015). Super-Resolution Microscopy Reveals Presynaptic Localization of the ALS/FTD Related Protein FUS in Hippocampal Neurons. *Frontiers in Cellular Neuroscience*, 9, 496. <https://doi.org/10.3389/fncel.2015.00496>
- Schoser, B. G., & Blotner, D. (1999). Matrix metalloproteinases MMP-2, MMP-7 and MMP-9 in denervated human muscle. *Neuroreport*, 10(13), 2795–2797. <https://doi.org/10.1097/00001756-199909090-00018>
- Schwartz, J. C., Ebmeier, C. C., Podell, E. R., Heimiller, J., Taatjes, D. J., & Cech, T. R. (2012). FUS binds the CTD of RNA polymerase II and regulates its phosphorylation at Ser2. *Genes and Development*, 26(24), 2690–2695. <https://doi.org/10.1101/gad.204602.112>
- Schwenk, B. M., Hartmann, H., Serdaroglu, A., Schludi, M. H., Hornburg, D., Meissner, F., ... Edbauer, D. (2016). TDP-43 loss of function inhibits endosomal trafficking and alters trophic signaling in neurons. *The EMBO Journal*, 35(21), 2350–2370. <https://doi.org/10.15252/emj.201694221>
- Scotter, E. L., Chen, H. J., & Shaw, C. E. (2015). TDP-43 Proteinopathy and ALS: Insights into Disease Mechanisms and Therapeutic Targets. *Neurotherapeutics*, 12(2), 352–363. <https://doi.org/10.1007/s13311-015-0338-x>
- Scotter, E. L., Vance, C., Nishimura, A. L., Lee, Y. B., Chen, H. J., Urwin, H., ... Shaw, C. E. (2014). Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. *Journal of Cell Science*, 127(6), 1263–1278. <https://doi.org/10.1242/jcs.140087>
- Seaman, M. N. J. (2012). The retromer complex – endosomal protein recycling and beyond. *Journal of Cell Science*, 125(20), 4693 LP – 4702. <https://doi.org/10.1242/jcs.103440>
- Sellier, C., Campanari, M., Julie Corbier, C., Gaucherot, A., Kolb-Cheynel, I., Oulad-Abdelghani, M., ... Charlet-Berguerand, N. (2016). Loss of C9 ORF 72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. *The EMBO Journal*, 35(12), 1276–1297. <https://doi.org/10.15252/emj.201593350>
- Sephton, C. F., Tang, A. A., Kulkarni, A., West, J., Brooks, M., Stubblefield, J. J., ... Yu, G. (2014). Activity-dependent FUS dysregulation disrupts synaptic homeostasis. *Proceedings of the National Academy of Sciences*, 111(44), E4769–E4778. <https://doi.org/10.1073/pnas.1406162111>
- Sephton, Chantelle F., Good, S. K., Atkin, S., Dewey, C. M., Mayer, P., Herz, J., & Yu, G. (2010). TDP-43 is a developmentally regulated protein essential for early embryonic development. *Journal of Biological Chemistry*, 285(9), 6826–6834. <https://doi.org/10.1074/jbc.M109.061846>
- Shahidullah, M., Le Marchand, S. J., Fei, H., Zhang, J., Pandey, U. B., Dalva, M. B., ... Levitan, I. B. (2013). Defects in synapse structure and function precede motor neuron degeneration in *Drosophila* models of FUS-related ALS. *Journal of Neuroscience*, 33(50), 19590–19598. <https://doi.org/10.1523/JNEUROSCI.3396-13.2013>
- Shang, Y., & Huang, E. J. (2016). Mechanisms of FUS mutations in familial amyotrophic lateral sclerosis. *Brain Research*, 1647, 65–78. <https://doi.org/10.1016/j.brainres.2016.03.036>
- Shanley, M. R., Hawley, D., Leung, S., Zaidi, N. F., Dave, R., Schlosser, K. A., ... Liu, M. (2015). LRRK2 Facilitates tau Phosphorylation through Strong Interaction with tau and cdk5. *Biochemistry*, 54(33), 5198–5208. <https://doi.org/10.1021/acs.biochem.5b00326>

- Sharma, A., Lyashchenko, A. K., Lu, L., Nasrabad, S. E., Elmaleh, M., Mendelsohn, M., ... Shneider, N. A. (2016a). ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nature Communications*, 7, 1–14. <https://doi.org/10.1038/ncomms10465>
- Sharma, A., Lyashchenko, A. K., Lu, L., Nasrabad, S. E., Elmaleh, M., Mendelsohn, M., ... Shneider, N. A. (2016b). ALS-associated mutant FUS induces selective motor neuron degeneration through toxic gain of function. *Nature Communications*, 7, 10465. Retrieved from <https://doi.org/10.1038/ncomms10465>
- Shaw, P. J., & Williams, R. (2000). Serum and cerebrospinal fluid biochemical markers of ALS. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders : Official Publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases*, 1 Suppl 2, S61-7.
- Shelkovernikova, T. A., Peters, O. M., Deykin, A. V., Connor-Robson, N., Robinson, H., Ustyugov, A. A., ... Ninkina, N. N. (2013). Fused in sarcoma (FUS) protein lacking nuclear localization signal (NLS) and major RNA binding motifs triggers proteinopathy and severe motor phenotype in transgenic mice. *Journal of Biological Chemistry*, 288(35), 25266–25274. <https://doi.org/10.1074/jbc.M113.492017>
- Shi, Y., Lin, S., Staats, K. A., Li, Y., Chang, W.-H., Hung, S.-T., ... Ichida, J. K. (2018). Haploinsufficiency leads to neurodegeneration in C9ORF72 ALS/FTD human induced motor neurons. *Nature Medicine*, (January 2017). <https://doi.org/10.1038/nm.4490>
- Shibata, N., & Kobayashi, M. (2008). The role for oxidative stress in neurodegenerative diseases. *Brain and Nerve*, Vol. 60, pp. 157–170. <https://doi.org/10.5607/en.2015.24.4.325>
- Shibuya, K., Misawa, S., Nasu, S., Sekiguchi, Y., Mitsuma, S., Beppu, M., ... Kuwabara, S. (2013). Split hand syndrome in amyotrophic lateral sclerosis: different excitability changes in the thenar and hypothenar motor axons. *Journal of Neurology, Neurosurgery & Psychiatry*, 84(9), 969 LP – 972. <https://doi.org/10.1136/jnnp-2012-304109>
- Shing, D. C., McMullan, D. J., Roberts, P., Smith, K., Chin, S. F., Nicholson, J., ... Coleman, N. (2003). FUS/ERG gene fusions in Ewing's tumors. *Cancer Research*, 63(15), 4568–4576.
- Siklós, L., Engelhardt, J., Harati, Y., Smith, R. G., Joó, F., & Appel, S. H. (1996). Ultrastructural evidence for altered calcium in motor nerve terminals in amyotrophic lateral sclerosis. *Annals of Neurology*, 39(2), 203–216. <https://doi.org/10.1002/ana.410390210>
- Siklós, L., Engelhardt, J. I., Alexianu, M. E., Gurney, M. E., Siddique, T., & Appel, S. H. (1998). Intracellular calcium parallels motoneuron degeneration in SOD-1 mutant mice. *Journal of Neuropathology and Experimental Neurology*, 57(6), 571–587. <https://doi.org/10.1097/00005072-199806000-00005>
- Simón-Sánchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., ... Gasser, T. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics*, 41(12), 1308–1312. <https://doi.org/10.1038/ng.487>
- Simpson, C. L., Lemmens, R., Miskiewicz, K., Broom, W. J., Hansen, V. K., van Vught, P. W. J., ... Al-Chalabi, A. (2008). Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration . *Human Molecular Genetics*, 18(3), 472–481. <https://doi.org/10.1093/hmg/ddn375>
- Simpson, E. P., Henry, Y. K., Henkel, J. S., Smith, R. G., & Appel, S. H. (2004). Increased lipid peroxidation in sera of ALS patients: A potential biomarker of disease burden. *Neurology*, 62(10), 1758–1765. <https://doi.org/10.1212/WNL.62.10.1758>
- Skourti-Stathaki, K., Proudfoot, N. J., & Gromak, N. (2011). Human Senataxin Resolves RNA/DNA Hybrids Formed at Transcriptional Pause Sites to Promote Xrn2-Dependent Termination. *Molecular Cell*, 42(6), 794–805. <https://doi.org/10.1016/j.molcel.2011.04.026>

- Smith, R. G., Henry, Y. K., Mattson, M. P., & Appel, S. H. (1998). Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Annals of Neurology*, *44*(4), 696–699. <https://doi.org/10.1002/ana.410440419>
- Snowden, J. S., Hu, Q., Rollinson, S., Halliwell, N., Robinson, A., Davidson, Y. S., ... Mann, D. M. A. (2011). The most common type of FTLN-FUS (aFTLN-U) is associated with a distinct clinical form of frontotemporal dementia but is not related to mutations in the FUS gene. *Acta Neuropathologica*, *122*(1), 99–110. <https://doi.org/10.1007/s00401-011-0816-0>
- Solnica-Krezel, L., Schier, A. F., & Driever, W. (1994). Efficient recovery of ENU-induced mutations from the zebrafish germline. *Genetics*, *136*(4), 1401–1420. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/8013916>
- Song, W., Song, Y., Kincaid, B., Bossy, B., & Bossy-Wetzel, E. (2013). Mutant SOD1G93A triggers mitochondrial fragmentation in spinal cord motor neurons: Neuroprotection by SIRT3 and PGC-1 α . *Neurobiology of Disease*, *51*, 72–81. <https://doi.org/10.1016/j.nbd.2012.07.004>
- Soo, K. Y., Halloran, M., Sundaramoorthy, V., Parakh, S., Toth, R. P., Southam, K. A., ... Atkin, J. D. (2015). Rab1-dependent ER–Golgi transport dysfunction is a common pathogenic mechanism in SOD1, TDP-43 and FUS-associated ALS. *Acta Neuropathologica*, *130*(5), 679–697. <https://doi.org/10.1007/s00401-015-1468-2>
- Soto, C. (2003). Unfolding the role of protein misfolding in neurodegenerative diseases. *Nature Reviews. Neuroscience*, *4*(1), 49–60. <https://doi.org/10.1038/nrn1007>
- Soussan, L., Burakov, D., Daniels, M. P., Toister-Achituv, M., Porat, A., Yarden, Y., & Elazar, Z. (1999). ERG30, a VAP-33-related protein, functions in protein transport mediated by COPI vesicles. *Journal of Cell Biology*, *146*(2), 301–311. <https://doi.org/10.1083/jcb.146.2.301>
- Spillantini, M. G., Crowther, R. A., Kamphorst, W., Heutink, P., & Van Swieten, J. C. (1998). Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. *American Journal of Pathology*, *153*(5), 1359–1363. [https://doi.org/10.1016/S0002-9440\(10\)65721-5](https://doi.org/10.1016/S0002-9440(10)65721-5)
- Spillantini, Maria Grazia, & Goedert, M. (2013). Tau pathology and neurodegeneration. *The Lancet Neurology*, *12*(6), 609–622. [https://doi.org/10.1016/S1474-4422\(13\)70090-5](https://doi.org/10.1016/S1474-4422(13)70090-5)
- Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., ... Shaw, C. E. (2008). TDP-43 Mutations in Familial and Sporadic Amyotrophic Lateral Sclerosis. *Science*, *319*(5870), 1668 LP – 1672. Retrieved from <http://science.sciencemag.org/content/319/5870/1668.abstract>
- Staff, N. P., Amrami, K. K., & Howe, B. M. (2015). Magnetic resonance imaging abnormalities of peripheral nerve and muscle are common in amyotrophic lateral sclerosis and share features with multifocal motor neuropathy. *Muscle & Nerve*, *52*(1), 137–139. <https://doi.org/10.1002/mus.24630>
- Stenmark, H. (2009, August). Rab GTPases as coordinators of vesicle traffic. *Nature Reviews Molecular Cell Biology*, Vol. 10, pp. 513–525. <https://doi.org/10.1038/nrm2728>
- Stoica, R., De Vos, K. J., Paillusson, S., Mueller, S., Sancho, R. M., Lau, K.-F., ... Miller, C. C. J. (2014). ER–mitochondria associations are regulated by the VAPB–PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nature Communications*, *5*, 3996.
- Stoica, R., Paillusson, S., Gomez-Suaga, P., Mitchell, J. C., Lau, D. H., Gray, E. H., ... Miller, C. C. (2016). ALS/FTD-associated FUS activates GSK-3 β to disrupt the VAPB–PTPIP51 interaction and ER–mitochondria associations. *EMBO Reports*, *17*(9), 1326–1342. <https://doi.org/10.15252/embr.201541726>
- Streisinger, G., Walker, C., Dower, N., Knauber, D., & Singer, F. (1981). Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). *Nature*, *291*(5813), 293–296.

<https://doi.org/10.1038/291293a0>

- Strong, M. J., Hudson, A. J., & Alvord, W. G. (1991). *Familial amyotrophic lateral sclerosis, 1850-1989: a statistical analysis of the world literature*. *18*(1), 45–58.
- Stys, P. K. (1998). Anoxic and ischemic injury of myelinated axons in CNS white matter: From mechanistic concepts to therapeutics. *Journal of Cerebral Blood Flow and Metabolism*, Vol. 18, pp. 2–25. <https://doi.org/10.1097/00004647-199801000-00002>
- Stys, P. K. (2005). General mechanisms of axonal damage and its prevention. *Journal of the Neurological Sciences*, *233*(1–2), 3–13. <https://doi.org/10.1016/j.jns.2005.03.031>
- Stys, P. K. (2007, July). Sodium channel blockers as neuroprotectants in neuroinflammatory disease: A double-edged sword. *Annals of Neurology*, *62*(1), 3–5. <https://doi.org/10.1002/ana.21188>
- Sullivan, P. M., Zhou, X., Robins, A. M., Paushter, D. H., Kim, D., Smolka, M. B., & Hu, F. (2016). The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. *Acta Neuropathologica Communications*, *4*(1), 51. <https://doi.org/10.1186/s40478-016-0324-5>
- Sun, S., Ling, S. C., Qiu, J., Albuquerque, C. P., Zhou, Y., Tokunaga, S., ... Cleveland, D. W. (2015). ALS-causative mutations in FUS/TLS confer gain and loss of function by altered association with SMN and U1-snRNP. *Nature Communications*, *6*, 1–14. <https://doi.org/10.1038/ncomms7171>
- Sun, Z., Diaz, Z., Fang, X., Hart, M. P., Chesi, A., Shorter, J., & Gitler, A. D. (2011). Molecular determinants and genetic modifiers of aggregation and toxicity for the als disease protein fus/tls. *PLoS Biology*, *9*(4). <https://doi.org/10.1371/journal.pbio.1000614>
- Sundaramoorthy, V., Walker, A. K., Yerbury, J., Soo, K. Y., Farg, M. A., Hoang, V., ... Atkin, J. D. (2013). Extracellular wildtype and mutant SOD1 induces ER-Golgi pathology characteristic of amyotrophic lateral sclerosis in neuronal cells. *Cellular and Molecular Life Sciences*, *70*(21), 4181–4195. <https://doi.org/10.1007/s00018-013-1385-2>
- Taetzsch, T., Tenga, M. J., & Valdez, G. (2017). Muscle Fibers Secrete FGFBP1 to Slow Degeneration of Neuromuscular Synapses during Aging and Progression of ALS. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(1), 70–82. <https://doi.org/10.1523/JNEUROSCI.2992-16.2016>
- Takahama, K., Takada, A., Tada, S., Shimizu, M., Sayama, K., Kurokawa, R., & Oyoshi, T. (2013). Regulation of telomere length by G-quadruplex telomere DNA- and TERRA-binding protein TLS/FUS. *Chemistry and Biology*, *20*(3), 341–350. <https://doi.org/10.1016/j.chembiol.2013.02.013>
- Talbot, J., & Maves, L. (2016, July 1). Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease. *Wiley Interdisciplinary Reviews: Developmental Biology*, Vol. 5, pp. 518–534. <https://doi.org/10.1002/wdev.230>
- Tamura, N., Kuwabara, S., Misawa, S., Kanai, K., Nakata, M., Sawai, S., & Hattori, T. (2006). Increased nodal persistent Na⁺ currents in human neuropathy and motor neuron disease estimated by latent addition. *Clinical Neurophysiology*, *117*(11), 2451–2458. <https://doi.org/https://doi.org/10.1016/j.clinph.2006.07.309>
- Tan, A. Y., & Manley, J. L. (2009). The TET family of proteins: Functions and roles in disease. *Journal of Molecular Cell Biology*, *1*(2), 82–92. <https://doi.org/10.1093/jmcb/mjp025>
- Tan, A. Y., Riley, T. R., Coady, T., Bussemaker, H. J., & Manley, J. L. (2012). TLS / FUS (translocated in liposarcoma / fused in sarcoma) regulates target gene transcription via single-stranded DNA response elements. *Pnas*, *109*(16), 6030–6035.

<https://doi.org/10.1073/pnas.1203028109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1203028109>

- Tan, W., Nanche, N., Bogush, A., Pedrini, S., Trotti, D., & Pasinelli, P. (2013). Small peptides against the mutant SOD1/Bcl-2 toxic mitochondrial complex restore mitochondrial function and cell viability in mutant SOD1-mediated ALS. *Journal of Neuroscience*, *33*(28), 11588–11598. <https://doi.org/10.1523/JNEUROSCI.5385-12.2013>
- Tashiro, Y., Urushitani, M., Inoue, H., Koike, M., Uchiyama, Y., Komatsu, M., ... Takahashia, R. (2012). Motor neuron-specific disruption of proteasomes, but not autophagy, replicates amyotrophic lateral sclerosis. *Journal of Biological Chemistry*, *287*(51), 42984–42994. <https://doi.org/10.1074/jbc.M112.417600>
- Teuling, E., Ahmed, S., Haasdijk, E., Demmers, J., Steinmetz, M. O., Akhmanova, A., ... Hoogenraad, C. C. (2007). Motor neuron disease-associated mutant vesicle-associated membrane protein-associated protein (VAP) B recruits wild-type VAPs into endoplasmic reticulum-derived tubular aggregates. *Journal of Neuroscience*, *27*(36), 9801–9815. <https://doi.org/10.1523/JNEUROSCI.2661-07.2007>
- Theuns, J., Verstraeten, A., Sleegers, K., Wauters, E., Gijssels, I., Smolders, S., ... Van Broeckhoven, C. (2014). Global investigation and meta-analysis of the C9orf72 (G₄C₂)_n repeat in Parkinson disease. *Neurology*, *83*(21), 1906 LP – 1913. <https://doi.org/10.1212/WNL.0000000000001012>
- Tolino, M., Köhrmann, M., & Kiebler, M. A. (2012). RNA-binding proteins involved in RNA localization and their implications in neuronal diseases. *European Journal of Neuroscience*, *35*(12), 1818–1836. <https://doi.org/10.1111/j.1460-9568.2012.08160.x>
- Tollervey, J. R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., ... Ule, J. (2011a). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nature Neuroscience*, *14*(4), 452–458. <https://doi.org/10.1038/nn.2778>
- Tollervey, J. R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., ... Ule, J. (2011b). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. *Nature Neuroscience*, *14*(4), 452–458. <https://doi.org/10.1038/nn.2778>
- Tradewell, M. L., Yu, Z., Tibshirani, M., Boulanger, M. C., Durham, H. D., & Richard, S. (2012). Arginine methylation by prmt1 regulates nuclear-cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations. *Human Molecular Genetics*, *21*(1), 136–149. <https://doi.org/10.1093/hmg/ddr448>
- Traynor, B. J., Codd, M. B., Corr, B., Forde, C., Frost, E., & Hardiman, O. (2000). Amyotrophic Lateral Sclerosis Mimic Syndromes. *Archives of Neurology*, *57*(1), 109. <https://doi.org/10.1001/archneur.57.1.109>
- Traynor, Bryan J., Codd, M. B., Corr, B., Forde, C., Frost, E., & Hardiman, O. M. (2000). Clinical Features of Amyotrophic Lateral Sclerosis According to the El Escorial and Airlie House Diagnostic Criteria. *Archives of Neurology*, *57*(8), 1171. <https://doi.org/10.1001/archneur.57.8.1171>
- Tsujimoto, Y., & Shimizu, S. (2007). Role of the mitochondrial membrane permeability transition in cell death. *Apoptosis*, *12*(5), 835–840. <https://doi.org/10.1007/s10495-006-0525-7>
- Tucker, R. P. (1990). The roles of microtubule-associated proteins in brain morphogenesis: a review. *Brain Research Reviews*, Vol. 15, pp. 101–120. [https://doi.org/10.1016/0165-0173\(90\)90013-E](https://doi.org/10.1016/0165-0173(90)90013-E)
- Turner, M R, Cagnin, A., Turkheimer, F. E., Miller, C. C. J., Shaw, C. E., Brooks, D. J., ... Banati, R. B. (2004). Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis:

- an [11C](R)-PK11195 positron emission tomography study. *Neurobiology of Disease*, 15(3), 601–609. <https://doi.org/10.1016/j.nbd.2003.12.012>
- Turner, Martin R, & Modo, M. (2010). Advances in the application of MRI to amyotrophic lateral sclerosis. *Expert Opinion on Medical Diagnostics*, 4(6), 483–496. <https://doi.org/10.1517/17530059.2010.536836>
- Udagawa, T., Fujioka, Y., Tanaka, M., Honda, D., Yokoi, S., Riku, Y., ... Sobue, G. (2015). FUS regulates AMPA receptor function and FTL/ALS-associated behaviour via GluA1 mRNA stabilization. *Nature Communications*, 6(May), 1–13. <https://doi.org/10.1038/ncomms8098>
- Umeda, T., Yamashita, T., Kimura, T., Ohnishi, K., Takuma, H., Ozeki, T., ... Mori, H. (2013). Neurodegenerative disorder FTDP-17-related tau intron 10 +16C → T mutation increases tau exon 10 splicing and causes tauopathy in transgenic mice. *The American Journal of Pathology*, 183(1), 211–225. <https://doi.org/10.1016/j.ajpath.2013.03.015>
- Urbani, A., & Belluzzi, O. (2000). Riluzole inhibits the persistent sodium current in mammalian CNS neurons. *European Journal of Neuroscience*, 12(10), 3567–3574. <https://doi.org/10.1046/j.1460-9568.2000.00242.x>
- Urushitani, M., Kurisu, J., Tsukita, K., & Takahashi, R. (2002). Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. *Journal of Neurochemistry*, 83(5), 1030–1042. <https://doi.org/10.1046/j.1471-4159.2002.01211.x>
- Vajda, A., McLaughlin, R. L., Heverin, M., Thorpe, O., Abrahams, S., Al-Chalabi, A., & Hardiman, O. (2017). Genetic testing in ALS: A survey of current practices. *Neurology*, 88(10), 991–999. <https://doi.org/10.1212/WNL.0000000000003686>
- Van Damme, P., Robberecht, W., & Van Den Bosch, L. (2017). Modelling amyotrophic lateral sclerosis: progress and possibilities. *Disease Models & Mechanisms*, 10(5), 537–549. <https://doi.org/10.1242/dmm.029058>
- Van Den Bosch, L., Vandenberghe, W., Klaassen, H., Van Houtte, E., & Robberecht, W. (2000). Ca²⁺-permeable AMPA receptors and selective vulnerability of motor neurons. *Journal of the Neurological Sciences*, 180(1–2), 29–34. [https://doi.org/10.1016/S0022-510X\(00\)00414-7](https://doi.org/10.1016/S0022-510X(00)00414-7)
- van Dis, V., Kuijpers, M., Haasdijk, E. D., Teuling, E., Oakes, S. A., Hoogenraad, C. C., & Jaarsma, D. (2014). Golgi fragmentation precedes neuromuscular denervation and is associated with endosome abnormalities in SOD1-ALS mouse motor neurons. *Acta Neuropathologica Communications*, 2(1). <https://doi.org/10.1186/2051-5960-2-38>
- Van Es, M. A., Veldink, J. H., Saris, C. G. J., Blauw, H. M., Van Vught, P. W. J., Birve, A., ... Van Den Berg, L. H. (2009). Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nature Genetics*, 41(10), 1083–1087. <https://doi.org/10.1038/ng.442>
- Van Hoecke, A., Schoonaert, L., Lemmens, R., Timmers, M., Staats, K. A., Laird, A. S., ... Robberecht, W. (2012). EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. *Nature Medicine*, 18(9), 1418–1422. <https://doi.org/10.1038/nm.2901>
- Van Langenhove, T., Piguet, O., Burrell, J. R., Leyton, C., Foxe, D., Abela, M., ... Hodges, J. R. (2017). Predicting Development of Amyotrophic Lateral Sclerosis in Frontotemporal Dementia. *Journal of Alzheimer's Disease*, 58(1), 163–170. <https://doi.org/10.3233/JAD-161272>
- Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K. J., Nishimura, A. L., Sreedharan, J., ... Shaw, C. E. (2009). Mutations in FUS, an RNA Processing Protein, Cause Familial Amyotrophic Lateral Sclerosis Type 6. *Science*, 323(5918), 1208–1211. <https://doi.org/10.1126/science.1165942>
- Vance, Caroline, Al-Chalabi, A., Ruddy, D., Smith, B. N., Hu, X., Sreedharan, J., ... Shaw, C. E.

- (2006). Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. *Brain*, 129(4), 868–876. <https://doi.org/10.1093/brain/awl030>
- Vance, Caroline, Scotter, E. L., Nishimura, A. L., Troakes, C., Mitchell, J. C., Kathe, C., ... Shaw, C. E. (2013). ALS mutant FUS disrupts nuclear localization and sequesters wild-type FUS within cytoplasmic stress granules. *Human Molecular Genetics*, 22(13), 2676–2688. <https://doi.org/10.1093/hmg/ddt117>
- Vance et al. (2009). *Mutations in FUS, an RNA Processing Protein, Cause Familial Amyotrophic Lateral Sclerosis Type 6*. 323(February), 1208–1212.
- Vande Velde, C., McDonald, K. K., Boukhedimi, Y., McAlonis-Downes, M., Lobsiger, C. S., Hadj, S. B., ... Cleveland, D. W. (2011). Misfolded SOD1 associated with motor neuron mitochondria alters mitochondrial shape and distribution prior to clinical onset. *PLoS ONE*, 6(7). <https://doi.org/10.1371/journal.pone.0022031>
- Vijayvergiya, C., Beal, M. F., Buck, J., & Manfredi, G. (2005). Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. *Journal of Neuroscience*, 25(10), 2463–2470. <https://doi.org/10.1523/JNEUROSCI.4385-04.2005>
- Vincenti, M. P., & Brinckerhoff, C. E. (2007). Signal transduction and cell-type specific regulation of matrix metalloproteinase gene expression: can MMPs be good for you? *Journal of Cellular Physiology*, 213(2), 355–364. <https://doi.org/10.1002/jcp.21208>
- Vucic, S., & Kiernan, M. C. (2006a). Axonal excitability properties in amyotrophic lateral sclerosis. *Clinical Neurophysiology*, 117(7), 1458–1466. <https://doi.org/https://doi.org/10.1016/j.clinph.2006.04.016>
- Vucic, S., & Kiernan, M. C. (2006b). Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. *Brain*, 129(9), 2436–2446. <https://doi.org/10.1093/brain/awl172>
- Vucic, S., & Kiernan, M. C. (2010). Upregulation of persistent sodium conductances in familial ALS. *Journal of Neurology, Neurosurgery & Psychiatry*, 81(2), 222 LP – 227. <https://doi.org/10.1136/jnnp.2009.183079>
- Vucic, S., Nicholson, G. A., & Kiernan, M. C. (2008). Cortical hyperexcitability may precede the onset of familial amyotrophic lateral sclerosis. *Brain*, 131(6), 1540–1550. <https://doi.org/10.1093/brain/awn071>
- Vucic, S., Rothstein, J. D., & Kiernan, M. C. (2014). Advances in treating amyotrophic lateral sclerosis: insights from pathophysiological studies. *Trends in Neurosciences*, 37(8), 433–442. <https://doi.org/10.1016/j.tins.2014.05.006>
- Wahl, M. C., Will, C. L., & Lührmann, R. (2009, February 20). The Spliceosome: Design Principles of a Dynamic RNP Machine. *Cell*, Vol. 136, pp. 701–718. <https://doi.org/10.1016/j.cell.2009.02.009>
- Wainger, B. J., Kiskinis, E., Mellin, C., Wiskow, O., Han, S. S. W., Sandoe, J., ... Woolf, C. J. (2014). Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Reports*, 7(1), 1–11. <https://doi.org/10.1016/j.celrep.2014.03.019>
- Walogorsky, M., Mongeon, R., Wen, H., Nelson, N. R., Urban, J. M., Ono, F., ... Brehm, P. (2012). Zebrafish model for congenital myasthenic syndrome reveals mechanisms causal to developmental recovery. *Proceedings of the National Academy of Sciences of the United States of America*, 109(43), 17711–17716. <https://doi.org/10.1073/pnas.1215858109>
- Walsh, M. J., Cooper-Knock, J., Dodd, J. E., Stopford, M. J., Mihaylov, S. R., Kirby, J., ... Hautbergue, G. M. (2015). Invited Review: Decoding the pathophysiological mechanisms that underlie RNA dysregulation in neurodegenerative disorders: A review of the current state of the

art. *Neuropathology and Applied Neurobiology*, 41(2), 109–134.
<https://doi.org/10.1111/nan.12187>

- Wang, J. W., Brent, J. R., Tomlinson, A., Shneider, N. A., & McCabe, B. D. (2011). The ALS-associated proteins FUS and TDP-43 function together to affect *Drosophila* locomotion and life span. *Journal of Clinical Investigation*, 121(10), 4118–4126. <https://doi.org/10.1172/JCI57883>
- Wang, W.-Y., Pan, L., Su, S. C., Quinn, E. J., Sasaki, M., Jimenez, J. C., ... Tsai, L.-H. (2013). Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. *Nature Neuroscience*, 16, 1383.
- Wang, W., Li, L., Lin, W. L., Dickson, D. W., Petrucelli, L., Zhang, T., & Wang, X. (2013a). The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. *Human Molecular Genetics*, 22(23), 4706–4719. <https://doi.org/10.1093/hmg/ddt319>
- Wang, W., Li, L., Lin, W. L., Dickson, D. W., Petrucelli, L., Zhang, T., & Wang, X. (2013b). The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. *Human Molecular Genetics*, 22(23), 4706–4719. <https://doi.org/10.1093/hmg/ddt319>
- Wang, W., Wang, L., Lu, J., Siedlak, S. L., Fujioka, H., Liang, J., ... Wang, X. (2016). The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. *Nature Medicine*, 22, 869.
- Wang, W. Y., Pan, L., Su, S. C., Quinn, E. J., Sasaki, M., Jimenez, J. C., ... Tsai, L. H. (2013). Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. *Nature Neuroscience*, 16(10), 1383–1391. <https://doi.org/10.1038/nn.3514>
- Wang, Xiangting, Arai, S., Song, X., Reichart, D., Du, K., Pascual, G., ... Kurokawa, R. (2008). Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. *Nature*, 454(7200), 126–130. <https://doi.org/10.1038/nature06992>
- Wang, Xueyin, Schwartz, J. C., & Cech, T. R. (2015). Nucleic acid-binding specificity of human FUS protein. *Nucleic Acids Research*, 43(15), 7535–7543. <https://doi.org/10.1093/nar/gkv679>
- Wang, Y., Wang, J., Gao, L., Lafyatis, R., Stamm, S., & Andreadis, A. (2005). Tau exons 2 and 10, which are misregulated in neurodegenerative diseases, are partly regulated by silencers which bind a SRp30c.SRp55 complex that either recruits or antagonizes htra2beta1. *The Journal of Biological Chemistry*, 280(14), 14230–14239. <https://doi.org/10.1074/jbc.M413846200>
- Waxman, S. G. (2006, December). Axonal conduction and injury in multiple sclerosis: The role of sodium channels. *Nature Reviews Neuroscience*, Vol. 7, pp. 932–941.
<https://doi.org/10.1038/nrn2023>
- Weidberg, H., & Elazar, Z. (2011). TBK1 mediates crosstalk between the innate immune response and autophagy. *Science Signaling*, 4(187), pe39. <https://doi.org/10.1126/scisignal.2002355>
- Weingarten, M. D., Lockwood, A. H., Hwo, S. Y., & Kirschner, M. W. (1975). A protein factor essential for microtubule assembly. *Proceedings of the National Academy of Sciences of the United States of America*, 72(5), 1858–1862. <https://doi.org/10.1073/pnas.72.5.1858>
- Weiss, M. D., Macklin, E. A., Simmons, Z., Knox, A. S., Greenblatt, D. J., Atassi, N., ... Cudkowicz, M. E. (2016). A randomized trial of mexiletine in ALS. *Neurology*, 86(16), 1474 LP – 1481. <https://doi.org/10.1212/WNL.0000000000002507>
- Wienholds, E., Schulte-Merker, S., Walderich, B., & Plasterk, R. H. A. (2002). Target-selected inactivation of the zebrafish rag1 gene. *Science (New York, N.Y.)*, 297(5578), 99–102. <https://doi.org/10.1126/science.1071762>
- Wienholds, E., van Eeden, F., Kosters, M., Mudde, J., Plasterk, R. H. A., & Cuppen, E. (2003). Efficient target-selected mutagenesis in zebrafish. *Genome Research*, 13(12), 2700–2707. <https://doi.org/10.1101/gr.1725103>

- Williams, A. H., Valdez, G., Moresi, V., Qi, X., McAnally, J., Elliott, J. L., ... Olson, E. N. (2009). MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. *Science*, 326(5959), 1549–1554. <https://doi.org/10.1126/science.1181046>
- Wils, H., Kleinberger, G., Janssens, J., Pereson, S., Joris, G., Cuijt, I., ... Kumar-Singh, S. (2010). TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proceedings of the National Academy of Sciences*, 107(8), 3858–3863. <https://doi.org/10.1073/pnas.0912417107>
- Wilson, B. W., Linkhart, T. A., Walker, C. R., & Nieberg, P. S. (1973). Tissue acetylcholinesterase in plasma of chick embryos and dystrophic chickens. *Journal of the Neurological Sciences*, 18(3), 333–350. [https://doi.org/10.1016/0022-510X\(73\)90082-8](https://doi.org/10.1016/0022-510X(73)90082-8)
- Wong, M., & Martin, L. J. (2010). Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice. *Human Molecular Genetics*, 19(11), 2284–2302. <https://doi.org/10.1093/hmg/ddq106>
- Wong, Y. C., & Holzbaur, E. L. F. (2014). Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proceedings of the National Academy of Sciences of the United States of America*, 111(42), E4439–48. <https://doi.org/10.1073/pnas.1405752111>
- Woo, J.-A. A., Liu, T., Trotter, C., Fang, C. C., De Narvaez, E., LePochat, P., ... Kang, D. E. (2017). Loss of function CHCHD10 mutations in cytoplasmic TDP-43 accumulation and synaptic integrity. *Nature Communications*, 8, 15558. <https://doi.org/10.1038/ncomms15558>
- Wood, J. G., Mirra, S. S., Pollock, N. J., & Binder, L. I. (1986). Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau). *Proceedings of the National Academy of Sciences of the United States of America*, 83(11), 4040–4043. <https://doi.org/10.1073/pnas.83.11.4040>
- Wu, H., Xiong, W. C., & Mei, L. (2010). To build a synapse: signaling pathways in neuromuscular junction assembly. *Development*, 137(7), 1017–1033. <https://doi.org/10.1242/dev.038711>
- Wu, L. S., Cheng, W. C., & Shen, C. K. J. (2012). Targeted depletion of TDP-43 expression in the spinal cord motor neurons leads to the development of amyotrophic lateral sclerosis-like phenotypes in mice. *Journal of Biological Chemistry*, 287(33), 27335–27344. <https://doi.org/10.1074/jbc.M112.359000>
- Wu, L. S., Cheng, W., Hou, S. C., Yan, Y. T., Jiang, S. T., & Shen, C. K. J. (2010). TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. *Genesis*, 48(1), 56–62. <https://doi.org/10.1002/dvg.20584>
- Xia, C. H., Roberts, E. A., Her, L. S., Liu, X., Williams, D. S., Cleveland, D. W., & Goldstein, L. S. B. (2003). Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. *Journal of Cell Biology*, 161(1), 55–66. <https://doi.org/10.1083/jcb.200301026>
- Xia, Q., Wang, H., Hao, Z., Fu, C., Hu, Q., Gao, F., ... Wang, G. (2016). TDP-43 loss of function increases TFEB activity and blocks autophagosome-lysosome fusion. *The EMBO Journal*, 35(2), 121–142. <https://doi.org/10.15252/embj.201591998>
- Xiao, S., Sanelli, T., Dib, S., Sheps, D., Findlater, J., Bilbao, J., ... Robertson, J. (2011). RNA targets of TDP-43 identified by UV-CLIP are deregulated in ALS. *Molecular and Cellular Neuroscience*, 47(3), 167–180. <https://doi.org/10.1016/j.mcn.2011.02.013>
- Xie, J., Marusich, M. F., Souda, P., Whitelegge, J., & Capaldi, R. A. (2007). The mitochondrial inner membrane protein Mitofilin exists as a complex with SAM50, metaxins 1 and 2, coiled-coil-helix coiled-coil-helix domain-containing protein 3 and 6 and DnaJC11. *FEBS Letters*, 581(18), 3545–3549. <https://doi.org/10.1016/j.febslet.2007.06.052>

- Xu, W., & Xu, J. (2018). C9orf72 Dipeptide Repeats Cause Selective Neurodegeneration and Cell-Autonomous Excitotoxicity in *Drosophila* Glutamatergic Neurons. *Journal of Neuroscience*, 38(35), 7741–7752. <https://doi.org/10.1523/JNEUROSCI.0908-18.2018>
- Xu, Y. F., Zhang, Y. J., Lin, W. L., Cao, X., Stetler, C., Dickson, D. W., ... Petrucelli, L. (2011). Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. *Molecular Neurodegeneration*, 6(1). <https://doi.org/10.1186/1750-1326-6-73>
- Yamaguchi, A., & Kitajo, K. (2012a). The Effect of PRMT1-Mediated Arginine Methylation on the Subcellular Localization, Stress Granules, and Detergent-Insoluble Aggregates of FUS/TLS. *PLoS ONE*, 7(11). <https://doi.org/10.1371/journal.pone.0049267>
- Yamaguchi, A., & Kitajo, K. (2012b). The Effect of PRMT1-Mediated Arginine Methylation on the Subcellular Localization, Stress Granules, and Detergent-Insoluble Aggregates of FUS/TLS. *PLoS ONE*, 7(11). <https://doi.org/10.1371/journal.pone.0049267>
- Yamanaka, K., Chun, S. J., Boillee, S., Fujimori-Tonou, N., Yamashita, H., Gutmann, D. H., ... Cleveland, D. W. (2008). Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature Neuroscience*, 11, 251.
- Yamazaki, T., Chen, S., Yu, Y., Yan, B., Haertlein, T. C., Carrasco, M. A., ... Reed, R. (2012). FUS-SMN Protein Interactions Link the Motor Neuron Diseases ALS and SMA. *Cell Reports*, 2(4), 799–806. <https://doi.org/10.1016/j.celrep.2012.08.025>
- Yan, J., Deng, H. X., Siddique, N., Fecto, F., Chen, W., Yang, Y., ... Siddique, T. (2010). Frameshift and novel mutations in FUS in familial amyotrophic lateral sclerosis and ALS/dementia. *Neurology*, 75(9), 807–814. <https://doi.org/10.1212/WNL.0b013e3181f07e0c>
- Yang, L., Embree, L. J., Tsai, S., & Hickstein, D. D. (1998). Oncoprotein TLS interacts with serine-arginine proteins involved in RNA splicing. *The Journal of Biological Chemistry*, 273(43), 27761–27764. <https://doi.org/10.1074/jbc.273.43.27761>
- Yang, Liuqing, Gal, J., Chen, J., & Zhu, H. (2014). Self-assembled FUS binds active chromatin and regulates gene transcription. *Proceedings of the National Academy of Sciences*, 111(50), 17809 LP – 17814. <https://doi.org/10.1073/pnas.1414004111>
- Yang, X., Li, W., Prescott, E. D., Burden, S. J., & Wang, J. C. (2000). DNA topoisomerase IIbeta and neural development. *Science (New York, N.Y.)*, 287(5450), 131–134. <https://doi.org/10.1126/science.287.5450.131>
- Yasuda, K., Zhang, H., Loisel, D., Haystead, T., Macara, I. G., & Mili, S. (2013). The RNA-binding protein Fus directs translation of localized mRNAs in APC-RNP granules. *Journal of Cell Biology*, 203(5), 737–746. <https://doi.org/10.1083/jcb.201306058>
- Yin, H. Z., Nalbandian, A., Hsu, C. I., Li, S., Llewellyn, K. J., Mozaffar, T., ... Weiss, J. H. (2012). Slow development of ALS-like spinal cord pathology in mutant valosin-containing protein gene knock-in mice. *Cell Death and Disease*, 3(8). <https://doi.org/10.1038/cddis.2012.115>
- Yin, X., Jin, N., Shi, J., Zhang, Y., Wu, Y., Gong, C. X., ... Liu, F. (2017). Dyrk1A overexpression leads to increase of 3R-tau expression and cognitive deficits in Ts65Dn Down syndrome mice. *Scientific Reports*, 7(1), 1–12. <https://doi.org/10.1038/s41598-017-00682-y>
- Yokota, O., Tsuchiya, K., Terada, S., Ishizu, H., Uchikado, H., Ikeda, M., ... Akiyama, H. (2008). Basophilic inclusion body disease and neuronal intermediate filament inclusion disease: A comparative clinicopathological study. *Acta Neuropathologica*, 115(5), 561–575. <https://doi.org/10.1007/s00401-007-0329-z>
- Yoshida, M. (2006). Cellular tau pathology and immunohistochemical study of tau isoforms in sporadic tauopathies. *Neuropathology*, 26(5), 457–470. <https://doi.org/10.1111/j.1440-1789.2006.00743.x>

- Yoshimura, A., Fujii, R., Watanabe, Y., Okabe, S., Fukui, K., & Takumi, T. (2006). Myosin-Va Facilitates the Accumulation of mRNA/Protein Complex in Dendritic Spines. *Current Biology*, 16(23), 2345–2351. <https://doi.org/10.1016/j.cub.2006.10.024>
- Yu, Y., & Reed, R. (2015). FUS functions in coupling transcription to splicing by mediating an interaction between RNAP II and U1 snRNP. *Proceedings of the National Academy of Sciences of the United States of America*, 112(28), 8608–8613. <https://doi.org/10.1073/pnas.1506282112>
- Yung, C., Sha, D., Li, L., & Chin, L. S. (2016). Parkin Protects Against Misfolded SOD1 Toxicity by Promoting Its Aggresome Formation and Autophagic Clearance. *Molecular Neurobiology*, 53(9), 6270–6287. <https://doi.org/10.1007/s12035-015-9537-z>
- Zerbes, R. M., Bohnert, M., Stroud, D. A., Von Der Malsburg, K., Kram, A., Oeljeklaus, S., ... Van Der Laan, M. (2012). Role of MINOS in mitochondrial membrane architecture: Cristae morphology and outer membrane interactions differentially depend on mitofilin domains. *Journal of Molecular Biology*, 422(2), 183–191. <https://doi.org/10.1016/j.jmb.2012.05.004>
- Zhang, G., Neubert, T. A., & Jordan, B. A. (2012). RNA binding proteins accumulate at the postsynaptic density with synaptic activity. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 32(2), 599–609. <https://doi.org/10.1523/JNEUROSCI.2463-11.2012>
- Zhang, H., Tan, C.-F., Mori, F., Tanji, K., Kakita, A., Takahashi, H., & Wakabayashi, K. (2008). TDP-43-immunoreactive neuronal and glial inclusions in the neostriatum in amyotrophic lateral sclerosis with and without dementia. *Acta Neuropathologica*, 115(1), 115–122. <https://doi.org/10.1007/s00401-007-0285-7>
- Zhang, J., Lefebvre, J. L., Zhao, S., & Granato, M. (2004). Zebrafish unplugged reveals a role for muscle-specific kinase homologs in axonal pathway choice. *Nature Neuroscience*, 7(12), 1303–1309. <https://doi.org/10.1038/nn1350>
- Zhang, K., Liu, Q., Shen, D., Tai, H., Liu, S., Wang, Z., ... Zhang, X. (2019). Mutation analysis of KIF5A in Chinese amyotrophic lateral sclerosis patients. *Neurobiology of Aging*, 73, 229.e1–229.e4. <https://doi.org/10.1016/j.neurobiolaging.2018.08.006>
- Zhen, Y., & Stenmark, H. (2015). Cellular functions of Rab GTPases at a glance. *Journal of Cell Science*, 128(17), 3171–3176. <https://doi.org/10.1242/jcs.166074>
- Zhou, Y., Liu, S., Liu, G., Öztürk, A., & Hicks, G. G. (2013a). ALS-Associated FUS Mutations Result in Compromised FUS Alternative Splicing and Autoregulation. *PLoS Genetics*, 9(10). <https://doi.org/10.1371/journal.pgen.1003895>
- Zhou, Y., Liu, S., Liu, G., Öztürk, A., & Hicks, G. G. (2013b). ALS-Associated FUS Mutations Result in Compromised FUS Alternative Splicing and Autoregulation. *PLoS Genetics*, 9(10). <https://doi.org/10.1371/journal.pgen.1003895>
- Zhou, Y., Liu, S., Öztürk, A., & Hicks, G. G. (2014). FUS-regulated RNA metabolism and DNA damage repair. *Rare Diseases*, 2(1), e29515. <https://doi.org/10.4161/rdis.29515>
- Zhou, Z. D., Saw, W. T., & Tan, E. K. (2017). Erratum to: Mitochondrial CHCHD-Containing Proteins: Physiologic Functions and Link with Neurodegenerative Diseases (*Molecular Neurobiology*, (2017), 54, 7, (5534–5546), 10.1007/s12035-016-0099-5). *Molecular Neurobiology*, 54(7), 5547–5549. <https://doi.org/10.1007/s12035-016-0160-4>
- Zinszner, H, Sok, J., Immanuel, D., Yin, Y., & Ron, D. (1997a). TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. *Journal of Cell Science*, 110 (Pt 1), 1741–1750.
- Zinszner, H, Sok, J., Immanuel, D., Yin, Y., & Ron, D. (1997b). TLS (FUS) binds RNA in vivo and engages in nucleo-cytoplasmic shuttling. *Journal of Cell Science*, 110 (Pt 15), 1741–1750. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/9264461>

- Zinszner, Helene, Albalat, R., & Ron, D. (1994). A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. *Genes and Development*, 8(21), 2513–2526. <https://doi.org/10.1101/gad.8.21.2513>
- Zon, L. I., & Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. *Nature Reviews Drug Discovery*, 4(1), 35–44. <https://doi.org/10.1038/nrd1606>
- Zondler, L., Feiler, M. S., Freischmidt, A., Ruf, W. P., Ludolph, A. C., Danzer, K. M., & Weishaupt, J. H. (2017). Impaired activation of ALS monocytes by exosomes. *Immunology and Cell Biology*, 95(2), 207–214. <https://doi.org/10.1038/icb.2016.89>
- Zondler, L., Müller, K., Khalaji, S., Bliedehäuser, C., Ruf, W. P., Grozdanov, V., ... Weishaupt, J. H. (2016). Peripheral monocytes are functionally altered and invade the CNS in ALS patients. *Acta Neuropathologica*, 132(3), 391–411. <https://doi.org/10.1007/s00401-016-1548-y>
- Zorova, L. D., Popkov, V. A., Plotnikov, E. Y., Silachev, D. N., Pevzner, I. B., Jankauskas, S. S., ... Zorov, D. B. (2018). Mitochondrial membrane potential. *Analytical Biochemistry*, 552, 50–59. <https://doi.org/10.1016/j.ab.2017.07.009>

Abbreviations

3

3R 3 tubulin-binding repetitions

4

4R 4 tubulin-binding repetitions

A

ACh acetylcholine

AChE Acetylcholinesterase

AChRs ACh receptors

ALS Amyotrophic Lateral Sclerosis

atp5a1 ATP synthase mitochondrial F1 complex alpha subunit

B

BIBD basophilic inclusion body disease

C

C9orf72 chromosome 9 open reading frame 72

CaM-KIIa Ca²⁺/calmodulin-dependent protein kinase IIa

caz cabeza

CHCHD10 Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 10

CLIP-Seq cross-linking immunoprecipitation (CLIP)-RNA sequencing

cox5ab cytochrome c oxidase subunit Va

CSF cerebrospinal fluid

D

dpf days post-fertilization

DPR dipeptide repeat proteins

E

EMG Electromyogram

ENU N-ethyl--N-nitrosourea

ER endoplasmic reticulum

ERK1/2 extracellular regulated kinases 1 and 2

EURALS European ALS Epidemiology Consortium

EWSR1 Ewing's sarcoma RNA binding protein 1

F

fALS familial ALS

FMR1 fragile X mental retardation 1

FP fasciculation potential

FTD Frontotemporal Dementia

FTLD frontotemporal lobar degeneration cases

FUS fused in sarcoma

H

HDAC4 histone deacetylase 4

hnRNP heterogeneous ribonuclear protein

I

iPS induced pluripotent stem-cell

K

KD knockdown

KIF5A kinesin-5A

KO knock out

L

LMNs lower motor neurons

lncRNAs long non-coding RNAs

LRRK2 Leucine-rich repeat kinase 2

M

MAPT microtubule-associated binding protein Tau

MBNL1 Muscle blind like splicing regulator 1

MICOS mitochondrial contact site

miRNA microRNA

MN motor neurons

MNDs Motor neuron diseases

MRI Magnetic Resonance Imaging

mRNP messenger ribonucleoprotein

N

NCS Nerve Conduction Study

NFTs neurofibrillary tangles

NIFID neuronal intermediate filament inclusion disease

NMJ neuromuscular junction

O

OPTN *optineurin*
Oxr1 *Oxidative Stress Resistance Protein 1*

P

polg1 *polymerase γ*
PRPH *peripherin*

R

ROS *reactive oxygen species*

S

SG *stress granules*
snRNP *small nuclear ribonucleoprotein*
SOD1 *superoxide dismutase 1*
SQSTM1/p62 *sequestosome 1/p62*

T

TAF-15 *Tata-binding protein-associated factor 2N*

TARDBP *transactive response DNA-binding protein*

TEER *touch-evoked escape response*
tk2 *Thymidine kinase*

TSC *terminal Schwann cells*

TUBA4A *tubulin β -4A*

U

UBQLN2 *ubiquilin-2*

UMNs *upper motor neurons*

UPS *ubiquitin proteasome system*

V

VCP *valosin-containing protein*

 Δ

$\Delta\Psi_m$ *mitochondrial membrane potential*

