Autophagy Dysfunction in ALS: from Transport to Protein Degradation

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Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting upper and lower motor neurons (MNs). Since the identification of the first ALS mutation in 1993, more than 40 genes have been associated with the disorder. The most frequent genetic causes of ALS are represented by mutated genes whose products challenge proteostasis, becoming unable to properly fold and consequently aggregating into inclusions that impose proteotoxic stress on affected cells. In this context, increasing evidence supports the central role played by autophagy dysfunctions in the pathogenesis of ALS. Indeed, in early stages of disease, high levels of proteins involved in autophagy are present in ALS MNs; but at the same time, with neurodegeneration progression, autophagy-mediated degradation decreases, often as a result of the accumulation of toxic protein aggregates in affected cells. Autophagy is a complex multistep pathway that has a central role in maintaining cellular homeostasis. Several proteins are involved in its tight regulation, and importantly a relevant fraction of ALS-related genes encodes products that directly take part in autophagy, further underlining the relevance of this key protein degradation system in disease onset and progression. In this review, we report the most relevant findings concerning ALS genes whose products are involved in the several steps of the autophagic pathway, from phagophore formation to autophagosome maturation and transport and finally to substrate degradation.

Keywords Amyotrophic lateral sclerosis · Autophagy · Mutations · Animal models

Introduction

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a rare adult motor neuron disease (MND) characterized by degeneration of upper and lower motor neurons (MNs), leading to progressive muscle atrophy and death within 3–5 years after symptom onset. Besides MNs, skeletal muscle (Dobrowolny et al. 2008; Onesto et al. 2011; Cicardi et al. 2018; Meroni et al. 2019) and glial cells (Trotti et al. 1999; Lobsiger et al. 2009; Philips et al. 2013) can be targeted by ALS. Neurons residing in the frontotemporal cortex might be affected, too, which may lead to the development of a mixed form of MND and frontotemporal dementia (FTD) referred to as ALS/FTD (Robberecht and Philips 2013). No drugs are currently available to treat or cure ALS (Taylor et al. 2016).

Most ALS cases (90%) are sporadic (sALS), while only 10% are characterized by familial inheritance (fALS). Clinical symptoms fully overlap between the two forms of the disease, but fALS tends to be more severe compared to sALS. Inherited ALS cases are associated to both loss-of-function (LOF) and aberrant gain-of-function (GOF) mutations, as well as to mixed LOF and GOF, that can be found in more than 40 genes. Nonetheless, around 30% of fALS genetic causes still need to be identified, indicating that ALS is a disease with a widely heterogeneous genetic background (Cristofani et al. 2020). Noteworthily, the wild-type forms of the gene products causing fALS display an aberrant behavior in sALS too, suggesting that the two forms of the disease probably share some pathogenetic mechanisms (Neumann et al. 2006).

ALS is caused by a combination of genetic, epigenetic, and environmental factors which result in different pathogenetic mechanisms capable of triggering neuronal damage (Morgan and Orrell 2016). To date, the precise etiology of ALS is still unknown, as well as the exact molecular mechanisms involved in the degeneration of motor neurons (Shaw
Autophagy includes three distinct pathways that promote and regulate the degradation of substrates via lysosomes, microautophagy (Schuck 2020), chaperone-mediated autophagy (CMA) (Kaushik and Cuervo 2018), and macroautophagy, that includes the chaperone assisted–selective autophagy or CASA (Cristofani et al. 2020). Here we will focus on macroautophagy (hereafter autophagy).

Autophagy is characterized by different steps: initiation, elongation, maturation, and degradation (Klionsky et al. 2021b). The initiation consists in the nucleation of the autophagic membrane (phagophore) and is regulated by different complexes. The first complex involved is the preinitiation complex, that is negatively regulated by the mammalian target of rapamycin (mTOR) pathway and is positively regulated by the AMP-activated protein kinase (AMPK) pathway. It is composed by autophagy-related proteins 13 and 101 (ATG13, ATG101), unc-51 like kinase 1/2 (ULK1/2), and FAK family-interacting protein of 200 kDa (FIP200). Another complex is the phosphoinositide 3-kinase (PI3K) complex composed by ATG14, vacuolar protein sorting-associated proteins 34 and 15 (VPS34, VPS15), and Beclin1 (BECN1) (Matsunaga et al. 2010; Cicchini et al. 2015). The contribution of ALS-related genes in this step is represented in Fig. 1.

On the forming phagophore are recruited other complexes that regulate its elongation and expansion into the autophagosome. These complexes are the ATG7-ATG3-microtubule-associated protein 1A/1B light chain 3B (MAP1LC3B, or simply LC3) complex that promotes the formation of the lipidated active form of LC3 (LC3-II), and the ATG12-ATG5-ATG16L1 complex (Itakura and Mizushima 2010). Autophagic substrate recognition, targeting, and engulfment are highly regulated. Ubiquitinated misfolded proteins and aggregates are specifically recognized by a complex of chaperones and co-chaperones known as the CASA complex. The components of this complex are heat shock protein B8 (HSPB8), Bcl2-associated athanogene 3 (BAG3), heat shock protein 70 (HSP70), and C-terminus of HSC70-interacting protein (CHIP). The substrates are recognized by HSPB8 and BAG3, and subsequently ubiquitinated by CHIP. The CASA complex is then routed to the forming autophagosome through a dynemin-mediated process regulated by BAG3-dynein interaction (Carra et al. 2008). Ubiquitinated substrates are recognized by autophagy receptors such as sequestosome 1 (SQSTM1/p62) or optineurin (OPTN) (Kraft et al. 2010). These proteins act as shuttles mediating substrates to phagophores. Indeed, autophagy receptors, thanks to the specific motifs present in their sequence such as LC3-interacting regions (LIRs), bind components of the autophagic machinery present in the forming autophagosome, such as LC3-II (Rogov et al. 2014). Subsequently to substrate engulfment, the
constituted autophagosome maturation may include fusion with an endosome and always terminates in fusion with a lysosome (Zhong et al. 2009). The importance of various ALS-associated genes in these steps has been highlighted in Figs. 2 and 3.

Strictly correlated to autophagy is the endocytosis pathway where the ALS-related genes described in Fig. 4 exert their function. Indeed, the formed endosome as previously described fuses with autophagosomes contributing to its maturation. The ALS-related genes contribution will be better explained in the dedicated sections.

In neurons, axonal transport plays a relevant role in promoting autophagosome maturation by guiding membrane fusion between autophagy intermediates and components of the endolysosomal pathway (Hollenbeck 2014). Fusion with lysosomes eventually results in the degradation of substrates, thanks to hydrolytic enzymes present in the lysosome lumen (Xu and Ren 2015).

### Table 1 Summary of autophagic genes role in ALS pathogenesis. LOF = loss of function, GOF = gain of function

| Gene       | Role in autophagy and in ALS pathogenesis                                                                 | References                                                                 |
|------------|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| ALS2       | Regulates endosomal maturation → truncating mutations cause rapid ALS2 degradation and abolish its guanine-nucleotide exchange factor activity for RAB5, leading to impairments in endosome maturation (LOF) | Hadano et al. (2001); Yamanaka et al. (2003); Cai et al. (2005)             |
| C9ORF72    | Regulates autophagy initiation and maturation → G4C2 hexanucleotide expansion leads to dipeptide repeats accumulation (GOF) and impaired ULK1 complex formation (LOF) | DeJesus-Hernandez et al. (2011); Koppers et al. (2015)                     |
| CHMP2B     | Modulates ESCRT-III complex assembly to form multivesicular bodies → truncating mutations disrupt autophagosome-lysosome fusion (GOF) | Lee et al. (2007); Han et al. (2012); West et al. (2020)                   |
| DCTN1      | Essential cofactor in dynein-mediated retrograde transport of autophagosomes and lysosomes → mutations are associated to decreased DCTN1 levels and immature autophagosome accumulation (LOF); protein accumulation (GOF) is still debated | Lai et al. (2007); Laird et al. (2008); Ikenaka et al. (2013); Stockmann et al. (2013) |
| FIG4       | Regulates PI(3,5)P2 levels together with PIKfyve and VAC14 to modulate late endosome maturation → mutations lead to enlarged endosomes accumulation (LOF) | Chow et al. (2007); Ferguson et al. (2009); Bharadwaj et al. (2016)         |
| KIF5A      | Transports lysosomes along axons → mutations might impair the autophagic flux (LOF)                         | Liu et al. (2021); Baron et al. (2022)                                     |
| OPTN       | Autophagy receptor → mutations prevent association with ubiquitinated substrates, including mitochondria, and disrupt myosin VI-mediated autophagosome-lysosome fusion (LOF) | Maruyama et al. (2010); Korac et al. (2013); Sundaramoorthy et al. (2015)   |
| SQSTM1/p62 | Autophagy receptor → mutations prevent association with ubiquitinated substrates or interaction with LC3-II (LOF) | Le Ber et al. (2013); Teyssou et al. (2013); Lattante et al. (2015); Goode et al. (2016) |
| TBK1       | Activates autophagy receptors through phosphorylation and promotes autophagosome formation and maturation → truncating mutations reduce TBK1 levels and abolish autophagy receptors activation (LOF) | Ryzhakov and Randow (2007); Freischmidt et al. (2015); Brenner et al. (2019); Duan et al. (2019) |
| TUBA4A     | Forms microtubules → mutations alter microtubule stability, therefore tampering with microtubule-based transport of autophagosomes and lysosomes (LOF), and lead to TUBA4A aggregation (GOF) | Howes et al. (2014); Rademakers and van Blitterswijk (2014); Smith et al. (2014) |
| UBQLN2     | Promotes the autophagic disposal of ubiquitinated ER proteins and regulates autophagy initiation and lysosomal acidification → mutations disrupt substrate recognition and increase autophagy activation while impairing lysosome-mediated degradation (LOF); UBQLN2 aggregation (GOF) is still debated | Deng et al. (2011); Wu et al. (2015); Şentürk et al. (2019)                 |
| VAPB       | Promotes autophagy and mitophagy initiation → mutations alter the autophagic flux (LOF); VAPB aggregation (GOF) is still debated | Teuling et al. (2007); Kuijpers et al. (2013a)                             |
| VCP        | Promotes the autophagic disposal of ubiquitinated substrates and aggresomes, operates in autophagy initiation and maturation, and regulates lysosome homeostasis → mutations aberrantly trigger autophagy but disrupt autophagosome-lysosome fusion (LOF) | Watts et al. (2004); Johnson et al. (2010); Nalbandian et al. (2012)         |
Fig. 1  Schematic representation of the ALS-related gene products involved in autophagy initiation (green) in their conventional intracellular functions.

Autophagy initiation

- SMCR8
- TBK1
- OPTN
- TBK1
- OPTN
- p62
- VCP
- ULK1
- ATG13
- FIP200
- ULK1
- ATG13
- FIP200
- VCP
- LC3-I
- LC3-II
- C9ORF72
- LC3-I
- LC3-II
- C9ORF72
- UBQLN2

Phagophore

Protein aggregate

Damaged mitochondrion

Fig. 2  Schematic representation of the ALS-related gene products involved in autophagosome elongation and maturation (green) in their conventional intracellular functions.

Autophagosome elongation - maturation

- UBQLN2
- LC3-II
- OPTN
- p62
- VCP
- LC3-II
- p62

Phagophore

Protein aggregate

Damaged mitochondrion

Autophagosome

Mature autophagosome

Endosome

Fig. 3  Schematic representation of the ALS-related gene products involved in autophagosome-lysosome fusion and degradation (green) in their conventional intracellular functions.

Autophagosome-lysosome fusion - degradation

- Ubiquitin
- SVIP
- UBQLN2
- C9ORF72
- KIF5A
- TUBA4A
- Dynein
- DCTN1
- HSP6
- PTPP51
- VCP
- VAPB

Mature autophagosome

Autolysosome

Mitochondrion

Endoplasmic reticulum

Nucleus

Springer
Autophagic Genes Involved in ALS

ALS2

Alsin (ALS2) is a guanine-nucleotide exchange factor (GEF) encoded by the gene ALS2 (chromosome 2q33). It is found in all tissues but particularly in neurons (Hadano et al. 2001; Otomo et al. 2003; Yamanaka et al. 2003). ALS2 comprises three GEF-specific domains: an N-terminal regulator of chromatin condensation–like domain (RCC1-like domain, or RLD), a central Db1-pleckstrin homology (DH/PH) domain, and a C-terminal vacuolar protein sorting 9 (VPS9) domain (Hadano et al. 2001; Yang et al. 2001).

ALS2 VPS9 domain mediates its homo-oligomerization and endosomal localization. ALS2 was indeed demonstrated to localize to structures positive for the early endosomal antigen 1 (EEA1) protein, a marker of early endosomes (Otomo et al. 2003; Topp et al. 2004; Kunita et al. 2004). The VPS9 domain is also essential for ALS2 to act as GEF for the small GTPase RAB5, a key player in endosomal membrane fusion (Fig. 4) (Otomo et al. 2003, 2011; Chandran et al. 2007). Transient ALS2 overexpression was shown to promote endosome enlargement and fusion through a RAB5-dependent mechanism in neuronal and non-neuronal cells, confirming ALS2 role in endosomal maturation (Kunita et al. 2004). Furthermore, ALS2 mutations were demonstrated to be associated with alterations in amphisome formation, and ALS2 downregulation was shown to cause a decrease in the autophagic clearance of substrates, suggesting that ALS2 is also involved in autophagosome maturation (Hadano et al. 2010; Otomo et al. 2011, 2012). Additionally, ALS2 was demonstrated to protect cultured murine MNs from the neurotoxic activity exerted by the ALS-related SOD1 mutants A4T, G85R, and G93R. It has been shown that ALS2 exerts such neuroprotective activity by directly binding to mutant SOD1 while contemporarily acting as GEF for the small GTPase Rac1 through its DH/PH domain. Indeed, Rac1 activation upon ALS2 binding triggers an antiapoptotic response mediated by the PI3K Akt3 that preserves neurons from death. Both Rac1 downregulation through RNA interference and deletion of ALS2 DH/PH domain fully abolish such neuroprotective function. Interestingly, ALS2 does not display the same activity against any other proteins implicated in neurodegeneration, such as Alzheimer’s disease-related amyloid-β and presenilin 1/2 mutants (Kanekura et al. 2004, 2005).

ALS2 mutations causing fALS are characterized by a recessive pattern of inheritance and produce premature stop codons in ALS2 sequence that abolish its VPS9 domain (Hadano et al. 2001; Yang et al. 2001; Kress et al. 2005; Sheerin et al. 2014). Truncated ALS2 isoforms are unstable and get rapidly eliminated, so that ALS2 pool is depleted in neurons harboring ALS2 isoforms (Yamanaka et al. 2003). Such loss of ALS2 function is expected to reduce active RAB5 levels, since ALS2-deprived neurons display decreased motility of RAB5-positive endosomes (Lai et al. 2009). Consistently, neurons isolated from Als2 knock-out mice show defective RAB5-dependent endosomal maturation leading to the accumulation of large, EEA1-positive vacuoles. The impairments in the early phases of endosomal trafficking and fusion caused by ALS2 loss increase MN susceptibility to oxidative stress and promote astrogliosis in the brain and in the spinal cord of ALS2-null mice (Cai et al. 2005; Hadano et al. 2006; Devon et al. 2008).
C9ORF72

C9ORF72 protein is encoded by chromosome 9 open reading frame 72 (C9ORF72), which presents 10 coding exons and two non-coding exons (Smeyers et al. 2021). C9ORF72 products are 3 transcript variants: a short transcript V1 and two transcripts V2 and V3 that share the coding of exons 2–11 (DeJesus-Hernandez et al. 2011; Smeyers et al. 2021). Interestingly for the development of animal models, the C9ORF72 human gene is conserved in primates but has a very low similarity in nematode and no orthologue in Drosophila (Therrien et al. 2013; Chen et al. 2017). C9ORF72 expression is found in most tissues including all brain regions, spinal cord, and immune system. At cellular level, C9ORF72 is localized in the cytoplasm or in organelles such as Golgi, mitochondria, lysosome, and other components of the endolysosome pathway. The V1 short isoform was also found localized in the nuclear membrane (Xiao et al. 2015; Atkinson et al. 2015; Aoki et al. 2017; Chitiprolu et al. 2018; Wang et al. 2021). To date, C9ORF72 has been found implicated in different pathways, including nucleocyttoplasmic import, stress granule (SG) formation and degradation or recovery, endosomal trafficking, axon growth, and autophagy regulation (Farg et al. 2014; Zhang et al. 2015; Yang et al. 2016; Amick et al. 2016; Sivadasan et al. 2016; Maharjan et al. 2017).

C9ORF72 regulates autophagy at different steps of its pathway as presented in Figs. 1 and 3. Its role in autophagy is supported by two cofactors, Smith–Magenis chromosome region 8 (SMCR8) and WD repeat domain 41 (WDR41), forming the C9ORF72-complex (Tang et al. 2020; Su et al. 2020). Both C9ORF72 and SMCR8 present a differentially expressed in normal and neoplastic cells (DENN) domain which functions as a GEF for RAB GTPases (Levine et al. 2013; Iyer et al. 2018). In autophagy initiation, its role is difficult to discriminate as C9ORF72 interacts with different complexes involved in this step. Firstly, C9ORF72 and SMCR8 interact with different members of the ULK1-complex (Yang et al. 2016). During starvation C9ORF72-complex is recruited by solute carrier family 6 member 1 (SLC66A1) to lysosomes, increasing the interaction with ULK1-complex (Amick et al. 2020). Moreover, the C9ORF72 complex promotes the interaction between RAB1 and ULK1-complex, enhancing its recruitment to forming autophagosomes (Webster et al. 2016). Another sign of C9ORF72-positive regulation of autophagy initiation stands in its interaction with RAB5 which promotes the delivery of the PI3K and the ATG7-ATG5-LC3 complex to autophagosome (Shi et al. 2018; Bingol 2018). The localization of C9ORF72 in forming autophagosomes was suggested to be mediated by SQSTM1/p62 or OPTN, which bind to C9ORF72-complex and RAB39b, another substrate of the complex (Sellier et al. 2016). While these interactions refer to a positive regulation of C9ORF72 complex in autophagy initiation, another mediates a negative regulation of autophagy. Indeed, signs of the interaction of C9ORF72 with mTOR complex 1 (mTORC1) were detected, which suggests a supporting role in mTORC1 function. C9ORF72 silencing decreases mTORC1 activity, promoting autophagy transcriptional factor regulators activation (Ji et al. 2017; Wang et al. 2020). C9ORF72 is also implicated in autophagosome maturation mainly by cooperating with RAB proteins, such as RAB7 and RAB11, which regulate microtubular transport of autophagosomes, late endosomes, multivesicular bodies, and the fusion with lysosomes (Hyttinen et al. 2013; Ao et al. 2014; Aoki et al. 2017; Tang et al. 2020).

C9ORF72 gene mutation consisting in a hexanucleotide expansion of the sequence GGGGCC (G4C2) present in the first intron has been associated to ALS and FTD in 2011 (Renton et al. 2011; DeJesus-Hernandez et al. 2011). The physiological size of the expansion can reach 24 repeats, whereas above 30 repeats it is considered pathological. The pathological length of expansion is very heterogeneous; indeed, patients generally present hundreds or even thousands of repeats (DeJesus-Hernandez et al. 2011; Gijselinck et al. 2016). Still, the length of the expansion can differ in brain and blood tissues of the same patient. This mosaicism is due to the instability of the repeat number (van Mossevelde et al. 2017). The mutation has an autosomal-dominant transmission and is associated to 40% of fALS, 80% of ALS/FTD, and 25% of familial FTD cases (Gijselinck et al. 2016; van Mossevelde et al. 2017). The mutation leads to both GOF and LOF that together concur in the onset of the pathology. The LOF is due to protein haploinsufficiency that is triggered by the decrease in gene transcription caused by altered RNA structures and by hypermethylation of
the promoter (Haeusler et al. 2014; Gjselinck et al. 2016; Esanov et al. 2017). The GOF is associated to the toxicity of the RNA repeats and the formation of abnormal dipeptide-repeat proteins (DRPs). The RNA repeats are transcribed bidirectionally forming sense and antisense transcripts. These transcripts are instable and form nuclear RNA foci that sequester RNA-binding proteins preventing their functionality (Gendron et al. 2013; Barker et al. 2017). In addition, guanosine-rich RNA repeats tend to fold in a stable secondary structure, known as G-quadruplex, that deleteriously interacts with splicing factors causing splicing errors (Reddy et al. 2013; Conlon et al. 2016). The translation of DPR sense and antisense transcripts occurs in an unconventional ATG-independent mechanism and results in the formation of five DRPs constituted of glycine-alanine (GA), glycine-proline (GP), glycine-arginine (GR), proline-arginine (PR), and proline-alanine (PA) (Zu et al. 2013). All DPRs are found in the brain tissue and spinal cord of patients and exert their toxicity by sequestering proteins, impairing ribosome biogenesis, and altering translation. Specifically, the DPRs poly-PR and poly-GR alter nuclear transport (Freibaum and Taylor 2017; Hayes et al. 2020). In addition, poly-GR also interferes with SGs formation and degradation (Chew et al. 2019). Both GOF and LOF concur in alteration of cellular proteostasis. A sign of altered proteostasis stands in TDP-43 mislocalization and aggregation, which is a hallmark of ALS and FTD C9orf72 patients (Cook et al. 2020). Another sign of altered proteostasis in C9orf72-patients is the presence of SQSTM1/p62 and ubiquitin-positive inclusions that frequently also contain DPRs (Mann et al. 2013).

C9orf72 animal models were developed to mimic LOF and GOF. For what concerns C9orf72 role in autophagy, transient reduction of C9orf72 expression in conditional knock-out mice in neurons does not cause alterations in behavior or motor phenotype (Koppers et al. 2015), whereas constitutive C9orf72 knock-out animals present dysregulation of the immune system probably associated to autophagy dysfunction, reduced survival, and mild motor and cognitive phenotypes (Jiang et al. 2016; Atanasio et al. 2016). Overall, data on animals support the theory that loss of C9orf72 function alone is not sufficient to develop ALS. On the other hand, models of GOF present neuronal alterations.

**CHMP2B**

Charged multivesicular body protein 2B (CHMP2B) is encoded by the gene *CHMP2B* (chromosome 3p11.2). It is an evolutionarily conserved protein playing a key role in the assembly of the endosomal sorting complex required for transport III (ESCRT-III; Fig. 4). The ESCRT-III machinery is implicated in the generation of multivesicular bodies (MVBs), endosomal sorting, and autophagy in several tissues, including all brain regions (Skibinski et al. 2005; Rusten and Stenmark 2009; Hurley and Hanson 2010; Henne et al. 2013).

When inactive, CHMP2B is found in an autoinhibited conformation masking the N-terminal sites required to recruit the ESCRT-III complex. Activation occurs through the interaction of CHMP2B C-terminal domain with the ATPase vacuolar protein sorting-associated protein 4 (VPS4) and triggers the assembly of the ESCRT-III and VPS4 complexes on the endosomal membrane. VPS4 ATPase activity is then required to let intraluminal vesicles bud from endosomal membranes, hence generating MVBs, and to finally dissociate ESCRT-III proteins from one another for MVB generation (Fig. 4). (Schmidt and Teis, 2012).

Apart from being involved in MVB maturation, CHMP2B also participates to lysosomal membrane repair and to autophagy together with the other components of the ESCRT-III machinery (Krasniak and Ahmad 2016; Radulovic et al. 2018). Loss of ESCRT genes was demonstrated to correlate with autophagosome accumulation in yeast (Roudier et al. 2005), *Drosophila* (Rusten et al. 2007), and mammals (Komatsu et al. 2006), implying a key role played by ESCRT proteins in autophagosome-lysosome fusion. Even though the mechanism through which CHMP2B is involved in autophagy is still not clearly defined, CHMP2B mutations are associated to important signs of autophagy impairment.

The first CHMP2B mutation linked to neurodegeneration was found in 1995 in a Danish family affected by autosomal-dominant FTD and was characterized as a substitution occurring at exon 6 splice acceptor site (Brown et al. 1995; Gydesen et al. 2002; Skibinski et al. 2005). Such mutation determines the production of two mutant CHMP2B forms, one including intron 5 in the protein sequence and producing a truncated CHMP2B isoform with a valine residue replacing the last 36 amino acids of the wild-type protein (CHMP2BIntron5) and the other generating a 29-amino acid nonsense sequence (CHMP2B410) (Skibinski et al. 2005). Additional CHMP2B mutations were later identified also in fALS and ALS/FTD (Parkinson et al. 2006; Cox et al. 2010; van Blitterswijk et al. 2012c; Narain et al. 2018).

Truncated CHMP2B mutants are incapable of autoinhibition and tend to associate to the rest of the ESCRT-III machinery at a higher frequency compared to wild-type CHMP2B. This abnormal interaction leads to the formation of aberrant complexes that remain associated to endosomal membranes, thus preventing autophagosome-lysosome fusion (Lee et al. 2007; Han et al. 2012). Indeed, CHMP2BIntron5 was shown to induce the accumulation of ubiquitin-positive puncta and/or SQSTM1/p62- and LC3-positive vacuoles in cells (Lee et al. 2007; Filimonenko et al. 2007; West et al. 2020). Autophagosome accumulation was also observed in murine and *Drosophila* CHMP2BIntron5 models (Ghazi-Noori et al. 2012; Vernay et al. 2016; West et al. 2020) and even in patient-derived fibroblasts and cortical tissue (Urwin et al.
Dynactin subunit 1 (DCTN1, or DCTN1) was shown to localize on accumulating endosomes positive for the small GTPases RAB4, RAB5, and RAB7 in both Drosophila and rat primary neurons (West et al. 2020). Similar observations were made in postmortem brains of fALS patients bearing CHMP2B mutations (T104N, I29V, Q206H), which displayed an accumulation of autophagosomes positive for SQSTM1/p62 and LC3-II (Parkinson et al. 2006; Cox et al. 2010), and the T140N CHMP2B mutant was also shown to localize with other ESCRT-III subunits in RAB5- and RAB7-positive endosomes in cortical neurons (Han et al. 2012). These data support the involvement of endolysosomal and autophagosomal activity dysfunction in CHMP2B-related neurodegeneration.

**DCTN1**

Dynactin subunit 1 (DCTN1, or p150Glued) is the largest subunit of the dynactin complex and is encoded by the gene DCTN1 (chromosome 2p13.1). The dynactin machinery is composed of at least 11 proteins organized into more than 20 subunits (Urnavicius et al. 2015). The dynactin complex interacts with dynein to increase its processivity in microtubule-based retrograde transport and acts as a multifunctional adaptor between dynein and specific cargos, including vesicles, organelles, cytosolic proteins, and mRNA (Karki and Holzbaur 1999; Culver-Hanlon et al. 2006). Inside the dynactin complex, DCTN1 forms dimers that interact with dynein intermediate chains and bind to microtubules through its N-terminal cytoskeletal-associated protein/glycine-rich (CAP-Gly) domain (Vaughan and Vallee 1995; Karki and Holzbaur 1995; Culver-Hanlon et al. 2006).

Considering autophagy, DCTN1 plays a central role in dynein-mediated retrograde transport of autophagosomes and lysosomes to the perinuclear region (Fig. 3). DCTN1 was indeed shown to interact with Hermansky-Pudlak syndrome 6 (HSP6), a protein that is strictly required to drive lysosomes to the microtubule organizing center for degradation (Li et al. 2014).

Based on the current knowledge, it is still unclear whether DCTN1 mutations promote ALS development through GOF or LOF mechanisms. The first pathogenic mutation ever identified in DCTN1 sequence was an autosomal dominant missense associated to a form of hereditary motor neuropathy that primarily affects lower MNs (HMN7B) (Puls et al. 2003). Such mutation produces a G59S substitution in DCTN1 CAP-Gly domain that alters the folding of DCTN1 microtubule-binding domain, reducing the affinity of the mutant protein for microtubules. G59S DCTN1 tends to misfold and form neurotoxic aggregates that were shown to sequester dynein, mitochondria, and TDP-43 (Puls et al. 2003, 2005; Levy et al. 2006; Deshimaru et al. 2021). Additional DCTN1 mutations lying both in DCTN1 CAP-Gly domain and in its dynein-binding domain have then been identified in ALS (Münch et al. 2004; Stockmann et al. 2013; Liu et al. 2014, 2017), including those forms with an FTD component (Münch et al. 2005). Several in vitro studies were performed to characterize the impact of ALS-related DCTN1 mutations on the protein. While some DCTN1 mutants did not evidence any alterations in protein conformation or distribution (Münch et al. 2004; Dixit et al. 2008; Stockmann et al. 2013), other variants identified in sALS patients were shown to form aggregates or abnormal filamentous structures upon overexpression in primary rat MNs (Stockmann et al. 2013). However, most of the DCTN1 mutations causing an aberrant protein behavior are characterized by uncertain patterns of inheritance and can be found also in control populations, suggesting that they might represent risk factors predisposing to ALS, rather than overt pathogenetic variants (Stockmann et al. 2013).

Analyses performed by Jiang and colleagues on postmortem samples revealed that both upper and lower MNs of sALS patients display lower DCTN1 mRNA levels compared to non-ALS controls starting from very early stages of disease progression (Jiang et al. 2005, 2007). Similarly, Kuzma-Kozakiewicz and colleagues evidenced increased DCTN1 mRNA levels but reduced DCTN1 protein levels in the motor cortex of sALS patients compared to the sensory cortex, suggesting that in sALS MNs DCTN1 depletion occurs and cannot be restored by gene expression upregulation, probably due to inefficient translation (Kuzma-Kozakiewicz et al. 2013). In contrast, while homozygous Dctn1 knock-out or G59S DCTN1 knock-in was proven to be embryonic lethal in mice, animals deprived of one Dctn1 allele did not develop MN degeneration (Lai et al. 2007), and neuron-specific Dctn1 knock-out mice only began manifesting neurodegeneration symptoms at 18 months of age (Yu et al. 2018). On the other hand, two heterozygous G59S DCTN1 knock-in strains generated by distinct research groups displayed halved DCTN1 protein levels and no sign of mutant protein aggregation in brain and spinal cord, suggesting that the G59S substitution might lead to rapid protein degradation in vivo, but at the same time, mice started manifesting muscle atrophy due to spinal MN loss at 10 months of age. These symptoms were accompanied by astrogliosis and by the accumulation of cytoskeletal and synaptic vesicle proteins at neuromuscular junctions, probably as a consequence of disrupted retrograde transport (Levy et al. 2006; Lai et al. 2007). Moreover, a third heterozygous G59S DCTN1 murine strain evidenced fast lower MN loss paralleled by impaired ER-to-Golgi vesicular trafficking and by the accumulation of LC3-II- and ubiquitin-positive DCTN1 aggregates in MN cell bodies hinting at an involvement of dysfunctional retrograde transport of autophagosomes in neuronal death (Laird et al. 2008). In line with these observations, knockout of the DCTN1 ortholog in C. elegans,
that further investigation is required.

cohort display reduced penetrance (Osmanovic et al. 2017), so ALS groups, and some of the variants isolated in the European patients, no deleterious to be responsible for 1–3% of ALS cases among European Osmanovic et al. 2017). While ALS-associated ropathies; notably, recessively inherited and were thus referred to as “pale tremor” mice. This phenotype displayed resting tremor accompanied by diluted pigmentation levels. Animals affected by this autosomal recessive mutation therefore resulting in a substantial reduction in Fig4 protein gene, which caused alterations in endolysosomal trafficking. Concerning pale tremor mice, the relevant reduction in Fig4 protein levels dependent on $ETn2\beta$ retrotransposition determined a strong decrease in P(3,5)P2 levels accompanied by the accumulation of large vacuoles harboring ubiquitinated proteins and positive for SQSTM1/p62, LC3-II, and the late-stage endosomal markers lysosomal-associated membrane proteins 1 and 2 (LAMP-1 and LAMP-2) in murine neurons and astrocytes (Ferguson et al. 2009; Lenk et al. 2011). Comparable accumulation of enlarged lysosomes causing the rapid development of severe brain degeneration was observed in both constitutive and neuron-specific Fig4 knock-out murine models (Ferguson et al. 2012), while in Drosophila Fig4 loss correlated with motility impairments and shorter lifespan (Bharadwaj et al. 2016; Kyotani et al. 2016). The decrease in P(3,5)P2 levels associated to Fig4 loss is hypothesized to depend on reduced PIKfyve activity, since Fig4 is required to stabilize the interaction between PIKfyve and VAC14 (Botelho et al. 2008).

FIG4

Factor-induced gene 4 (FIG4) is a magnesium-dependent phosphatase encoded by the gene FIG4 (chromosome 6q21). FIG4 mediates phosphatidylinositol-3,5-bisphosphate (PI(3,5)P2) conversion to phosphatidylinositol-3-phosphate (PI(3)P) on the cytosolic side of endosomal membranes, where FIG4 localizes through the interaction with the scaffolding protein VAC14 (Rudge et al. 2004; Sbrissa et al. 2007; Jin et al. 2008). PI(3)P and its derivatives are central players in endosomal transport and autophagy, with different PI3-phosphate pools generated by distinct kinases and phosphatases localized to different endosomal compartments (Di Paolo and De Camilli 2006). Specifically, PI(3,5)P2 is produced by the kinase I-phosphatidylinositol 3-phosphate 5-kinase (PIKfyve), which joins the complex formed by FIG4 and VAC14 on endosomal membranes, and this modified phospholipid acts as a key signal for the retrograde trafficking of endosomes (Sbrissa et al. 2007; Ferguson et al. 2009). Conversion of PI(3)P to PI(3,5)P2 is also essential for endosomal maturation since PI(3,5)P2 modulates cargo degradation in late endosomes/lysosomes (Odorizzi et al. 1998; Rutherford et al. 2006; Zhang et al. 2007). Therefore, the PIKfyve-FIG4-VAC14 ternary complex plays a central role in regulating endosomal metabolism (Fig. 4).

The first mutation identified in FIG4 is an insertion of the 5.5-kb retrotransposon $ETn2\beta$ into intron 18 of Fig4 murine gene, which caused alterations in Fig4 mRNA processing, therefore resulting in a substantial reduction in Fig4 protein levels. Animals affected by this autosomal recessive mutation displayed resting tremor accompanied by diluted pigmentation and were thus referred to as “pale tremor” mice. This phenotype is consistent with Charcot-Marie-Tooth (CMT) axonal neuropathies; notably, recessively inherited FIG4 mutations were subsequently identified in CMT4J patients (Chow et al. 2007). ALS-associated FIG4 mutations include truncating mutations, missenses, and mutations in splice sites and are all predicted to cause loss of protein function, similarly to that observed in pale tremor mice and CMT4J patients (Chow et al. 2007, 2009; Osmanovic et al. 2017). While FIG4 mutations are reported to be responsible for 1–3% of ALS cases among European patients, no deleterious FIG4 variants have been found in larger ALS groups, and some of the variants isolated in the European cohort display reduced penetrance (Osmanovic et al. 2017), so that further investigation is required.

Animal models have been exploited to better characterize the role of FIG4 mutations in endolysosomal trafficking. Concerning pale tremor mice, the relevant reduction in Fig4 protein levels dependent on $ETn2\beta$ retrotransposition determined a strong decrease in P(3,5)P2 levels accompanied by the accumulation of large vacuoles harboring ubiquitinated proteins and positive for SQSTM1/p62, LC3-II, and the late-stage endosomal markers lysosomal-associated membrane proteins 1 and 2 (LAMP-1 and LAMP-2) in murine neurons and astrocytes (Ferguson et al. 2009; Lenk et al. 2011). Comparable accumulation of enlarged lysosomes causing the rapid development of severe brain degeneration was observed in both constitutive and neuron-specific Fig4 knock-out murine models (Ferguson et al. 2012), while in Drosophila Fig4 loss correlated with motility impairments and shorter lifespan (Bharadwaj et al. 2016; Kyotani et al. 2016). The decrease in P(3,5)P2 levels associated to Fig4 loss is hypothesized to depend on reduced PIKfyve activity, since Fig4 is required to stabilize the interaction between PIKfyve and VAC14 (Botelho et al. 2008).

KIF5A

Kinesin 5A (KIF5A) is a neuron-specific kinesin heavy chain encoded by the gene KIF5A (chromosome 12q13.3) (Aizawa et al. 1992). Kinesins are ATP-dependent molecular motors that transport cargo along microtubule tracks in the anterograde direction, from the center of the cell to its periphery (Vale et al. 1985; Brady 1985). KIF5A comprises an N-terminal motor domain involved in microtubule binding and ATP hydrolysis, a stalk domain required for homodimerization and interaction with kinesin light chains, and a C-terminal tail domain which mediates interaction with cargos and adapter proteins (Miki et al. 2005; Hirokawa and Noda 2008). Among KIF5A cargos, lysosomes can be found, conferring KIF5A a role in the autophagic pathway (Fig. 3) (Liu et al. 2021).

KIF5A was found mutated in ALS in 2018, with genome-wide analyses identifying both low- and high-risk mutations (Brenner et al. 2018; Nicolas et al. 2018). Mutations in KIF5A had been previously linked to other neurodegenerative or neurodevelopmental disorders. Interestingly, mutations targeting different KIF5A domains give rise to distinct phenotypes, with minimal overlapping. Specifically, mutations targeting KIF5A motor or stalk domains cause hereditary spastic paraplegia and CMT2 (Reid et al. 2002; Fichera et al. 2004; Crimella et al. 2012), while mutations falling in its C-terminal tail are associated to neonatal intractable myoclonus as well as ALS (Rydzanicz et al. 2017; Brenner et al. 2018; Nicolas et al. 2018).

Recently, de novo frameshift mutations causing exon 27 skipping ($\Delta$Exon27) and downstream elongation of the KIF5A tail have been reported to abolish KIF5A ability to perform autoinhibition, leading to aberrant mitochondrial
transport along axons, and to enhance mutant KIF5A interaction with SQSTM1/p62 (Baron et al. 2022). Therefore, neurodegeneration caused by ΔExon27 KIF5A mutations seems to be linked to a toxic GOF disrupting neuronal trafficking and homeostasis. Since lysosomes are part of KIF5A cargos (Liu et al. 2021), the axonal transport alterations implicated in KIF5A-related ALS pathogenesis might tamper with the autophagic flux, too. Further studies are required to better elucidate the contribution of KIF5A mutations to ALS.

**OPTN**

OPTN is a multifunctional adaptor protein encoded by the *OPTN* gene (chromosome 10p13). It is highly expressed in brain and skeletal muscle (Rezaie et al. 2002; De Marco et al. 2006) and takes part in a wide variety of cellular processes, such as NF-κB activation, viral sensing, Golgi maintenance, and autophagy (Markovinovic et al. 2017). OPTN acts as an autophagy receptor by binding substrates, including damaged mitochondria, through its C-terminal ubiquitin-binding region of ABIN proteins and NEMO (UBAN) domain and delivers them to phagophores by interacting with LC3, thanks to its LIR domain (Figs. 1 and 2) (Wild et al. 2011; Wong and Holzbaur 2014). Subsequently, it links to myosin VI to promote autophagosome fusion with lysosomes (Tumbarello et al. 2012).

OPTN role as an autophagy receptor makes it a neuroprotective factor. Indeed, OPTN is sequestered into aggregates formed by mutant proteins in several neurodegenerative disorders (Maruyama et al. 2010; Hortobágyi et al. 2011; Osawa et al. 2011), and its deletion promotes aggregates accumulation (Korac et al. 2013). Additionally, OPTN interacts with SOD1 aggregates through its C-terminal coiled-coil domain, therefore in an ubiquitin-independent fashion (Korac et al. 2013).

*OPTN* mutations were initially associated to primary open-angle glaucoma (Rezaie et al. 2002; Minegishi et al. 2016) and were found to impair the autophagic flux. For example, retinal cells harboring OPTN E50K were shown to be characterized by defects in phagophore formation and by autophagy inhibition upon amino acid starvation (Chalasani et al. 2014). For what concerns ALS, more than 40 *OPTN* variants have been reported both in sALS and in fALS, with a limited number of mutations being shared between populations of different ethnicity (Maruyama et al. 2010; Del Bo et al. 2011; Tümner et al. 2012; van Blitterswijk et al. 2012b; Iida et al. 2012; Beeldman et al. 2015; Gotkine et al. 2021). ALS-related *OPTN* mutations are mainly missenses, truncations, and exon 5 deletions. A LOF mechanism is hypothesized for truncating mutations since mRNA decay and loss of OPTN immunoreactivity were reported in patient spinal cord (Maruyama et al. 2010; Iida et al. 2012; Gotkine et al. 2021). Regarding missense variants, they tend to cluster around OPTN UBAN domain (Maruyama et al. 2010).

The best characterized one is E478G OPTN, which is defective in ubiquitin binding (Wild et al. 2011) and was indeed reported to lose the ability to associate with polyubiquitinated inclusions and mitochondria to drive them to autophagy (Korac et al. 2013; Wong and Holzbaur 2014). The E478G mutant also sequesters wild-type OPTN into oligomers upon overexpression, limiting the OPTN pool available to guide phagophore formation (Shen et al. 2015) and forming inclusion bodies in sALS neurons (Maruyama et al. 2010).

Furthermore, E478G and G398X OPTN were shown to be unable to bind to myosin VI, determining the accumulation of immature autophagosomes triggering ER and Golgi stress (Sundaramoorthy et al. 2015).

Animal models recapitulate most observations on ALS-associated *OPTN* mutations made in vitro. OPTN ortholog knock-down in zebrafish is associated to motor axonopathy symptoms resembling models of SOD1 pathology (Korac et al. 2013). Moreover, 470T Optn mice mimic human OPTN truncations and reduced protein levels following mRNA decay are reported for such animal model (Munitic et al. 2013). Finally, the murine equivalent of the human E478G mutation (D477N Optn) is similarly unable to bind ubiquitin (Gleason et al. 2011). Despite the wide variety of data collected about *OPTN* mutations in ALS, the pathogenetic mechanisms causing autophagy impairments lying behind them are still to be fully characterized.

**SQSTM1/p62**

SQSTM1/p62 is a multifunctional adaptor protein encoded by the gene *SQSTM1* (chromosome 5q35.3). It is highly expressed in spinal cord MNs (Keller et al. 2012) and is implicated in several cellular processes, including NF-κB activation, apoptosis, and proteostasis maintenance (Rea et al. 2013). Concerning the latter process, SQSTM1/p62 acts as an autophagy receptor similarly to OPTN, therefore by sequestering ubiquitinated proteins via its C-terminal ubiquitin-binding domain (UBD), named ubiquitin-associated (UBA) domain, and contemporarily interacting with LC3 through its LIR to deliver substrates to autophagosomes (Figs. 1 and 2) (Björkøy et al. 2005; Pankiv et al. 2007; Ichimura et al. 2008). The N-terminal Phox and Bem1p-1 (PB1) ubiquitin-like domain allows SQSTM1/p62 to oligomerize, and both the PB1 and the UBA domains are involved in the interaction with other autophagy receptors, like OPTN and neighbor of BRCA1 gene 1 (NBR1), required to better coordinate autophagosome formation (Johansen and Lamark 2011). SQSTM1/p62 is also able to drive ubiquitinated substrates to proteasomal degradation, thanks to its ubiquitin-like domains (Seibenhener et al. 2004; Babu et al. 2005; Geetha et al. 2008). Evidence supports the hypothesis that SQSTM1/p62 might be a neuroprotective factor, once again similarly to OPTN.
it is upregulated at the onset of neuronal apoptosis, when ubiquitinated proteins accumulate in response to cell damage (Kuusisto et al. 2001). Moreover, it is found within ubiquitin-positive inclusions formed by mutant proteins in several neurodegenerative disorders (Zatolukal et al. 2002; Arai et al. 2003; Mizuno et al. 2006; King et al. 2013), underlining the central role this autophagy receptor plays in PQC. SQSTM1/p62 is also responsible for driving mutant SOD1 to autophagosomes through an ubiquitin-independent interaction mediated by its SOD1 mutant interaction region (SMIR) (Gal et al. 2009). Lastly, mice deprived of Sqstm1 display tau hyperphosphorylation and memory impairments (Babu et al. 2008), both hallmarks of Alzheimer’s disease, while Sqstm1 knock-down in zebrafish determines the development of locomotory impairments associated with autophagy defects and MN axon shortening (Lattante et al. 2015).

SQSTM1 mutations were firstly identified in 2002 in patients suffering from Paget disease of bone (PDB), a chronic progressive skeletal disorder. PDB-causing SQSTM1 mutations mainly target SQSTM1/p62 UBA domain, with the P392L substitution being the most frequent among them (Laurin et al. 2002). Mutations spanning the whole SQSTM1 sequence, including its promoter, have thereafter been related to fALS, sALS, and ALS/FTD (Rubino et al. 2012; Teyssou et al. 2013; Hirano et al. 2013; Shimizu et al. 2013; Le Ber et al. 2013; Chen et al. 2014; Kwok et al. 2014), and in most cases they have been associated with TDP-43 pathology (van der Zee et al. 2014). Since many ALS-related SQSTM1 variants were also found at relatively high frequencies in control populations, it is still unclear whether they are actually causative of the phenotype, as it remains to be determined how mutations in the same gene give rise to such distinct phenotypes as ALS and PDB (Rea et al. 2014). Nonetheless, the body of evidence connecting ALS-associated SQSTM1 mutations to specific impairments of SQSTM1/p62 functions in autophagy is growing. For example, the L341V substitution in SQSTM1/p62 LIR sequence was shown to cause a strong decrease in its interaction with LC3-II, thus tampering with the ability of the autophagy receptor to deliver cargo to forming phagophores (Goode et al. 2016). Additionally, some studies have confirmed the presence of SQSTM1/p62-positive inclusions along with increased levels of the protein in spinal MNs of SQSTM1 mutation carriers and found frontal cortical atrophy associated with these aggregates, thus providing further evidence of pathologic overlap between ALS and FTLD (Teyssou et al. 2013; Le Ber et al. 2013).

Until now, mechanistic studies on SQSTM1 mutations have mainly concentrated on the P392L substitution. Overexpression of P392L SQSTM1/p62 was shown to enhance autophagosome formation in the osteoclasts of mice harboring the murine equivalent of the P392L mutation (Daroszewska et al. 2011). Additionally, rescue of the locomotor defects displayed by zebrafish upon Sqstm1 downregulation could be achieved through overexpression of human wild-type SQSTM1/p62, but not of the P392L mutant. Amelioration of the phenotype was also evidenced upon administration of the autophagy inductor rapamycin, which further supports the hypothesis of a LOF being connected to the P392L substitution (Lattante et al. 2015). Interestingly, the P392L mutation was later found in ALS patients (Teyssou et al. 2013; Le Ber et al. 2013; Kwok et al. 2014), but its effect on MNs has not been characterized yet.

Finally, Sqstm1 depletion was demonstrated to exacerbate H46R SOD1 mice ALS phenotype, with shorter lifespan and accelerated MN degeneration and weight loss (Hadano et al. 2016).

TBK1

TANK-binding kinase 1 (TBK1) is a serine/threonine kinase encoded by the gene TBKI (chromosome 12q14.2). TBK1 is highly expressed in neuronal cells belonging to the cerebral cortex, the hippocampus, and the lateral ventricle (Uhlén et al. 2015).

TBK1 was first isolated as OPTN binding partner through yeast two-hybrid screening (Morton et al. 2008). In fact, it phosphorylates OPTN in its UBAN domain, promoting its binding to LC3-II and to ubiquitinated substrates to enhance the autophagic flux (Oakes et al. 2017). OPTN phosphorylation also enforces its role in TBK1 activation, which generates a positive feedback loop between TBK1 and OPTN activation (Lazarou et al. 2015; Heo et al. 2015). TBK1 exerts a similar modulatory activity on nuclear domain 10 protein 52 (NDP52) and SQSTM1/p62, two other autophagy adaptors, increasing their affinity for the ubiquitin chains of their UBDs through phosphorylation (Thurston et al. 2009; Pilli et al. 2012; Li et al. 2018a). TBK1-mediated OPTN, NDP52, and SQSTM1/p62 phosphorylation also promotes their association with damaged mitochondria to support mitophagy (Lazarou et al. 2015; Heo et al. 2015; Richter et al. 2016; Moore and Holzbaur 2016). Additionally, this kinase targets the C9ORF72-coupled GEF SMCR8, leading to C9ORF72 activation and therefore promoting autophagy initiation (Fig. 1) (Sellier et al. 2016). Moreover, TBK1 plays a role in autophagosome maturation, probably by interacting with the small GTPase RAB8b. TBK1 silencing was indeed shown not to interfere with autophagosome formation but to prevent autophagosome-lysosome fusion (Wang et al. 2018). Finally, microtubule-binding proteins required to trigger retrograde transport of autophagosomes and autolysosome formation are among TBK1 substrates, too (Oakes et al. 2017).
TBK1 mutations were firstly identified in ALS in 2015 through whole-exome sequencing of large patient cohorts of European ethnicity. Subsequently, additional studies confirmed the role of TBK1 in ALS pathogenesis, including ALS/FTD (Le Ber et al. 2015; Williams et al. 2015; Shu et al. 2016; Tsai et al. 2016; Borghero et al. 2016; van Rheenen et al. 2016). To date, more than 90 TBK1 mutations have been reported (Abramzon et al. 2020), and while they are rare in sALS patients, they account for around 3–4% of fALS and ALS/FTD cases (Cirulli et al. 2015; Freischmidt et al. 2015; Gijselinck et al. 2015). Most identified TBK1 variants are missense mutations with uncharacterized pathogenic effects (Oakes et al. 2017). On the other hand, nonsense and frameshift TBK1 mutations associated to the deletion of its second coiled-coil domain (CCD2) are reported to alter TBK1 ability to interact with adaptor proteins that regulate its localization and activation of downstream signaling pathways (Ryzhakov and Randow 2007). Nonsense and frameshift TBK1 mutations also correlate with reduced TBK1 expression as a consequence of mRNA decay (Freischmidt et al. 2015; Brenner et al. 2019). Thus, the pathogenetic mechanisms underlying TBK1-related ALS cases are considered to depend on the loss of TBK1 function. Abolishment of the interaction between TBK1 and OPTN by TBK1 mutations seems to play a prominent role in ALS pathogenesis. Indeed, an ALS-related TBK1 mutant deprived of amino acids 690–713 in the CCD2 domain was shown to be unable to bind OPTN when transiently overexpressed in HEK293T cells while preserving interaction with other TBK1 protein partners (Freischmidt et al. 2015). The same was demonstrated for additional TBK1 mutants (Richter et al. 2016; Moore and Holzbaur 2016; Li et al. 2018a; de Majo et al. 2018). Moreover, E696K TBK1 was shown to correlate with decreased recruitment of OPTN and LC3 to damaged mitochondria, leading to mitochondrial dysfunction and accumulation (Moore and Holzbaur 2016). Finally, TBK1 mutations were shown to correlate with the formation of inclusions positive for TDP-43, ubiquitin, and SQSTM1/p62 in MNs and glial cells of ALS and FTD patients (Van Mossevelde et al. 2016), further reflecting autophagy disruption. Taken together, these data support the hypothesis that the concerted activity of TBK1 and OPTN in autophagy and mitophagy is essential to prevent neurodegeneration.

Animal models have been generated to better investigate TBK1 role in ALS. While heterozygous Tbk1 knock-out mice did not develop MN degeneration or autophagy impairments (Brenner et al. 2019), loss of murine Tbk1 resulted embryonic lethal (Bonnard et al. 2000), and animals undergoing neuron-specific Tbk1 deletion displayed loss of cortical synapses, dendrite dysmorphism, and accumulation of neurofibrillary tangles correlating with motor and cognitive defects reminiscent of ALS/FTD (Duan et al. 2019). Additionally, loss of one Tbk1 allele in a G93A SOD1 murine model accelerated autophagy dysfunction and muscle denervation during the early stage of ALS, but at the same time, it extended mouse survival by reducing neuroinflammation at later stages (Brenner et al. 2018). A comparable disease course was observed in G93A SOD1 mice harboring Tbk1 mutations that impair its kinase activity (Gerbino et al. 2020). Therefore, these findings seem to indicate that loss of TBK1 function does not only lead to ALS development but can also modify the course of the pathology.

**TUBA4A**

α-tubulin isoform 4a (TUBA4A) is encoded by the gene TUBA4A (chromosome 2q35). It is ubiquitously expressed but particularly enriched in neurons (Rustici et al. 2013; Smith et al. 2014). α- and β-tubulins heterodimerize to form microtubules, cytoskeletal scaffolds along which kinesin- and dynein-mediated transport occurs in cells (Fig. 3).

TUBA4A mutations were firstly identified in familial ALS and ALS/FTD in 2014 based on exome sequencing performed on a large cohort of European and American patients (Smith et al. 2014). In the following years, additional TUBA4A mutations were found in other European and Asian cohorts (Dols-Icardo et al. 2016; Perrone et al. 2017; Li et al. 2018b) and even among sporadic ALS and ALS/FTD patients (Pensato et al. 2015). Differently from other tubulin genes that are highly expressed during brain development and that are therefore found mutated in neurodevelopmental disorders, TUBA4A expression increases with aging (Tischfield et al. 2011; Hershenson et al. 2013), which might explain why its mutations are associated to late-onset neurodegeneration (Smith et al. 2014; Clark et al. 2016) and may account for TUBA4A contributions to ALS progression. The frequency of TUBA4A mutations is anyway low in all tested populations so far, making them a rare cause of ALS (Chia et al. 2018).

Most TUBA4A mutations found in ALS cluster in the protein domain involved in the interaction with other tubulins and with the molecular motors kinesin and dynein (Howes et al. 2014) and are therefore predicted to tamper with microtubule stability and microtubule-based transport in cells. To date, the best characterized ALS-associated TUBA4A mutant is the truncated W407X variant, which displays impaired α/β-tubulin dimer formation and incorporation into microtubules accompanied by aggregation propensity when expressed in primary MNs (Smith et al. 2014).

Concerning autophagy, disruption of microtubule dynamics related to TUBA4A mutations might negatively reflect on autolysosome formation, which is dependent on dynein-mediated retrograde transport (Rademakers and van Blitterswijk 2014). Mutant TUBA4A aggregation might further worsen autophagy impairment; however, up to now, no data are available to confirm this hypothesis except for the
fALS-associated W407X mutant (Smith et al. 2014). The generation of animal models could help in characterizing the pathogenetic mechanisms associated to TUBA4A mutations in ALS.

**UBQLN2**

Ubiquilin-2 (UBQLN2) is one of the four members of the ubiquilin (UBQLN) family in the human genome encoded by the UBQLN2 gene located on chromosome Xp11.21 (Kaye and Shows 2000). It is expressed in various tissues, but the highest expression levels are found in muscles and brain (Lin et al. 2021). UBQLN2 localizes mainly in the cytoplasm where it functions in the maintenance of proteostasis. UBQLN2 presents an ubiquitin-like (UBL) domain at the N-terminus, which interacts with the regulatory cap of the proteasome, and a ubiquitin domain at the C-terminus, which recognizes polyubiquitin chains present on substrates; the UBQLN2 central domain is characterized by the presence of four stress-induced protein 1 (STI-1)-like motifs, which bind to heat shock proteins through a still unclear mechanism, and a proline-rich repeat domain containing 12 PXX repeats whose function is unknown (Kaye et al. 2000; Walters et al. 2002; Ko et al. 2004; Deng et al. 2011).

UBQLN2 activity mainly consists in promoting the disposal of misfolded or redundant proteins through the UPS or autophagy. The UBL and UBA domains permit the shuffling of ubiquitinated proteins to the UPS. UBQLN2 substrates are cytosolic and ER proteins. Indeed, through its binding with ER membrane proteins as ubiquitin regulatory X domain-containing protein 8 protein (Ubxd8) and homocysteine-induced ER protein (Herp), UBQLN2 specifically promotes the disposal of altered ER proteins. UBQLN2 interaction with autophagy is more complex. In the first place, UBQLN2 is implicated in autophagy initiation; indeed, it regulates mTORC1 activity. Loss of UBQLN2 activity prevents mTORC1-mediated autophagy inhibition resulting in an increased autophagy induction (Şentürk et al. 2019). Furthermore, UBQLN2 interacts indirectly with LC3 through its UBA domain promoting autophagosome formation (N’Diaye et al. 2009), and it was found also to interact with OPTN, confirming a role of UBQLN2 in the first steps of the autophagic pathway (Figs. 1 and 2) (Osaka et al. 2015).

Recent data showed a role of UBQLN2 also in lysosomal activity by regulating its acidification. Indeed, different groups presented two parallel mechanisms through which UBQLN2 interacts with subunits of v-ATPase, promoting its formation (Fig. 3) (Şentürk et al. 2019; Wu et al. 2020). Thus, silencing or mutations of UBQLN2 gene are associated to an increase in autophagy induction that is paralleled with a blockage of autophagosomal disposal due to a loss of lysosome activity (Şentürk et al. 2019).

UBQLN2 mutations were associated in 2011 to a dominantly inherited, X-linked form of ALS and ALS/FTD (Deng et al. 2011). To date, more than 20 mutations have been detected mainly in the PXX domain, while fewer are present in STI-1-like motifs or between domains [as reviewed in (Renaud et al. 2019)]. UBQLN2 mutations impair cellular homeostasis with different mechanisms: by triggering proteasome impairment, preventing the delivery to the proteasome of polyubiquitinated substrates, which accumulate leading to further cellular alterations (Chang and Monteiro 2015); by altering different steps of autophagy as previously described; by interacting with TDP-43 and its C-terminal fragments, triggering their aggregation and formation of cytoplasmic inclusions (Cassel and Reitz 2013; Picher-Martel et al. 2015); and by promoting neuroinflammation through direct interaction with NF-κB, an inflammation transcriptional factor regulator, or through a TDP-43-dependent process (Swarup et al. 2011). Different pathological mechanisms associated with UBQLN2 mutations result in colocalization of UBQLN2 and TDP-43 cytoplasmic inclusions in the brain and the spinal cord of patients. Of interest, UBQLN2 colocalization with TDP-43 was also present in the spinal cord of sALS patients, underling a central role for UBQLN2 in ALS pathology (Fecto and Siddique 2011).

In vivo models were developed to better understand UBQLN2 pathology. The first model generated was a mouse strain harboring the P497H UBQLN2 substitution under the human UBQLN2 promoter which expressed low protein levels (Gorrie et al. 2014). Because of the limited mutant UBQLN2 expression, this model presented cognitive impairments and hippocampal inclusions but did not show MNs loss. Subsequently, two rat models harboring the same mutation but expressed at higher levels were developed. These models presented impaired autophagy and endocytosis associated with neuronal loss (Wu et al. 2015; Chen et al. 2018). Also, transgenic mice expressing the P520T substitution, which is highly aggressive in humans causing an early ALS/FTD onset (Deng et al. 2011), failed to present neurodegeneration, but only displayed alterations in neuronal proteostasis (Sharkey et al. 2020). Furthermore, mice and rat knock-out models showed late or no neurodegenerative phenotype. Together, these data suggest that UBQLN2 pathology is triggered by both GOF and LOF.

**VAPB**

VAMPs-associated protein B (VAPB) is a member of the evolutionarily conserved VAP protein family (Skehel et al. 1995) encoded by the gene VAPB (chromosome 20q13.32). VAPs localize on the membrane of the ER and of ER-Golgi intermediates, in close association with vesicle-associated membrane proteins (VAMPs). There, VAPs act as adaptors for the recruitment to the ER surface of cytosolic proteins mainly identified by the two phenylalanines in an acidic tract (FFAT) motif (Loewen et al. 2021).
VAP proteins comprise an N-terminal major sperm protein (MSP) domain (Kaiser et al. 2005) involved in target proteins recruitment, a central coiled-coil domain, and a C-terminal hydrophobic sequence anchoring VAPs to the ER membrane. Among VAP interactors, many proteins allowing to put the ER in contact with other organelles can be found. The interaction sites that form through this process are known as membrane contact sites (MCS) and represent regions of communication between organelles achieved in the absence of membrane fusion (Prinz et al. 2020).

Based on these features, VAPB takes part in several cellular processes, among which autophagy can be found. For example, VAPB triggers mitophagy by interacting with the mitochondrial protein tyrosine phosphatase interacting protein 51 (PTPIP51), thus promoting autophagosome formation at the ER-mitochondria interface (Fig. 3) (Gomez-Suaga et al. 2017). Additionally, VAPB regulates autophagy through its interaction with RAB3 GTPase-activating protein 1 (RAB3GAP1), which plays a role in autophagy initiation (Spang et al. 2014).

The autosomal dominant P56S substitution was the first and best characterized VAPB mutation ever associated to ALS (Nishimura et al. 2004; Funke et al. 2010; Di et al. 2016; Guber et al. 2018). Other VAPB mutations were thereafter associated to FALS (Chen et al. 2010; van Blitterswijk et al. 2012a; Sun et al. 2017). The observations made in cellular and animal models of P56S VAPB pathology suggest that both LOF and GOF mechanisms might be implicated in neurodegeneration. Nevertheless, data collected in ALS patient-derived cell models are in contrast with the GOF hypothesis. The P56 VAPB residue is evolutionarily conserved and lies in the MSP domain, in close proximity to the FFAT binding site (Nishimura et al. 2004). The P56S substitution almost entirely prevents VAPB binding to its FFAT-containing cytosolic partners; this contemporarily induces mutant VAPB aggregation both in cellular (Nishimura et al. 2004; Kanekura et al. 2006; Teuling et al. 2007; Suzuki et al. 2009) and in murine ALS models (Tudor et al. 2010; Qiu et al. 2013; Kuijpers et al. 2013a; Aliaga et al. 2013). Aggregating P56S VAPB also sequesters into its inclusions both wild-type VAPB (Kanekura et al. 2006; Teuling et al. 2007; Suzuki et al. 2009) and other proteins involved in membrane trafficking and MCS formation (De Vos et al. 2012; Kuijpers et al. 2013b; Hua et al. 2017). Moreover, increased SQSTM1/p62 and LC3 levels were detected in the spinal cord of P56S VAPB mice, possibly indicating alterations in the autophagic flux (Larroquette et al. 2015). This observation, coupled with the fact that P56S VAPB accumulation was shown to determine profound rearrangement of the affected ER sites in cells (Fasana et al. 2010; Papiani et al. 2012), might provide a link between disrupted VAPB function, ER stress, and autophagy impairments. Indeed, the reduction in ER-mitochondria MCS observed upon VAPB depletion resulted in an enhancement of the autophagic flux in HEK293 cells, probably due to impaired ER-to-mitochondria calcium transfer triggering mitophagy (Gomez-Suaga et al. 2017). At the same time, reduced VAPB protein levels and no signs of VAPB aggregation were detected in sALS spinal cord MNs (Teuling et al. 2007; Anagnostou et al. 2010) and in P56S VAPB fibroblasts, iPSCs, and iPSC-derived MNs (Mitne-Neto et al. 2011). Additionally, despite harboring MN inclusions, most P56S VAPB murine models (Tudor et al. 2010; Qiu et al. 2013; Kuijpers et al. 2013a) did not develop ALS-like motor symptoms, except for the ones overexpressing the highest levels of the transgenic protein (Aliaga et al. 2013). The same occurred for Drosophila models harboring the P56S substitution (Sanhueza et al. 2014; Moustaqim-Barrette et al. 2014). At the same time, though, VAPB-null mice develop mild motor dysfunction (Kabashi et al. 2013), suggesting that loss of VAPB function alone might not be sufficient to justify the onset of ALS symptoms in patients. Overall, the contrasting evidence coming from cellular and animal models indicates that further investigation is required to better elucidate the role of VAPB mutations in ALS pathogenesis.

VCP

Valosin-containing protein (VCP) is an ATPase associated with diverse cellular activities (AAA+ encoded by VCP gene (chromosome 9p13.3). VCP is ubiquitously expressed in tissues (Koller and Brownstein 1987). At cellular level, VCP localizes mainly found in the cytoplasm, while a smaller fraction binds to organelles or localizes in the nucleus (Acharya et al. 1995; Latterich et al. 1995; Madeo et al. 1998; Xu et al. 2011; Ramanathan and Ye 2012). VCP functions assembling in a homo-hexamer. Each monomer presents an N-terminal domain that interacts with adaptors and cofactors; two ATPase domains, D1 and D2, that function respectively in the formation of the homo-hexamer and in accomplishing VCP activity; and a C-terminal domain that cooperates with D2 activity and binds to a small subset of VCP partners (Huyton et al. 2003; Niwa et al. 2012; Chou et al. 2014).

VCP mechanism of action is to identify and segregate ubiquitinated proteins from different cellular compartment and to enhance their degradation through the UPS or the autophagic pathway. Thanks to the binding of various cofactors and adaptors, VCP has a key role in the maintenance of cellular homeostasis, regulating different cellular pathways as endoplasmic reticulum-associated degradation (Stein et al. 2014), organelle degradation (Tanaka et al. 2010; Papadopoulos et al. 2017), ribosome-associated degradation (Verma et al. 2013), regulation of autophagy (Hill et al. 2021), chaperone activity (Hirabayashi et al. 2001), chromatin-associated degradation (Meerang et al. 2011), NF-κB activation (Dai et al. 1998), and membrane fusion (Amenta et al. 1978). Most of VCP activity...
is aimed at regulating and preserving cellular proteostasis. To contribute to the maintenance of proteostasis, VCP is involved in the routing of misfolded proteins and aggregates to the UPS or autophagy for their disposal (Figs. 1 and 2).

VCP role in addressing substrates to autophagy is still not completely clear. However, it was established that VCP interacts with different partners involved in aggresome formation and promotes substrates routing to autophagosomes (Iwata et al. 2005; Boyault et al. 2006). Besides this, VCP regulates different steps of the autophagic pathway [as reviewed in (Ferrari et al. 2022)]. VCP participates in the activation of transcriptional regulators of autophagy, such as NF-κB and TFEB. In particular, VCP promotes the degradation of IκBα that releases NF-κB permitting its activation (Dai et al. 1998). On the other hand, VCP mechanism in the regulation of TFEB is unknown, but when lysosomal damage is induced, the silencing or inhibition of VCP activity stabilizes TFEB activation, suggesting that VCP modulates TFEB action (Arhzaouy et al. 2019). In addition, VCP is involved in autophagy initiation and maturation (Figs. 1 and 2). Recent data showed that VCP promotes the formation of the initiation complex ATG14-VPS34-VPS15-BECN1 through two distinct pathways (Hill et al. 2021). Different studies show that VCP mutation impacts on autophagosome maturation; indeed, VCP silencing or mutations are correlated with an increase in LC3-II and SQSTM1 protein levels and accumulation of autophagosomes with increased size (Ju et al. 2009; Tresse et al. 2010). Finally, VCP has a key role in regulating lysosome stability and disposal. Studies on muscle tubular lysosomes show that VCP, supported by its cofactor small VCP interacting protein (SVIP), preserves lysosome stability (Fig. 3). Indeed, the inhibition or downregulation of VCP causes lysosomal fragmentation (Johnson et al. 2015). VCP, together with its cofactors phospholipase A2 activating protein (PLAA), UBX domain-containing protein 6 (UBXD1), and ubiquitin thioesterase OTU1 (YOD1), is also essential for damaged lysosome degradation. Indeed, they promote the removal of ubiquitinated proteins exposed on damaged lysosome membrane, an essential step in lysosome disposal (Papadopoulos et al. 2017).

The essential involvement of VCP in proteostasis is also demonstrated by cellular alterations of VCP-patients affected tissue. VCP mutations have been mainly associated in 2004 to inclusion bodies myopathy Paget disease and frontotemporal dementia (IBMPFD), a multisystem proteinopathy, and in 2010 to fALS (Watts et al. 2004; Johnson et al. 2010). To date, more than 40 missense mutations, localized at the interface between N-terminal and D1 domains, were associated to IBMPFD, and almost 20 mutations, localized at N-terminal and D1 interface and in D2, were associated to ALS (Johnson et al. 2010; Mehta et al. 2013; Kenna et al. 2013). VCP-patients present different phenotypes, yet they display similar alterations at cellular level. In skeletal muscles of VCP-patients inclusions positive to ubiquitin and VCP, damaged lysosomes and rimmed vacuoles can be found (Watts et al. 2004; Kimonis and Watts 2005; Ritson et al. 2010). Similarly, in affected neurons cytoplasmic inclusions positive to VCP and ubiquitin are also found (Kimonis and Watts 2005). Moreover, in both muscles and neurons, TDP-43 mislocalizes and forms inclusions (Ritson et al. 2010).

Animal models recapitulate most observations made on VCP mutations in vitro and in VCP-patients. VCP mouse models with VCP mutations or conditional knockout (Watts et al. 2004; Johnson et al. 2010) present lower survival with TDP-43 pathology in spinal cord and/or in skeletal muscle, muscle weakness, increase in fibers size that harbor ubiquitinated protein inclusions, and increased autophagy activity (Yin et al. 2012; Nalbandian et al. 2012). VCP mutants-induced neurodegeneration is also visible in Drosophila VCP models that overexpress mutations of TER94 (VCP ortholog in Drosophila), such as R152H, R188Q, and A229E (corresponding to human VCP mutations R155H, R191Q, and A232E). These models present disruption in nervous system and in muscle tissue that additionally shows specific lysosomal alterations (Chang et al. 2011; Johnson et al. 2015). Together, data obtained from animal models show an impairment in the autophagic pathway, reinforcing VCP essential role in this pathway.

Conclusions
Research over the past decades has highlighted the central role played by the autophagic pathway in ALS. Autophagy has a determinant role in the disposal of toxic proteins, including the aberrant aggregates formed by mutated proteins in ALS, and damaged organelles, and its failure in keeping under control protein misfolding upon saturating conditions is strictly associated to ALS progression. For this reason, the modulation of the autophagic pathway has been studied by different groups and has been outlined as a target to ameliorate ALS-related pathological conditions. Moreover, as this review summarizes, several ALS-associated mutations are found in genes implicated in all different steps of the autophagic pathway, from transport to protein degradation. Despite the efforts in studying ALS, to date no cure is available, and no therapies that drastically ameliorate life-span have been discovered yet. Thus, by analyzing the physiological role of ALS-related genes involved in autophagy and by evaluating the main experimental data linking mutations in these genes to ALS, it is clear that not all the implications of autophagy dysfunction in ALS have been completely unraveled yet. Indeed, in several cases, the molecular mechanisms explaining the pathogenicity of autophagy-related gene mutations found in ALS are still to be fully deciphered. In some cases, in vitro and cellular analyses might contrast with
observations made in patients or in animal models, indicating that further studies are required to fully elucidate the connection between genes involved in the various steps of autophagy and ALS. In addition, this review highlights that alterations in autophagy are directly linked with ALS progression and suggests that a better functionality of autophagy could ameliorate the phenotype. Indeed, various studies on modulation of autophagy have been carried out and are ongoing with promising results. Compounds that positively modulate autophagy, such as trehalose, rapamycin, and colchicine, have been tested in animals or are already in clinical trial (Castillo et al. 2013; Mandrioli et al. 2018, 2019). These compounds stimulate autophagy through different mechanisms that have still to be completely clarified. Potentiation of the autophagic flux in ALS is surely positive in presence of a LOF of autophagy or in presence of impairment of organelles or accumulation of protein aggregates. Contrary, the implication of a potentiation of autophagy in presence of alterations derived by mutations of autophagic components has to be carefully analyzed. In this context, a better understanding of ALS-related genes function and contribution in each step of autophagy is fundamental to fully comprehend the pathological mechanisms and to display novel rescue strategies.

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Declarations

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References

Abramzon YA, Fratta P, Traynor BJ, Chia R (2020) The overlapping genetics of amyotrophic lateral sclerosis and frontotemporal dementia. Front Neurosci 14:42. https://doi.org/10.3389/fnins.2020.00042

Acharya U, Jacobs R, Peters JM et al (1995) The formation of Golgi stacks from vesiculated Golgi membranes requires two distinct fusion events. Cell 82:895–904. https://doi.org/10.1016/0092-8674(95)90269-4

Aizawa H, Sekine Y, Takemura R et al (1992) Kinesin family in murine central nervous system. J Cell Biol 119:1287–1296. https://doi.org/10.1083/jcb.119.5.1287

Aliaga L, Lai C, Yu J et al (2013) Amyotrophic lateral sclerosis-related VAPB P56S mutation differentially affects the function and survival of corticospinal and spinal motor neurons. Hum Mol Genet 22:4293–4305. https://doi.org/10.1093/hmg/ddt279

Amenta JS, Hlviko TJ, McBee AG et al (1978) Specific inhibition by NH4Cl of autophagy-associated proteolysis in cultured fibroblasts. Exp Cell Res 115:357–366. https://doi.org/10.1016/0014-4827(78)90289-6

Amick I, Rocznia-Ferguson A, Ferguson SM (2016) C9orf72 binds SMCR8, localizes to lysosomes, and regulates mTORC1 signaling. Mol Biol Cell 27:3040–3051. https://doi.org/10.1091/mbc.e16-01-0003

Amick J, Tharkeshwar AK, Talaia G, Ferguson SM (2020) PQLC2 recruits the C9orf72 complex to lysosomes in response to catonic amino acid starvation. J Cell Biol. https://doi.org/10.1083/jcb.201906076

Anagnostou G, Akbar MT, Paul P et al (2010) Vesicle associated membrane protein B (VAPB) is decreased in ALS spinal cord. Neurobiol Aging 31:969–985. https://doi.org/10.1016/j.neurobiolaging.2008.07.005

Ao X, Zou L, Wu Y (2014) Regulation of autophagy by the Rab GTPase network. Cell Death Differ 21:348–358. https://doi.org/10.1038/cdd.2013.187

Aoki Y, Manzano R, Lee Y et al (2017) C9orf72 and RAB7L1 regulate vesicle trafficking in amyotrophic lateral sclerosis and frontotemporal dementia. Brain 140:887–897. https://doi.org/10.1093/brain/awx024

Arai T, Nonaka T, Hasegawa M et al (2003) Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. Neurosci Lett 342:41–44. https://doi.org/10.1016/s0304-3900(03)00216-7

Arhzaouy K, Papadopoulos C, Schulze N et al (2019) VCP maintains lysosomal homeostasis and TFE4 activity in differentiated skeletal muscle. Autophagy 15:1082–1099. https://doi.org/10.1080/15548672.2019.13569933

Atanasio A, Decman V, White D et al (2016) C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production and glomerulonephropathy in mice. Sci Rep 6:23204. https://doi.org/10.1038/srep23204

Atkinson RAK, Fernandez-Martos CM, Atkin JD et al (2015) C9ORF72 expression and cellular localization over mouse development. Acta Neuropathol Commun 3:59. https://doi.org/10.1001/jnci.2011.40478-015-0238-7

Babu JR, Geevath T, Wooten MW (2005) Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. J Neurochem 94:192–203. https://doi.org/10.1111/j.1471-4159.2005.03181.x
paraplegia. Neurology 63:1108–1110. https://doi.org/10.1212/01.wnl.0000383731.60693.42
Filimonenko M, Staffers S, Raiborg C et al (2007) Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol 179:485–500. https://doi.org/10.1083/jcb.200702115
Freibaum BD, Taylor JP (2017) The role of dipeptide repeats in C9orf72-related ALS-FTD. Front Mol Neurosci. https://doi.org/10.3389/fnmol.2017.00035
Freischmidt A, Wieland T, Richter B et al (2015) Haploinsufficiency of TBK1 causes familial ALS and frontotemporal dementia. Nat Neurosci 18:631–636. https://doi.org/10.1038/nn.4000
Funke AD, Esser M, Krüttgen A et al (2010) The p. P56S mutation in the VAPB gene is not due to a single founder: the first European case. Clin Genet 77:302–303
Gal J, Ström A-L, Kwinter DM et al (2009) Sequestosome 1/p62 links familial ALS mutant SOD1 to LC3 via an ubiquitin-independent mechanism. J Neurochem 111:1062–1073. https://doi.org/10.1111/j.1471-4159.2009.06388.x
Geetha T, Seibenhener ML, Chen L et al (2008) p62 serves as a shuttling factor for TrkA interaction with the proteasome. Biochem Biophys Res Commun 374:33–37. https://doi.org/10.1016/j.bbrc.2008.06.082
Gendron TF, Bieniek KF, Zhang Y-J et al (2013) Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in C9FTD/ALS. Acta Neuropathol 126:829–844. https://doi.org/10.1007/s00401-013-1192-8
Gerbino V, Kaunga E, Ye J et al (2020) The loss of TBK1 kinase activity in motor neurons or in all cell types differentially impacts ALS disease progression in SOD1 mice. Neuron 106:789-805. e5. https://doi.org/10.1016/j.neuron.2020.03.005
Ghazi-Noori S, Froud KE, Mizielska S et al (2012) Progressive neuronal inclusion formation and axonal degeneration in CHMP2B mutant transgenic mice. Brain 135:819–832. https://doi.org/10.1093/brain/aww006
Gijselink I, van Mossevelde S, van der Zee J et al (2016) The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter. Mol Psychiatry 21:1112–1124. https://doi.org/10.1038/mp.2015.159
Gijselink I, Van Mossevelde S, Van der Zee J et al (2015) Loss of TBK1 is a frequent cause of frontotemporal dementia in a Belgian cohort. Neurology 85:216–2125. https://doi.org/10.1212/WNL.0000000000002220
Gleason CE, Ordureau A, Gourlay R et al (2011) Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon β. J Biol Chem 286:35663–35674. https://doi.org/10.1074/jbc.M111.267567
Gomez-Suaga P, Paillusson S, Stoica R et al (2017) The ER-mitochondria tethering complex VAPB-PTP1P51 regulates autophagy. Curr Biol 27:371–385. https://doi.org/10.1016/j.cub.2016.12.038
Goode A, Butler K, Long J et al (2016) Defective recognition of LC3B by mutant SQSTM1/p62 implicates impairment of autophagy as a pathogenic mechanism in ALS-FTLD. Autophagy 12:1094–1104. https://doi.org/10.1080/15548627.2016.1170257
Gorrie GH, Fecto F, Radzicki D et al (2014) Dendritic spinopathy in transgenic mice expressing ALS/dementia-linked mutant UBQLN2. Proc Natl Acad Sci 111:14524–14529. https://doi.org/10.1073/pnas.1405471111
Gotkine M, deMajo M, Wong CH et al (2021) A recessive S174X mutation in Optineurin causes amyotrophic lateral sclerosis through a loss of function via allele-specific nonsense-mediated decay. Neurobiol Aging 106:351.e1-351.e6. https://doi.org/10.1016/j.neurobiolaging.2021.05.009
Guber RD, Schindler AB, Budron MS et al (2018) Nucleocytoplasmic transport defect in a North American patient with ALS8. Ann Clin Transl Neurol 5:369–375. https://doi.org/10.1002/acn2.515
Gydesen S, Brown JM, Brun A et al (2002) Chromosome 3 linked frontotemporal dementia (FTD-3). Neurology 59:1585–1594. https://doi.org/10.1212/01.wnl.0000034763.54161.1f
Hadano S, Benn SC, Kakuta S et al (2006) Mice deficient in the Rab5 guanine nucleotide exchange factor AL2/salsin exhibit age-dependent neurological deficits and altered endosome trafficking. Hum Mol Genet 15:233–250. https://doi.org/10.1093/hmg/ddi440
Hadano S, Hand CK, Osuga H et al (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29:166–173. https://doi.org/10.1038/ng1001-166
Hadano S, Mitsui S, Pan L et al (2016) Functional links between SQSTM1 and ALS2 in the pathogenesis of ALS: cumulative impact on the protection against mutant SOD1-mediated motor dysfunction in mice. Hum Mol Genet 25:3321–3340. https://doi.org/10.1093/hmg/ddw180
Hadano S, Otomo A, Kunita R et al (2010) Loss of ALS2/Alisin exacerbates motor dysfunction in a SOD1-expressing mouse ALS model by disturbing endolysosomal trafficking. PLoS ONE 5:e9805. https://doi.org/10.1371/journal.pone.0009805
Haeseler AR, Donnelly CJ, Periz G et al (2014) C9orf72 nucleotide repeat structures initiate molecular cascades of disease. Nature 507:195–200. https://doi.org/10.1038/nature13124
Hane N-H, Rya H-H, Jun M-H et al (2012) The functional analysis of the CHMP2B missense mutation associated with neurodegenerative diseases in the endo-lysosomal pathway. Biochem Biophys Res Commun 421:544–549. https://doi.org/10.1016/j.bbrc.2012.04.041
Hayes LR, Duan L, Bowen K et al (2020) C9orf72 arginine-rich dipeptide repeat proteins disrupt karyopherin-mediated nuclear import. Elife. https://doi.org/10.7554/eLife.51685
Henne WM, Stemmark H, Emil SD (2013) Molecular mechanisms of the membrane sculpting ESCRT pathway. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a016766
Heo J-M, Ordureau A, Paulo JA et al (2015) The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDPS2 recruitment and TBK1 Activation to Promote Mitophagy. Mol Cell 60:7–20. https://doi.org/10.1016/j.molcel.2015.08.016
Hernández J, Menacccci NE, Davis M et al (2013) Mutations in the autoregulatory domain of β-tubulin 4a cause hereditary dystonia. Ann Neurol 73:546–553. https://doi.org/10.1002/ana.23832
Hill SM, Wrobel L, Ashkenazi A et al (2021) VCP/p97 regulates bec-1. Dev Cell 29:505–506. https://doi.org/10.1016/j.devcel.2021.05.016
Hortobágyi T, Troakes C, Nishimura AL et al (2011) Optineurin inclu- sions occur in a minority of TDP-43 positive ALS and FTLD-TDP cases and are rarely observed in other neurodegenerative 
Spang N., Feldmann A., Huesmann H. et al. (2014) RAB3GAP1 and RAB3GAP2 modulate basal and rapamycin-induced autophagy. Autophagy 10:2297–2309. https://doi.org/10.4161/15548627.2014.994359

Stein A., Ruggiano A., Carvalho P., Rapoport T. (2014) Key steps in ERAD of luminal ER proteins reconstituted with purified components. Cell 158:1375–1388. https://doi.org/10.1016/j.cell.2014.07.050

Stockmann M., Meyer-Ohlendorf M., Achberger K. et al. (2013) The dynactin p150 subunit: cell biology of sequence changes found in ALS/MND and Parkinsonian syndromes. J. Neurotransm. (vienna) 120:785–798. https://doi.org/10.1007/s00702-012-0910-x

Su M.-Y., Fromm S A., Zoncu R., Harley JH. (2020) Structure of the C9orf72 ARF GAP complex that is haploinsufficient in ALS and FTD. Nature 585:251–255. https://doi.org/10.1038/s41586-020-2633-x

Sun Y.-M., Dong Y., Wang J. et al. (2017) A novel mutation of VAPB in one Chinese familial amyotrophic lateral sclerosis pedigree and its clinical characteristics. J Neurol 264:2387–2393. https://doi.org/10.1007/s00415-017-8628-3

Sundaramoorthy V., Walker AK., Tan V. et al. (2015) Defects in optineurin- and myosin VI-mediated cellular trafficking in amyotrophic lateral sclerosis. Hum Mol Genet 24:3830–3846. https://doi.org/10.1039/c4hm01216d

Suzuki H., Kanekura K., Levine TP. et al. (2009) ALS-linked P56S-VAPB, an aggregated loss-of-function mutant of VAPB, predisposes motor neurons to ER stress-related death by inducing aggregation of co-expressed wild-type VAPB. J Neurochem 108:973–985. https://doi.org/10.1111/j.1471-4159.2008.05857.x

Swarp V., Phaneuf D., Dupré N. et al. (2011) Derelegation of TDP-43 in amyotrophic lateral sclerosis triggers nuclear factor κB-mediated pathogenic pathways. J Exp Med 208:2429–2447. https://doi.org/10.1084/jem.20111313

Tanaka A., Cleland MM., Xu S. et al. (2010) Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. J Cell Biol 191:1367–1380. https://doi.org/10.1083/jcb.201007013

Tang D., Sheng J., Xu L. et al. (2020) Cryo-EM structure of C9ORF72–SMCR8–WDR41 reveals the role as a GAP for Rab8a and Rab11a. Proc Natl Acad Sci 117:9876–9883. https://doi.org/10.1073/pnas.2002110117

Taylor JP., Brown RH., Cleveland DW. (2016) Decoding ALS: from genes to mechanism. Nature 539:197–206. https://doi.org/10.1038/nature20413

Teuling E., Ahmed S., Haasdijk E. et al. (2007) Motor neuron disease-associated mutant vesicle-associated membrane protein-associated protein (VAP) B recruits wild-type VAPs into endoplasmic reticulum-derived tubular aggregates. J Neurosci 27:9801–9815. https://doi.org/10.1523/JNEUROSCI.2661-07.2007

Teyssou E., Takeda T., Lebon V. et al. (2013) Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: genetics and neuropathology. Acta Neuropathol 125:511–522. https://doi.org/10.1007/s00401-013-1090-0

Therrien M., Rouleau GA., Dion PA., Parker JA. (2013) Deletion of C9ORF72 results in motor neuron degeneration and stress sensitivity in C. elegans. PLoS One 8:e83450. https://doi.org/10.1371/journal.pone.0083450

Thurston TLM., Ryzhakov G., Bloor S. et al. (2009) The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. Nat Immunol 10:1215–1221. https://doi.org/10.1038/ni.1800

Tischfield MA., Cederquist GY., Gupta MLJ., Engle EC. (2011) Phyloptic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. Curr Opin Genet Dev 21:286–294. https://doi.org/10.1016/j.gde.2011.01.003

Topp JD., Gray NW., Gerard RD., Horazdovsky BF. (2004) Alsin is a Rab5 and Rac1 guanine nucleotide exchange factor. J Biol Chem 279:24612–24623. https://doi.org/10.1074/jbc.M313504200

Tresse E., Salomons FA., Vesa J. et al. (2010) VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBM/FRD. Autophagy 6:217–227. https://doi.org/10.4161/aut.6.2.11014

Trotti D., Rolfs A., Danbolt NC. et al. (1999) Erratum: SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. Nat Neurosci 2:848–848. https://doi.org/10.1038/12227

Tsai P.-C., Liu Y.-C., Lin K-P. et al. (2016) Mutational analysis of TBK1 in Taiwanese patients with amyotrophic lateral sclerosis. Neurobiol Aging 40:191.e11–191.e16. https://doi.org/10.1016/j.neurobiaging.2015.12.022

Tudor EL., Galtry CM., Perkinton MS. et al. (2010) Amyotrophic lateral sclerosis mutant vesicle-associated membrane protein-associated protein-B transgenic mice develop TAR-DNA-binding protein-43 pathology. Neuroscience 167:774–785. https://doi.org/10.1016/j.neuroscience.2010.02.035

Tumbarello DA., Waxse BJ., Arden SD. et al. (2012) Autophagy receptors link myosin VI to autophagosomes to mediate Tom1-dependent autophagosome maturation and fusion with the lysosome. Nat Cell Biol 14:1024–1035. https://doi.org/10.1038/ncb2589

Tümer Z., Bertelsen B., Gredal O. et al. (2012) Novel heterozygous nonsense mutation of the OPTN gene segregating in a Danish family with ALS. Neurobiol Aging 33:208.e1–5. https://doi.org/10.1016/j.neurobiaging.2011.07.001

Uhlén M., Fagerberg L., Hallström BM. et al. (2015) Proteomics. A tissue-based map of the human proteome. Sci 347:1260419. https://doi.org/10.1126/science.1260419

Urvainicius L., Zhang K., Diamant AG. et al. (2015) The structure of optineurin mutations in sporadic amyotrophic lateral sclerosis mutant vesicle-associated membrane protein-associated protein-B transgenic mice develop TAR-DNA-binding protein-43 pathology. Neuroscience 167:774–785. https://doi.org/10.1016/j.neuroscience.2015.07.004

Van Blitterswijk M., van Es MA., van der Zee J., Gijselinck I. et al. (2016) Clinical phenotype. Curr Opin Genet Dev 44:117–124. https://doi.org/10.1016/j.gde.2016.02.008

Van Blitterswijk M., Vlam L., van Es MA. et al. (2012a) VAPB and C9orf72 mutations in familial amyotrophic lateral sclerosis patient. Neurobiol Aging 33:2950.e1–4. https://doi.org/10.1016/j.neurobiaging.2012.07.004

Van Blitterswijk M., van Vught PWJ., van Es MA. et al. (2012b) Novel optineurin mutations in sporadic amyotrophic lateral sclerosis patients. Neurobiol Aging 33:1016.e1–7. https://doi.org/10.1016/j.neurobiaging.2011.05.019

Van Blitterswijk M., Vlam L., van Es MA et al. (2012c) Genetic overlap between apparently sporadic motor neuron diseases. PLoS ONE 7:e48983. https://doi.org/10.1371/journal.pone.0048983

Van der Zee J., Van Langenhove T., Kovacs GG. et al. (2014) Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration. Acta Neuropathol 128:397–410. https://doi.org/10.1007/s00401-014-1298-7

Van Mossevelde S., van der Zee J., Cruits M., van Broeckhoven C. (2017) Relationship between C9orf72 repeat size and clinical phenotype. Curr Opin Genet Dev 44:117–124. https://doi.org/10.1016/j.gde.2017.02.008

Van Mossevelde S., van der Zee J., Gijselinck I et al. (2016) Clinical features of TBK1 carriers compared with C9orf72, GRN and non-mutation carriers in a Belgian cohort. Brain 139:452–467. https://doi.org/10.1093/brain/aww358

Van Rheenen W., Shatunov A., Dekker AM et al. (2016) Genome-wide association analyses identify new risk variants and the
genetic architecture of amyotrophic lateral sclerosis. Nat Genet 48:1043–1048. https://doi.org/10.1038/ng.3622

Vaughan KT, Vallee RB (1995) Cytoplasmic dynein binds dynactin through a direct interaction between the intermediate chains and p150(Glued). J Cell Biol 131:1507–1516. https://doi.org/10.1083/jcb.131.6.1507

Verma R, Oania RS, Kolawa NJ, Deshaies RJ (2013) Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. Elife 2:e00308. https://doi.org/10.7554/eLife.00308

Vernay A, Therreau L, Blot B et al (2016) A transgenic mouse expressing CHMP2B intron5 mutant in neurons develops histological and behavioural features of amyotrophic lateral sclerosis and frontotemporal dementia. Hum Mol Genet 25:3341–3360. https://doi.org/10.1093/hmg/ddw182

Walters KJ, Kleijn MF, Goh AM et al (2002) Structural studies of the interaction between ubiquitin family proteins and proteasome subunit Ssa. Biochemistry 41:1767–1777. https://doi.org/10.1021/bi011892y

Wang J, Hussain T, Yue R et al (2018) MicroRNA-199a inhibits cellular autophagy and downregulates IFN-β expression by targeting TBK1 in Mycobacterium bovis infected cells. Front Cell Infect Microbiol 8:238. https://doi.org/10.3389/fcimb.2018.00238

Wang M, Wang H, Tao Z et al (2020) C9orf72 associates with inactive Rag GTases and regulates mTORC1-mediated autophagosomal and lysosomal biogenesis. Aging Cell. https://doi.org/10.1111/agec.13126

Wang T, Liu H, Itoh K et al (2021) C9orf72 regulates energy homeostasis by stabilizing mitochondrial complex I assembly. Cell Metab 33:531-546.e9. https://doi.org/10.1016/j.cmet.2021.01.005

Watts GDJ, Wymer J, Kovach MJ et al (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 36:377–381. https://doi.org/10.1038/ng1332

Webster CP, Smith EF, Bauer CS et al (2016) The C9orf72 protein interacts with Rab1a and the ULK 1 complex to regulate initiation of autophagy. EMBO J 35:1656–1676. https://doi.org/10.15222/embj.201694401

West RH, Ugboce C, Fort-Aznar L, Sweeney ST (2020) Neuroprotective activity of ursodeoxycholic acid in CHMP2B(intron5) models of frontotemporal dementia. Neurobiol Dis 144:105047. https://doi.org/10.1016/j.nbd.2020.105047

Wild P, Farhan H, McEwan DG et al (2011) Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science 333:228–233. https://doi.org/10.1126/science.1205405

Williams KL, McCann EP, Ffita JA et al (2015) Novel Tbk1 truncating mutation in a familial amyotrophic lateral sclerosis patient of Chinese origin. Neurobiol Aging 36:3334.e1–3334.e5. https://doi.org/10.1016/j.neurobiolaging.2015.08.013

Wong YC, Holzbaur ELF (2014) Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. Proc Natl Acad Sci U S A 111:E4439–48. https://doi.org/10.1073/pnas.1405752111

Wu JJ, Cai A, Greenslade JE et al (2020) ALS/FTD mutations in UBQLN2 impede autophagy by reducing ubiquophasomal acidification through loss of function. Proc Natl Acad Sci U S A 117:15230–15241. https://doi.org/10.1073/pnas.1917371117

Wu Q, Liu M, Huang C et al (2015) Pathogenic Ubqln2 gains toxic properties to induce neuron death. Acta Neuropathol 129:417–428. https://doi.org/10.1007/s00401-014-1367-y

Xiao S, MacNair L, McGoldrick P et al (2015) Isoform-specific antibodies reveal distinct subcellular localizations of C9orf72 in amyotrophic lateral sclerosis. Ann Neurol 78:568–583. https://doi.org/10.1002/ana.24469

Xu H, Ren D (2015) Lysosomal physiology. Annu Rev Physiol 77:57–80. https://doi.org/10.1146/annurev-physiol-021014-071649

Xu S, Peng G, Wang Y et al (2011) The AAA-ATPase p97 is essential for outer mitochondrial membrane protein turnover. Mol Cell 22:291–300. https://doi.org/10.1010/mcb.E10-09-0748

Yamanaka K, Miller TM, McAlonis-Downes M et al (2006) Progressive spinal axonal degeneration and slowness in ALS2-deficient mice. Ann Neurol 60:95–104. https://doi.org/10.1002/ana.20888

Yamanaka K, Vande Velde C, Eymard-Pierre E et al (2003) Unstable mutants in the peripheral endosomal membrane component ALS2 cause early-onset motor neuron disease. Proc Natl Acad Sci U S A 100:16041 LP – 16046. https://doi.org/10.1073/pnas.2635267100

Yang M, Liang C, Swaminathan K et al (2016) A C9ORF72/SMC8-containing complex regulates ULK1 and plays a dual role in autophagy. Sci Adv. https://doi.org/10.1126/sciadv.1601167

Yang Y, Hentati A, Deng H-X et al (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet 29:160–165. https://doi.org/10.1038/ng1001-160

Yin HZ, Nalbandian A, Hsu C-I et al (2012) Slow development of ALS-like spinal cord pathology in mutant valosin-containing protein gene knock-in mice. Cell Death Dis 3:e374–e374. https://doi.org/10.1038/cddis.2012.115

Yu J, Lai C, Shim H et al (2018) Genetic ablation of dynactin p150(Glued) in postnatal neurons causes preferential degeneration of spinal motor neurons in aged mice. Mol Neurodegener 13:10. https://doi.org/10.1186/s13024-018-0242-z

Zatloukal K, Stumptner C, Fuchsbichler A et al (2002) p62 Is a common component of cytoplasmic inclusions in protein aggregation diseases. Am J Pathol 160:255–263. https://doi.org/10.1016/S0002-9440(00)04369-6

Zhang K, Donnelly CJ, Haeusler AR et al (2015) The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature 525:56–61. https://doi.org/10.1038/nature14973

Zhang Y, Zolov SN, Chow CY et al (2007) Loss of Vac14, a regulator of the signaling lipid phosphatidylinositol 3,5-bisphosphate, results in neurodegeneration in mice. Proc Natl Acad Sci U S A 104:17518–17523. https://doi.org/10.1073/pnas.0702275104

Zhong Y, Wang QJ, Li X et al (2009) Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1–phosphatidylinositol-3-kinase complex. Nat Cell Biol 11:468–476. https://doi.org/10.1038/ncb1854

Zu T, Liu Y, Bañez-Coronzol M et al (2013) RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. PNAS 110:E4968 LP-E4977. https://doi.org/10.1073/pnas.1315438110

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