We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Autophagy-Lysosome Dysfunction in Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration

Peter M. Sullivan, Xiaolai Zhou and Fenghua Hu

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69371>

Abstract

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two devastating neurodegenerative diseases. Several lines of evidence suggest that these diseases are part of a continuum with common genetic factors. As researchers uncover more genes associated with ALS/FTLD, studies have shown that majority of these genes regulate lysosome-related processes. Lysosomes play important roles in clearing damaged organelles and proteins through the autophagy-lysosome pathway and clearing extracellular debris by the endolysosomal pathway. Disruption of both the autophagy and endolysosomal pathways has been implicated in ALS/FTLD pathogenesis.

Keywords: autophagy, lysosome, amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), neurodegeneration, progranulin (PGRN), TMEM106B, C9orf72, OPTN, p62, TBK1, ubiquilin2 (UBQLN2), TDP-43, FUS, tau, VCP, CHMP2B

1. Introduction

Proper degradation machinery is necessary for neuronal survival, and disruption of lysosomal function is sufficient to cause neurodegeneration [1–4]. To recycle cellular material, cells use two major pathways: autophagy for damaged organelles and long-lived proteins and the ubiquitin-proteasome system (UPS) for short-lived proteins [5, 6]. Autophagy consists of three pathways and each of them ultimately delivers cellular contents to the lysosome for degradation. The pathways are chaperone-mediated autophagy (CMA), which uses HSC70 to recognize

specific misfolded proteins; microautophagy, which directly invaginates material into the lysosome; and macroautophagy, which is responsible for the degradation of organelles, protein aggregates, and large protein complexes. Macroautophagy (hereafter referred to as autophagy) is the most common pathway. The autophagy pathways and molecular mechanisms have been recently reviewed elsewhere [7, 8]. The presence of protein aggregates in most neurodegenerative diseases suggests common underlying problem in protein degradation systems. Here, we summarize the connection between the autophagy-lysosome pathway and two neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) [9].

ALS is characterized by the loss of upper and lower motor neurons resulting in progressive weakness and ultimately paralysis. Patients survive a median of 3–5 years from disease onset [10]. FTLD is characterized by the degeneration of neurons in the frontal cortex and anterior temporal lobes. This degeneration leads to changes in behavior and language impairment. The subtypes of FTLD can be distinguished by the prominent symptoms, which reflect the area affected by neuron loss [11, 12]. The subtypes are behavioral variant frontotemporal dementia (bvFTD), semantic dementia (SD), and primary nonfluent aphasia (PNFA). Behavioral variant frontotemporal dementia, the most common subtype, is characterized by changes in behavior such as disinhibition, loss of empathy, impaired social skills, and decline in personality. SD is characterized by impaired language comprehension, and PNFA disrupts speech production [9]. These subtypes often overlap and can additionally include Parkinson's disease-like symptoms. Patients survive for a median of 7–11 years after diagnosis. There are no treatments for FTLD [9]. ALS and FTLD symptoms are often present in the same patient with an indication that these diseases have shared etiology [13, 14].

Each disease is also subdivided by molecular pathology depending on the primary components of inclusion bodies, such as Tau, TDP-43 (TAR DNA-Binding Protein 43), FUS (fused in sarcoma), SOD1 (superoxide dismutase 1) and C9 or f72 dipeptide repeats (DPRs) [9, 15]. In 2006, both ALS and FTLD were found to have neuronal inclusions composed largely of TDP-43, an RNA-binding protein, that are also ubiquitin and p62-positive, suggesting that these aggregates were tagged for degradation [16–18]. Additionally, genetic mutations that can lead to the development of both ALS and FTLD have since been discovered. Thus, these two diseases are linked by clinical concurrence, molecular pathology, and genetic overlap [13, 14, 19].

As many new genes have been identified for FTLD and ALS in the last decade, studies have revealed a common theme of these genes functioning in the lysosomal network (**Figure 1**). Some mutations, such as *GRN*, *TMEM106B*, *CHMP2B*, and valosin-containing protein (*VCP*) are associated with disrupted lysosomes and multivesicular bodies (MVB). Other mutations, such as in *p62/SQSTM1*, *OPTN*, ubiquilin2 (*UBQLN2*), and TANK-binding kinase (*TBK1*) directly disrupt selective autophagy and therefore prevent cargo from being degraded. The rest of the mutations have a more complex relationship with autophagy and lysosome function, such as mutations in the RNA-binding proteins TDP-43 and *FUS*. Here, we will discuss the genetic causes of ALS and FTLD in more detail with specific emphasis on lysosomal and autophagy impairment (**Figure 1**).

Figure 1. Functions of the ALS/FTLD genes in the autophagy-lysosome pathway. Many genes associated with ALS/FTLD play critical roles in the endosome-lysosomal pathway, regulate lysosomal functions, or affect autophagy pathway directly or indirectly.

2. Mutations affecting the endolysosome pathway: progranulin (PGRN), TMEM106B, CHMP2B, and VCP

2.1. Progranulin

The most common cause of familial FTLD with ubiquitin-positive aggregates is mutation of the *GRN* gene, which accounts for 10% of all FTLD cases and ~25% of familial FTLD [20–22]. About 70 mutations in the *GRN* gene have been linked to FTLD, most of which have been shown or predicted to decrease PGRN protein level or disrupt secretion of PGRN [20–24]. While FTLD is caused by haploinsufficiency of PGRN, a more severe neurodegeneration is caused by homozygous loss of PGRN. This complete loss of PGRN results in neuronal ceroid lipofuscinosis (NCL), a type of lysosome storage disorder (LSD) characterized by the build-up of autofluorescent lipofuscin [25, 26]. These findings suggest that loss of function mutations in the *GRN* gene causes neurodegenerative diseases in a dose-dependent manner and PGRN is important for lysosome function.

The function of PGRN is still under investigation: it is known to be a secreted glycoprotein comprised of 7.5 granulin repeats with pleiotropic roles, including protein homeostasis, inflammation, and neuronal survival and outgrowth [27]. Recently, several lines of evidence suggest that it plays a vital role in lysosome function. First, *GRN* has been found to be regulated with other lysosomal genes [28]. Furthermore, *GRN* mRNA and PGRN protein levels are upregulated in response to lysosome or autophagy inhibition [29]. Finally, PGRN was found to be delivered to the lysosome [30, 31]. PGRN reaches the lysosome through at two independent pathways. In one pathway, PGRN's extreme C-terminus binds the sorting receptor sortilin, which carries PGRN to the lysosome [30, 32]. In the second pathway, PGRN binds prosaposin, and they are

transported to the lysosome together by the cation-independent mannose-6-phosphate receptor (CI-M6PR) and low-density lipoprotein receptor-related protein 1 (LRP1) [31].

Mouse models of PGRN deficiency have consistently found increased levels of ubiquitin and p62, an adaptor for delivering cargo to the autophagosome [33], buildup of lipofuscin and its protein components saposin D and SCMAS and electron-dense storage granules, all of which suggest lysosome impairment [34–36]. Several models also found aggregation of TDP-43, similar to what is seen in FTLD patients [34, 37, 38]. Furthermore, PGRN-deficient mouse models also phenocopy FTLD symptoms such as decreased social interaction and mild learning/memory defects [35, 38–40]. The presence of clear lysosomal problems in mouse models and in patients with complete loss of PGRN suggests that PGRN is necessary for lysosome function. FTLD patients with GRN mutations also exhibit typical pathological features of NCL pathology [36], suggesting FTLD and NCL caused by PGRN mutations are pathologically linked and lysosomal dysfunction is one of the underlying disease mechanisms for FTLD-GRN. However, how PGRN regulates lysosomal function remains to be investigated.

2.2. TMEM106B

Another gene associated with FTLD is *TMEM106B*, which is the only identified risk factor for FTLD with *GRN* mutations [41–44]. *TMEM106B* was also found to increase risk in patients with *C9orf72* hexanucleotide repeat expansions [45, 46]. The *TMEM106B* SNP associated with FTLD increases the mRNA and protein levels of TMEM106B [36, 44, 47]. TMEM106B is a single pass, type II transmembrane protein that localizes to the late endosome and lysosome [47–49]. Cellular studies on TMEM106B have pointed to roles in lysosome trafficking and lysosomal stress response [50, 51]. Overexpression of TMEM106B in cells disrupts lysosome morphology and function [47, 48]. Furthermore, when a transgenic TMEM106B mouse line was crossed with a PGRN deficient mouse line, the lysosome abnormalities and lipofuscin accumulation seen in PGRN deficient mice were exacerbated [52]. The connection between TMEM106B's role at the lysosome and a risk factor for FTLD with *GRN* mutations further highlights the importance of the lysosome pathway in FTLD etiology.

2.3. CHMP2B

The sole mutation identified to cause FTLD with ubiquitin-positive aggregates, but tau, TDP-43, and FUS negative inclusions, occurs in the gene *CHMP2B* [53, 54]. *CHMP2B* has also been found to cause rare cases of ALS [55]. CHMP2B functions in the ESCRT-III complex, involved in MVB formation to deliver cargo from endocytic pathway to lysosomes [56, 57]. The mutations identified create an early termination of the protein, resulting in an unregulated CHMP2B truncation that is unable to recruit VPS4 to recycle the ESCRT-III complex to new sites of MVB formation [58, 59]. With ESCRT-III still engaged on the MVB, MVB-lysosome fusion cannot take place [54, 60–62]. Furthermore, *CHMP2B* mutations impair autophagosome maturation, possibly through the disruption of amphisome formation between autophagosome and late endosomes [63–66]. Mouse models of *CHMP2B* mutations replicate both ALS and FTLD pathology, whereas *CHMP2B* knockout mice do not show neurodegenerative

phenotypes, implicating a gain of function disease mechanism [67–70]. Similar to the PGRN deficiency mouse models, *CHMP2B* mutations cause protein inclusions and accumulation of autofluorescent aggregates in the frontal cortex, reminiscent of lysosome storage disorders [71]. Thus, FTLN-associated mutations in *CHMP2B* impair the endolysosomal pathway, which may cause additional defects in autophagy [66, 69], providing additional evidence that disruption of the autophagy-lysosome pathway may drive ALS and FTLN.

2.4. VCP

Valosin-containing protein (VCP) has been implicated in several diseases including FTLN [22, 72–76], ALS [77], and Charcot Marie Tooth disease, a genetic peripheral nerve disorder [78]. VCP is an AAA⁺-ATPase that delivers and unfolds ubiquitinated proteins, as well as endoplasmic reticulum-associated protein degradation (ERAD) substrates, at the proteasome [79–83]. Furthermore, VCP binds to clathrin and EEA1 to regulate the size and selectivity of endosomes [83–85]. Pharmacological inactivation of VCP as well as VCP knockdown inhibits MVB formation and blocks autophagosome maturation, resulting in accumulated LC3-II, ubiquitin, and p62 levels along with cytoplasmic TDP-43 aggregation [86–88]. Disease-associated mutants of VCP present similar phenotypes in transgenic mouse models, whereas complete loss of VCP is embryonic lethal [86, 89–91]. Finally, VCP mutants inhibit the autophagic turnover of stress granules, which may be relevant to the accumulation of TDP-43-positive aggregates found in patients with VCP mutations [76, 92, 93]. The precise mechanism that halts autophagosome maturation in VCP mutations remains unclear, though MVB dysfunction may play a role [66]. VCP's role in MVB formation and autophagic flux suggest that loss of VCP function may cause ALS, FTLN, and other related neurodegenerative diseases by impairing the autophagy-lysosome pathway.

3. Autophagy adaptor proteins

Further evidence that ALS and FTLN are linked to autophagy and lysosome disruption comes from mutations that directly affect several autophagy adaptor proteins and their regulation. Genetic mutations in the adaptor proteins *p62/SQSTM1*, *UBQLN2*, and *OPTN* have been shown to contribute to rare cases of ALS [94–99] and FTLN [100, 101]. All these adaptor proteins contain an ubiquitin-associated (UBA) domain, which is able to bind polyubiquitin conjugated proteins that are tagged for degradation by either the UPS or autophagy. The autophagy adaptors then associate with LC3 on the autophagosome to deliver the cargo for degradation through autophagy-lysosome pathway.

3.1. p62/SQSTM1

p62/SQSTM1 (p62)-positive inclusions have been observed in patient tissue samples in both ALS and FTLN [18, 102–104]. The association of p62 with inclusions suggests that the inclusion body has been targeted for degradation and the accumulation of such inclusions suggests defects with their turnover [33, 105, 106]. p62 bridges autophagy substrates to the

autophagosome by interacting with ubiquitinated proteins via its UBA domain [107] and LC3 with its LC3-interacting region (LIR) [33, 108, 109].

p62 is activated by phosphorylation at Ser407 by ULK1, allowing further phosphorylation by casein kinase 2 or TANK-binding kinase 1 (TBK1), which increases p62's affinity for polyubiquitinated cargo [110–113]. p62 acts within the selective autophagy system by aggregating proteins and organelles together for the autophagosome to enclose [106, 114]. These aggregated cargos are then subject to autophagy [115, 116]. While p62 accumulation and association with protein aggregates broadly suggests a defect in autophagy, mutations in p62 directly link selective autophagy impairment to neurodegeneration.

The *p62* mutations identified in ALS and FTLN patients disrupt aggregate formation or decrease the amount of p62 protein produced, leading to loss of function [117–119]. Homozygous mutation of *p62* causes adolescence/childhood-onset neurodegeneration with a defect in mitochondrial depolarization response due to impaired autophagy [120]. Thus, a loss of normal p62 function in autophagy leads to neurodegeneration in a dose-dependent manner, with earlier onset correlating to lower levels of functional p62.

In addition to its role in autophagy, p62 also links ubiquitinated cargo to the proteasome through its UBA domain [106] and mediates the degradation of the protein via the UPS, indicating that p62 plays multiple roles in proteostasis [121].

3.2. Ubiquilin2

Another adaptor protein implicated in ALS and FTLN is ubiquilin2 (UBQLN2) [95, 122]. Similar to p62, UBQLN2 is able to recognize ubiquitinated proteins and bind them via its UBA domain [123]. The UBA domain is also required for UBQLN2 to associate with the autophagosome, though unlike p62 and OPTN, UBQLN2 does not directly recognize LC3 [124, 125].

Knockdown of UBQLN2 in culture reduced autophagosome formation and inhibited lysosomal degradation of mitochondria [124, 125]. This loss of UBQLN2 also sensitizes cells to starvation-induced death in an autophagy-dependent manner [124]. Interestingly, UBQLN2 binds directly to TDP-43 holo-protein and C-terminal fragments and may regulate the levels of TDP-43 in the cell independent of ubiquitin [126]. Indeed, overexpression of UBQLN2 in culture can reduce aggregation of TDP-43 [126].

Many of the disease-associated mutations map to the proline-rich domain in *UBQLN2*, which is important in mediating protein-protein interactions [95, 127]. Furthermore, mutations in *UBQLN2* have a reduced binding to hnRNPA1, a RNA-binding protein associated with stress granules. Interestingly, mutations in hnRNPA1 are also associated with ALS and these mutations also disrupt its interaction with UBQLN2 [128], confirming that the interaction of autophagy adaptors with stress granules is important for neuronal survival.

UBQLN2 knockout in a rodent model showed no neuronal loss, implying that loss of function is not the disease mechanism or that other autophagy adaptors are able to compensate for its loss *in vivo*. Transgenic animals with the ALS/FTLD-associated *UBQLN2* mutations produce ubiquitin, p62, and UBQLN2-positive puncta accompanied by neuronal loss, cognitive defects, and

motor impairment [129–131]. Increased expression of the wild-type UBQLN2 also causes neurodegeneration in a rodent model [132]. Thus, unlike mutations in *p62*, *UBQLN2* mutations appear to have a gain of function mechanism that impairs proper protein degradation by autophagy.

In addition to its function in the autophagy pathway, UBQLN2 binds to the proteasome through its ubiquitin-like (UBL) domain to deliver polyubiquitinated proteins and ERAD substrates to the proteasome for degradation [133]. A role of UBQLN2 in delivering protein aggregates to proteasome-mediated degradation via HSP70 has been recently demonstrated [134]. UBQLN2 also function together with other ALS/FTLD-related proteins, such as regulating endosome constitution with OPTN [135] and delivering ERAD substrates to the proteasome with VCP [136].

3.3. Optineurin (OPTN)

Rare mutations in *OPTN* are also associated with both ALS [97, 99] as well as FTLD [101]. These mutations are expected to decrease the level of OPTN protein, suggesting a loss of function resulting in disease [101]. In total, 1–4% of familial ALS cases are linked to mutations in *OPTN* [137]. OPTN, like *p62* and *UBQLN2*, binds to polyubiquitin-labeled proteins via a UBA domain [138]. OPTN also binds LC3 through an LIR to connect cargo to autophagosomes. Damaged mitochondria specifically recruit OPTN to induce mitophagy [139]. In support of a loss of function model for OPTN, depletion of OPTN in zebrafish causes motor defects [140].

OPTN also interacts with several other proteins associated with ALS. The E3 ubiquitin ligase HACE1 ubiquitinates OPTN to promote binding to *p62*, which forms a complex that enhances autophagic flux [141]. Similarly, phosphorylation of OPTN by TBK1 increases the interaction of OPTN and *p62* to the same effect [138, 142]. OPTN also binds directly to SOD1 aggregates independently of ubiquitination. Mutations in *OPTN* do not affect this interaction, but do impair autophagic clearance of SOD1 protein aggregates through an unknown mechanism [138, 140].

Mutation in *OPTN* had previously been linked to primary open-angle glaucoma (POAG) where these mutations were shown to decrease basal autophagy and inhibit autophagic flux upon autophagy induction [143]. Thus, mutations in *OPTN* have clear links to multiple neurodegenerative disease with consistent impairment in the autophagy pathway. How mutations in the same gene and similar cellular impairments can lead to distinct clinical outcomes remains unclear.

3.4. TBK1

TBK1 has recently been associated with both ALS and FTLD [96, 98, 101, 110, 111, 144–147]. TBK1 has functions in autophagy and in inflammation [148]. Regarding its function in autophagy, TBK1 phosphorylates *p62* and OPTN to increase their binding to LC3 and ubiquitin, respectively [138, 142]. Many of the discovered disease-associated mutations are expected to decrease TBK1 protein level, suggesting a loss of function model [96, 101].

While TBK1 interacts with both *p62* and OPTN, TBK1 and OPTN share several additional connections. Like OPTN, some mutations in TBK1 also cause glaucoma [149].

Furthermore, the mutation in OPTN that causes POAG enhances the binding of OPTN to TBK1, which may sequester TBK1 and prevent it from carrying out its normal function [142]. Finally, both TBK1 and OPTN are required specifically for mitophagy, with depletion of either component or expression of an ALS-associated mutant impairing mitophagy [150]. Taken together, mutations in *TBK1* cause decreased protein expression and defects in p62 and OPTN regulation again supporting a role of autophagy in preventing ALS and FTLT.

4. C9orf72

The most common known cause of both ALS and FTLT was discovered to be a hexanucleotide intronic repeat expansion in the gene *C9orf72* [151–153]. This repeat expansion is found in 18–25% of familial FTLT, 40% of familial ALS, and 4–8% of sporadic ALS and FTLT combined [154, 155]. While patients with *C9orf72* mutations display TDP-43-positive aggregates, they also have separate inclusions unique to this genetic mutation. These ubiquitin, p62, and occasionally UBQLN2-positive inclusions also contain dipeptide repeats generated from the repeat expansion [156–160]. Three molecular mechanisms of disease have been proposed: toxic gain of function of RNA repeats, gain of function of dipeptide repeats (DPRs) produced by repeat-associated non-ATG translation, and haploinsufficiency of the *C9orf72* protein.

RNA-repeats transcribed from the repeat expansion form nuclear foci and sequester many RNA-binding proteins, including several RNA-binding proteins already implicated in ALS and FTLT [151, 161–163]. In addition the RNA foci disrupt nucleocytoplasmic transport [164, 165]. Furthermore, five distinct DPRs are translated and can also alter nucleocytoplasmic transport - [167, 168] as well as disrupt membrane-less, phase-separated organelles such as the nucleolus, nuclear pore, and stress granules [169]. Nuclear translocation of TDP-43 has been shown to be blocked by both RNA repeats and DPRs [166–168], allowing TDP-43 to accumulate and aggregate in the cytosol, which is observed in ALS/FTLT with *C9orf72* mutations.

Haploinsufficiency was also proposed as a disease mechanism [153, 151, 170–172]. Early *C9orf72*-depletion models in *Caenorhabditis elegans* and zebrafish showed motor dysfunction, supporting this model [173, 174]. However, a neuronal-specific *C9orf72* knockout mouse showed no such phenotype [175]. Complete *C9orf72* knockout mice also do not show much neurodegeneration, but instead exhibit severe immune problems similar to autoimmune disorders [176–181].

Interestingly, *C9orf72* has been reported to play a role in autophagy and lysosome regulation. While many of the reports suggest that *C9orf72* and its binding partners, SMCR8 and WDR41, play a role in regulating autophagy initiation or maturation, likely via the FIP200/ULK1 complex, the precise mechanism remains uncertain [179, 182–186]. Other reports suggest that *C9orf72* plays a role in mammalian Target of Rapamycin (mTOR) and Transcription Factor EB (TFEB) signaling [186, 187], in stress granule assembly [188], or in actin dynamics [189].

5. RNA-binding proteins

The RNA-binding proteins TDP-43 and FUS have been closely associated with ALS and FTLN. Pathogenic TDP-43 or FUS aggregates are present in both conditions, though mutations in these genes result primarily in ALS [190]. Both proteins travel between the nucleus and cytoplasm as they regulate gene splicing, mRNA stability and trafficking, and stress granule dynamics [191, 192].

As both TDP-43 and FUS regulate the RNA from thousands of genes, many cellular problems could be anticipated. However, several lines of evidence have pointed out a role in regulating and challenging the autophagy pathway [193].

5.1. TDP-43

The identification of TDP-43 as the main component of protein aggregates in both ALS and FTLN spurred the awareness that ALS and FTLN had some underlying similarities [16, 17]. Interestingly, mutations in *TARDBP* (TAR DNA binding protein), the gene encoding TDP-43, lead overwhelmingly to ALS or ALS/FTLN, but not to FTLN alone [194, 195]. While soluble TDP-43 can be cleared by chaperone-mediated autophagy through its interaction with Hsc70 [196], TDP-43-positive stress granules and aggregates are cleared by macroautophagy [197, 198].

In addition being a substrate of autophagy, TDP-43 may play a direct role in regulating autophagy through its transcriptional regulation of *ATG7* [199]. As TDP-43 is sequestered in protein aggregates, it can no longer regulate *ATG7* transcription, impairing autophagy initiation, and further promoting TDP-43 accumulation [198, 199]. In a similar manner, TDP-43 also regulates the mRNA for Regulatory-Associated Protein of mTOR (RPTOR) and Dynactin subunit 1 (DCTN1) [197]. *RPTOR* encodes a component of the mTOR complex, and loss of *RPTOR* due to TDP-43 loss of function upregulates lysosome and autophagy biogenesis [197]. However, TDP-43 loss of function also results in reduced *DCTN1* mRNA, which encodes dynactin, a key component of autophagosome-lysosome fusion, leading to the accumulation of autophagosomes, preventing the turnover of aggregated TDP-43 [197].

TDP-43 additionally plays an important role in stress granule dynamics and mutations in *TARDBP* have been shown to increase the stability of stress granules, possibly allowing them to become irreversible protein aggregates [198, 200–203]. In support of this prolonged stress granule hypothesis, mutations in VCP decrease stress granule turnover by autophagy, leading to TDP-43-positive inclusion [92].

The interaction of TDP-43 with autophagy suggests a complex regulatory balance between the two under normal conditions. In disease states, a feedforward mechanism of TDP-43 sequestration into stress granules and aggregates followed by impaired autophagy could drive pathogenesis of ALS and FTLN [9, 202].

5.2. FUS

Like *TARDBP*, mutations in *FUS* have been linked more closely to ALS, though positive protein aggregates for FUS appear in both ALS and FTLN [9]. FUS-positive inclusions account for about 5–10% of FTLN cases [9] and 1% of ALS cases [15]. Several proposed mechanisms link FUS to disruption of the autophagy-lysosome pathway. First, the presence of FUS-positive aggregates in both familial and sporadic cases of ALS and FTLN suggests FUS may be particularly susceptible to aggregation. FUS is also involved in autoregulation, which could allow for a feedforward cycle of increased FUS production followed by cytosolic accumulation and aggregation [198, 204].

Additionally, mutations in *FUS* have been linked to altered stress granule dynamics [205, 206]. FUS-positive stress granules were found to be degraded by autophagy; however, stress granules containing mutant FUS were more stable and prevented stress granules disassembly [198]. As with TDP-43, stabilized stress granules may promote insoluble aggregate formation [202, 207–209]. This increases the burden on the autophagy pathway and may drive further cell damage. A recent study also found that ALS-associated mutant *FUS* was able to inhibit the early steps of autophagosome formation, leading to impaired autophagy flux [210]. Many of these studies found that enhancing autophagy, genetically or pharmaceutically, was able to reduce FUS-positive inclusions and prevent cellular toxicity [198, 205, 210]. While less well understood than TDP-43, the RNA-binding protein FUS seems to play a similar cellular role as TDP-43, including regulating the dynamics of stress granules. Besides increased burden on autophagy due to stabilized stress granules, FUS may also play a more direct role in autophagy impairment.

6. Microtubule-associated protein tau

Thirty percent of familial FTLN cases are caused by mutations in Microtubule-Associated Protein Tau (MAPT), encoding the protein tau [211]. These cases are characterized by the presence of tau aggregates positive for ubiquitin and p62, suggesting impaired degradation of accumulated tau [121, 212]. Genetic disruption of autophagy cargo selection is sufficient to cause aggregation of pathogenic tau [213]. The tau protein is mostly well-known for its association with Alzheimer's disease, when it also forms aggregates and is accompanied by neurodegeneration of the hippocampus [214]. How Alzheimer's disease and FTLN patients have overlapping cellular pathology but develop different clinical symptoms remains unclear.

Full length tau can be degraded by the UPS in an ubiquitin-dependent and independent manner [121, 215, 216], whereas misfolded or phosphorylated tau is sent to the autophagy pathway [217]. Generally, tau aggregation and toxicity correlates with autophagy activity, where enhanced autophagy rescues neurodegeneration and impairment exacerbates the symptoms [218–221]. Likewise, modulating TFEB to increase lysosome biogenesis prevents the accumulation of tau [222].

Tau is a microtubule-binding protein that helps to stabilize axonal microtubules [223, 224]. Small increases in unbound tau induces aggregation, suggesting that even mild impairment

of the UPS or autophagy-lysosome pathway could lead to pathological tau accumulation [225, 226]. In support of this idea, Niemann-Pick disease, another lysosome storage disorder, also develops tau aggregates [227, 228]. These studies suggest that tau clearance is highly dependent on autophagy and lysosome function and disruption of this pathway may drive tau aggregation. Furthermore, tau has a role in microtubule stability and disrupted cytoskeletal dynamics and trafficking have also been proposed as a disease mechanism. Since lysosomes, endosomes, MVB, and autophagosomes all move along microtubules, any disruptions would affect their ability to maintain proteostasis [229].

7. Discussion

ALS and FTLN are distinct clinical disorders that share overlapping symptoms, pathology, and genetics. Many of the causative genetic mutations and risk factors result in disruption of the lysosome-autophagy pathway (**Figure 1**). Some disease-associated mutants or alleles directly impact lysosomal function through yet unknown mechanisms, such as *PGRN* and *TMEM106B*, or through disruption of the late stages of the endolysosome pathway, as *VCP* and *CHMP2B* mutations are proposed to do. Beyond the lysosome, there are also many mutations in adaptor proteins that impair selective autophagy, including *p62/SQSTM1*, *OPTN*, and *UBQLN2*. The misregulation of these adaptors is sufficient to induce neurodegeneration, as seen with *TBK1* mutants. Finally, some mutations have a more intricate relationship to the autophagy-lysosome pathway that future research will have to address, including *C9orf72* protein, repeat-associated RNA foci, and dipeptide repeats, as well as the microtubule-binding protein tau and the RNA-binding proteins TDP-43 and FUS.

Identifying the underlying cellular problems that lead to disease is an important step in being able to distinguish disorders and subtypes that may ultimately require distinct diagnosis and treatment. The genetic analysis of ALS and FTLN has improved our understanding of this disease spectrum and may inform us of the broad problems that underlie both familial and sporadic ALS and FTLN. The consistent impairment of cellular clearance pathways by ALS and FTLN-associated mutations points to a disease mechanism that is likely to be shared in undiscovered genetic causes, as well as environmental risk factors, that account for the cases of ALS and FTLN that have no known cause.

Acknowledgements

This work is supported by funding to F.H. from the Alzheimer's Association, the Association of Frontotemporal Dementia (AFTD), and the Muscular Dystrophy Association and NINDS (R21 NS081357-01, R01NS088448-01) and by funding to P.M.S. from the Henry and Samuel Mann Outstanding Graduate Student Award and to X.Z. from the Weill Institute Fleming Postdoctoral fellowship.

Author details

Peter M. Sullivan, Xiaolai Zhou and Fenghua Hu*

*Address all correspondence to: fh87@cornell.edu

Department of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA

References

- [1] Mizushima N, Hara T. Intracellular quality control by autophagy: How does autophagy prevent neurodegeneration? *Autophagy*. 2006;**2**(4):302-304
- [2] Lee S, Sato Y, Nixon RA. Lysosomal proteolysis inhibition selectively disrupts axonal transport of degradative organelles and causes an Alzheimer's-like axonal dystrophy. *The Journal of Neuroscience*. 2011;**31**(21):7817-7830
- [3] Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature*. 2006;**441**:880-884
- [4] Nedelsky NB, Todd PK, Taylor JP. Autophagy and the ubiquitin-proteasome system: Collaborators in neuroprotection. *Biochimica et Biophysica Acta Molecular Basis of Disease*. 2008;**1782**(12):691-699
- [5] Mizushima N. Autophagy : Process and function. *Genes & Development*. 2007;**21**: 2861-2873
- [6] Wong E, Cuervo AM. Integration of clearance mechanisms : The proteasome and autophagy. *Cold Spring Harbor Perspectives in Biology*. 2010;**2**(12):a006734
- [7] Feng Y, Yao Z, Klionsky DJ. How to control self-digestion: Transcriptional, post-transcriptional, and post-translational regulation of autophagy. *Trends in Cell Biology*. 2015;**25**:354-363
- [8] Carroll B, Hewitt G, Korolchuk VI. Autophagy and ageing: Implications for age-related neurodegenerative diseases. *Essays in Biochemistry*. 2013;**55**:119-131
- [9] Gotzl JK, Lang CM, Haass C, Capell A. Impaired protein degradation in FTLD and related disorders. *Ageing Research Reviews*. 2016;**32**:122-139
- [10] Morgan S, Orrell RW. Pathogenesis of amyotrophic lateral sclerosis. *British Medical Bulletin*. 2016;**119**:87-97
- [11] Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. *Neurology*. 2002;**58**(11):1615-1621

- [12] Neary D, Snowden JS, Mann DM. Classification and description of frontotemporal dementias. *Annals of the New York Academy of Sciences*. 2000;**920**:46-51
- [13] Hardy J, Rogaeva E. Motor neuron disease and frontotemporal dementia: Sometimes related, sometimes not. *Experimental Neurology*. 2014;**262**(PB):75-83
- [14] Janssens J, Van Broeckhoven C. Pathological mechanisms underlying TDP-43 driven neurodegeneration in FTLD-ALS spectrum disorders. *Human Molecular Genetics*. 2013;**22**(R1):77-87
- [15] Guerrero EN, Wang H, Mitra J, Hegde PM, Stowell SE, Liachko NF, et al. TDP-43/FUS in motor neuron disease: Complexity and challenges. *Progress in Neurobiology*. 2016;**145-146**:78-97
- [16] Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;**314**(5796):130-133
- [17] Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochemical and Biophysical Research Communications*. 2006;**351**(3):602-611
- [18] Tanji K, Zhang HX, Mori F, Kakita A, Takahashi H, Wakabayashi K. P62/sequestosome 1 binds to TDP-43 in brains with frontotemporal lobar degeneration with TDP-43 inclusions. *Journal of Neuroscience Research*. 2012;**90**(10):2034-2042
- [19] Ng ASL, Rademakers R, Miller BL. Frontotemporal dementia: A bridge between dementia and neuromuscular disease. *Annals of the New York Academy of Sciences*. 2015;**1338**(1):71-93
- [20] Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*. 2006;**442**(7105):916-919
- [21] Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature*. 2006;**442**(August):920-924
- [22] Sieben A, Van Langenhove T, Engelborghs S, Martin JJ, Boon P, Cras P, et al. The genetics and neuropathology of frontotemporal lobar degeneration. *Acta Neuropathologica*. 2012;**124**(3):353-372
- [23] Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Human Molecular Genetics*. 2006;**15**(20):2988-3001
- [24] Shankaran SS, Capell A, Hruscha AT, Fellerer K, Neumann M, Schmid B, et al. Missense mutations in the progranulin gene linked to frontotemporal lobar degeneration with ubiquitin-immunoreactive inclusions reduce progranulin production and secretion. *The Journal of Biological Chemistry*. 2008;**283**(3):1744-1753

- [25] Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, et al. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. *The American Journal of Human Genetics*. 2012;**90**(6):1102-1107
- [26] Canafoglia L, Morbin M, Scaioli V, Pareyson D, D'Incerti L, Fugnanesi V, et al. Recurrent generalized seizures, visual loss, and palinopsia as phenotypic features of neuronal ceroid lipofuscinosis due to progranulin gene mutation. *Epilepsia*. 2014;**55**(6):56-59
- [27] Kleinberger G, Capell A, Haass C, Van Broeckhoven C. Mechanisms of granulin deficiency: Lessons from cellular and animal models. *Molecular Neurobiology*. 2013;**47**(1):337-360
- [28] Belcastro V, Siciliano V, Gregoretti F, Mithbaokar P, Dharmalingam G, Berlingieri S, et al. Transcriptional gene network inference from a massive dataset elucidates transcriptome organization and gene function. *Nucleic Acids Research*. 2011;**39**(20):8677-8688
- [29] Capell A, Liebscher S, Fellerer K, Brouwers N, Willem M, Lammich S, et al. Rescue of progranulin deficiency associated with frontotemporal lobar degeneration by alkalizing reagents and inhibition of vacuolar ATPase. *The Journal of Neuroscience*. 2011;**31**(5):1885-1894
- [30] Hu F, Padukkavidana T, Vægter CB, Brady OA, Zheng Y, Mackenzie IR, et al. Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. *Neuron*. 2010;**68**(4):654-667
- [31] Zhou X, Sun L, de Oliveira FB, Qi X, Brown WJ, Smolka MB, et al. Prosaposin facilitates sortilin-independent lysosomal trafficking of progranulin. *The Journal of Cell Biology*. 2015;**210**(6):991-1002
- [32] Zheng Y, Brady OA, Meng PS, Mao Y, Hu F. C-Terminus of Progranulin Interacts with the Beta-Propeller Region of Sortilin to Regulate Progranulin Trafficking. *PLoS ONE*. 2011;**6**(6):e21023.
- [33] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy*[S]. *The Journal of Biological Chemistry*. 2007;**282**(33):24131-24145
- [34] Tanaka Y, Chambers JK, Matsuwaki T, Yamanouchi K, Nishihara M. Possible involvement of lysosomal dysfunction in pathological changes of the brain in aged progranulin-deficient mice. *Acta Neuropathologica Communications*. 2014;**2**:78.
- [35] Wils H, Kleinberger G, Pereson S, Janssens J, Capell A, Van Dam D, et al. Cellular ageing, increased mortality and FTLT-TDP-associated neuropathology in progranulin knock-out mice. *The Journal of Pathology*. 2012;**228**(1):67-76
- [36] Götzl JK, Mori K, Damme M, Fellerer K, Tahirovic S, Kleinberger G, et al. Common pathobiochemical hallmarks of progranulin-associated frontotemporal lobar degeneration and neuronal ceroid lipofuscinosis. *Acta Neuropathologica*. 2014;**127**(6):845-860
- [37] Ahmed Z, Sheng H, Xu Y, Lin W-L, Innes AE, Gass J, et al. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. *American Journal of Pathology*. 2010;**177**(1):311-324

- [38] Yin F, Dumont M, Banerjee R, et al. Behavioral deficits and progressive neuropathology in progranulin-deficient mice: A mouse model of frontotemporal dementia. *the Federation of American Societies for Experimental Biology journal*. 2010;**24**(12):4639-4647
- [39] Ghoshal N, Dearborn JT, Wozniak DF, Cairns NJ. Core features of frontotemporal dementia recapitulated in progranulin knockout mice. *Neurobiology of Disease*. 2012;**45**(1):395-408
- [40] Petkau TL, Neal SJ, Milnerwood A, Mew A, Hill AM, Orban P, et al. Synaptic dysfunction in progranulin-deficient mice. *Neurobiology of Disease*. 2012;**45**(2):711-722
- [41] Cruchaga C, Graff C, Chiang H-H, Wang J, Hinrichs AL, Spiegel N, et al. Association of TMEM106B gene polymorphism with age at onset in granulin mutation carriers and plasma granulin protein levels. *Archives of Neurology*. 2011;**68**(5):581-586
- [42] Finch N, Carrasquillo MM, Baker M, Rutherford NJ, Coppola G, DeJesus-Hernandez M, et al. TMEM106B regulates progranulin levels and the penetrance of FTL in GRN mutation carriers. *Neurology*. 2011;**76**(5):467-474
- [43] Van Der Zee J, Van Langenhove T, Kleinberger G, Sleegers K, Engelborghs S, Vandenberghe R, et al. TMEM106B is associated with frontotemporal lobar degeneration in a clinically diagnosed patient cohort. *Brain*. 2011;**134**(3):808-815
- [44] Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang L-S, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nature Genetics*. 2010;**42**(3):234-239
- [45] Gallagher MD, Suh E, Grossman M, Elman L, McCluskey L, Van Swieten JC, et al. TMEM106B is a genetic modifier of frontotemporal lobar degeneration with C9orf72 hexanucleotide repeat expansions. *Acta Neuropathologica*. 2014;**127**(3):407-418
- [46] Ash PEA, Bieniek KF, Gendron TF, Caulfield T, Lin WL, DeJesus-Hernandez M, et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron*. 2013;**77**:639-646
- [47] Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, et al. TMEM106B, the risk gene for frontotemporal dementia, is regulated by the microRNA-132/212 cluster and affects progranulin pathways. *The Journal of Neuroscience*. 2012;**32**(33):11213-11227
- [48] Brady OA, Zheng Y, Murphy K, Huang M, Hu F. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. *Human Molecular Genetics*. 2013;**22**(4):685-695
- [49] Lang CM, Fellerer K, Schwenk BM, Kuhn PH, Kremmer E, Edbauer D, et al. Membrane orientation and subcellular localization of transmembrane protein 106B (TMEM106B), a major risk factor for frontotemporal lobar degeneration. *The Journal of Biological Chemistry*. 2012;**287**(23):19355-19365
- [50] Stagi M, Klein ZA, Gould TJ, Bewersdorf J, Strittmatter SM. Lysosome size, motility and stress response regulated by fronto-temporal dementia modifier TMEM106B. *Molecular and Cellular Neurosciences*. 2014;**61**:226-240

- [51] Schwenk BM, Lang CM, Hogg S, Tahirovic S, Orozco D, Rentzsch K, et al. The FTLD risk factor TMEM106B and MAP6 control dendritic trafficking of lysosomes. *The European Molecular Biology Organization Journal*. 2014;**33**(5):450-467
- [52] Zhou X, Sun L, Brady OA, Murphy KA, Hu F. Elevated TMEM106B levels exaggerate lipofuscin accumulation and lysosomal dysfunction in aged mice with progranulin deficiency. *Acta Neuropathologica Communications*. 2017;**5**(1):9
- [53] Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nature Genetics*. 2005;**37**(8):806-808
- [54] Urwin H, Josephs KA, Rohrer JD, MacKenzie IR, Neumann M, Authier A, et al. FUS pathology defines the majority of tau-and TDP-43-negative frontotemporal lobar degeneration. *Acta Neuropathologica*. 2010;**120**(1):33-41
- [55] Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, et al. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology*. 2006;**67**(6):1074-1077
- [56] Tsang HTH, Connell JW, Brown SE, Thompson A, Reid E, Sanderson CM. A systematic analysis of human CHMP protein interactions: Additional MIT domain-containing proteins bind to multiple components of the human ESCRT III complex. *Genomics*. 2006;**88**(3):333-346
- [57] Fader CM, Colombo MI. Autophagy and multivesicular bodies: Two closely related partners. *Cell Death and Differentiation*. 2009;**16**(1):70-78
- [58] Shim S, Kimpler LA, Hanson PI. Structure/function analysis of four core ESCRT-III proteins reveals common regulatory role for extreme C-terminal domain. *Traffic*. 2007;**8**(8):1068-1079
- [59] van der Zee J, Urwin H, Engelborghs S, Bruyland M, Vandenberghe R, Dermaut B, et al. CHMP2B C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. *Human Molecular Genetics*. 2008;**17**(2):313-322
- [60] Stuchell-Brereton MD, Skalicky JJ, Kieffer C, Karren MA, Ghaffarian S, Sundquist WI. ESCRT-III recognition by VPS4 ATPases. *Nature*. 2007;**449**(7163):740-744
- [61] Obita T, Saksena S, Ghazi-Tabatabai S, Gill DJ, Perisic O, Emr SD, Williams RL. Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. *Nature*. 2007;**449**:735-740
- [62] Wollert T, Wunder C, Lippincott-schwartz J, Hurley JH. Membrane scission by the ESCRT-III complex. *Nature*. 2009;**458**(7235):172-177
- [63] Lu Y, Zhang Z, Sun D, Sweeney ST, Gao FB. Syntaxin 13, a genetic modifier of mutant CHMP2B in frontotemporal dementia, is required for autophagosome maturation. *Molecular Cell*. 2013;**52**(2):264-271

- [64] West RJH, Lu Y, Marie B, Gao FB, Sweeney ST. Rab8, POSH, and TAK1 regulate synaptic growth in a *Drosophila* model of frontotemporal dementia. *The Journal of Cell Biology*. 2015;**208**(7):931-947
- [65] Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerød L, Fisher EMC, et al. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. *The Journal of Cell Biology*. 2007;**179**(3):485-500
- [66] Lee JA, Liu L, Gao FB. Autophagy defects contribute to neurodegeneration induced by dysfunctional ESCRT-III. *Autophagy*. 2009;**5**:1070-1072
- [67] Ghazi-Noori S, Froud KE, Mizielinska S, Powell C, Smidak M, Fernandez De Marco M, et al. Progressive neuronal inclusion formation and axonal degeneration in CHMP2B mutant transgenic mice. *Brain*. 2012;**135**(3):819-832
- [68] Krasniak CS, Ahmad ST. The role of CHMP2B Intron5 in autophagy and frontotemporal dementia. *Brain Research*. 2016;**1649**:151-157
- [69] Vernay A, Therreau L, Blot B, Risson V, Dirrig-Grosch S, Waegaert R, et al. A transgenic mouse expressing CHMP2B Intron5 mutant in neurons develops histological and behavioural features of amyotrophic lateral sclerosis and frontotemporal dementia. *Human Molecular Genetics*. 2016;**25**(15):3341-3360
- [70] Nielsen TT, Mizielinska S, Hasholt L, Isaacs AM, Nielsen JE. Reversal of pathology in CHMP2B-mediated frontotemporal dementia patient cells using RNA interference. *The Journal of Gene Medicine*. 2012;**14**(8):521-529
- [71] Clayton EL, Mizielinska S, Edgar JR, Nielsen TT, Marshall S, Norona FE, et al. Frontotemporal dementia caused by CHMP2B mutation is characterised by neuronal lysosomal storage pathology. *Acta Neuropathologica*. 2015;**130**(4):511-523
- [72] Watts GDJ, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nature Genetics*. 2004;**36**(4):377-381
- [73] Guyant-Maréchal L, Laquerrière A, Duyckaerts C, Dumanchin C, Bou J, Dugny F, et al. Valosin-containing protein gene mutations: Clinical and neuropathologic features. *Neurology*. 2006;**67**(4):644-651
- [74] Forman MS, Mackenzie IR, Cairns NJ, Swanson E, Boyer PJ, Drachman DA, et al. Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. *Journal of Neuropathology and Experimental Neurology*. 2006;**65**(6):571-581
- [75] Schröder R, Watts GDJ, Mehta SG, Evert BO, Broich P, Fließbach K, et al. Mutant valosin-containing protein causes a novel type of frontotemporal dementia. *Annals of Neurology*. 2005;**57**(3):457-461
- [76] Neumann M, Mackenzie IR, Cairns NJ, Boyer PJ, Markesbery WR, Smith CD, et al. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *Journal of Neuropathology and Experimental Neurology*. 2007;**66**(2):152-157

- [77] Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron*. 2010;**68**(5):857-864
- [78] Gonzalez MA, Feely SM, Speziani F, Strickland AV, Danzi M, Bacon C, et al. A novel mutation in VCP causes Charcot-Marie-Tooth Type 2 disease. *Brain*. 2014;**137**(11):2897-2902
- [79] Jentsch S, Rumpf S. Cdc48 (p97): A “molecular gearbox” in the ubiquitin pathway? *Trends in Biochemical Sciences*. 2007;**32**(1):6-11
- [80] Rabinovich E, Kerem A, Fröhlich K-U, Diamant N, Bar-Nun S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Molecular and Cellular Biology*. 2002;**22**(2):626-634
- [81] Song C, Wang Q, Song C, Rogers TJ. Valosin-containing protein (VCP/p97) is capable of unfolding polyubiquitinated proteins through its ATPase domains. *Biochemical and Biophysical Research Communications*. 2015;**463**(3):453-457
- [82] Lim PJ, Danner R, Liang J, Doong H, Harman C, Srinivasan D, et al. Ubiquilin and p97/VCP bind erasin, forming a complex involved in ERAD. *The Journal of Cell Biology*. 2009;**187**(2):201-217
- [83] Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature*. 2001;**414**(6864):652-656
- [84] Pleasure IT, Black MM, Keen JH. Valosin-containing protein, VCP, is a ubiquitous clathrin-binding protein. *Nature*. 1993;**365**(6445):459-462
- [85] Ramanathan HN, Ye Y. The p97 ATPase associates with EEA1 to regulate the size of early endosomes. *Cell Research*. 2012;**22**(2):346-359
- [86] Ju JS, Fuentealba RA, Miller SE, Jackson E, Piwnicka-Worms D, Baloh RH, et al. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *The Journal of Cell Biology*. 2009;**187**(6):875-888
- [87] Tresse E, Salomons FA, Vesa J, Bott LC, Kimonis V, Yao TP, et al. VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. *Autophagy*. 2010;**6**(2):217-227
- [88] Ritz D, Vuk M, Kirchner P, Bug M, Schütz S, Hayer A, et al. Endolysosomal sorting of ubiquitylated caveolin-1 is regulated by VCP and UBXD1 and impaired by VCP disease mutations. *Nature Cell Biology*. 2011;**13**(9):1116-1123
- [89] Badadani M, Nalbandian A, Watts GD, et al. VCP Associated Inclusion Body Myopathy and Paget Disease of Bone Knock-In Mouse Model Exhibits Tissue Pathology Typical of Human Disease. *PLoS ONE*. 2010;**5**(10):e13183.
- [90] Custer SK, Neumann M, Lu H, Wright AC, Taylor JP. Transgenic mice expressing mutant forms VCP/p97 recapitulate the full spectrum of IBMPFD including degeneration in muscle, brain and bone. *Human Molecular Genetics*. 2010;**19**(9):1741-1755

- [91] Müller JMM, Deinhardt K, Rosewell I, Warren G, Shima DT. Targeted deletion of p97 (VCP/CDC48) in mouse results in early embryonic lethality. *Biochemical and Biophysical Research Communications*. 2007;**354**(2):459-465
- [92] Buchan JR, Kolaitis RM, Taylor JP, Parker R. XEukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell*. 2013;**153**(7):1461-1474
- [93] Weihl CC, Temiz P, Miller SE, Watts G, Smith C, Forman M, et al. TDP-43 accumulation in inclusion body myopathy muscle suggests a common pathogenic mechanism with frontotemporal dementia. *Journal of Neurology Neurosurgery and Psychiatry*. 2008;**79**(10):1186-1189
- [94] Fecto F. Mutations in familial and sporadic amyotrophic lateral sclerosis. *Archives of Neurology*. 2011;**68**(11):1440-1446
- [95] Deng H-X, Chen W, Hong S-T, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature*. 2011;**477**(7363):211-215
- [96] Freischmidt A, Müller K, Ludolph AC, Weishaupt JH, Andersen PM. Association of mutations in TBK1 with sporadic and familial amyotrophic lateral sclerosis and frontotemporal dementia. *JAMA Neurology*. 2017;**74**(1):110-113
- [97] Weishaupt JH, Waibel S, Birve A, Volk AE, Mayer B, Meyer T, et al. A novel optineurin truncating mutation and three glaucoma-associated missense variants in patients with familial amyotrophic lateral sclerosis in Germany. *Neurobiology of Aging*. 2013;**34**(5):1516.e9-1516.e15
- [98] Williams KL, McCann EP, Fifita JA, Zhang K, Duncan EL, Leo PJ, et al. Novel TBK1 truncating mutation in a familial amyotrophic lateral sclerosis patient of Chinese origin. *Neurobiology of Aging*. 2015;**36**(12):3334.e1-3334.e5
- [99] Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, et al. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*. 2010;**465**(7295):223-236
- [100] Kovacs GG, van der Zee J, Hort J, Kristoferitsch W, Leitha T, Höftberger R, et al. Clinicopathological description of two cases with SQSTM1 gene mutation associated with frontotemporal dementia. *Neuropathology*. 2016;**36**(1):27-38
- [101] Pottier C, Bieniek KF, Finch NC, van de Vorst M, Baker M, Perkersen R, et al. Whole-genome sequencing reveals important role for TBK1 and OPTN mutations in frontotemporal lobar degeneration without motor neuron disease. *Acta Neuropathologica*. 2015;**130**(1):77-92
- [102] Arai T, Nonaka T, Hasegawa M, Akiyama H, Yoshida M, Hashizume Y, et al. Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. *Neuroscience Letters*. 2003;**342**(1-2):41-44
- [103] Kuusisto E, Kauppinen T, Alafuzoff I. Use of p62/SQSTM1 antibodies for neuropathological diagnosis. *Neuropathology and Applied Neurobiology*. 2008;**34**(2):169-180

- [104] Nakano T, Nakaso K, Nakashima K, Ohama E. Expression of ubiquitin-binding protein p62 in ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic lateral sclerosis with dementia: Analysis of five autopsy cases with broad clinicopathological spectrum. *Acta Neuropathologica*. 2004;**107**(4):359-364
- [105] Korolchuk VI, Menzies FM, Rubinsztein DC. A novel link between autophagy and the ubiquitin-proteasome system. *Autophagy*. 2009;**5**(6):862-863
- [106] Seibenhener M, Babu J. Sequestosome 1/p62 Is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Molecular and Cellular Biology*. 2004;**24**(18):8055-8068
- [107] Rea SL, Walsh JP, Layfield R, Ratajczak T, Xu Jiak J. New insights into the role of sequestosome 1/p62 mutant proteins in the pathogenesis of paget's disease of bone. *Endocrine Reviews*. 2013;**34**(4):501-524
- [108] Rogov V, Dötsch V, Johansen T, Kirkin V. Interactions between autophagy receptors and Ubiquitin-like proteins form the molecular basis for selective autophagy. *Molecular Cell*. 2014;**53**(2):167-178
- [109] Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. *Autophagy*. 2011;**7**(3):279-296
- [110] Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Molecular Cell*. 2011;**44**(2):279-289
- [111] Pilli M, Arko-Mensah J, Ponpuak M, Roberts E, Master S, Mandell MA, et al. TBK-1 Promotes Autophagy-Mediated antimicrobial defense by controlling autophagosome maturation. *Immunity*. 2012;**37**(2):223-234
- [112] Lim J, Lachenmayer ML, Wu S, Liu W, Kundu M, Wang R, et al. Proteotoxic stress induces phosphorylation of p62/SQSTM1 by ULK1 to regulate selective autophagic clearance of protein aggregates. *PLoS Genetics*. 2015;**11**(2):e1004987
- [113] Ro SH, Semple IA, Park H, Park H, Park HW, Kim M, et al. Sestrin2 promotes Unc-51-like kinase 1 mediated phosphorylation of p62/sequestosome-1. *The FEBS Journal*. 2014;**281**(17):3816-3827
- [114] Paine MG, Babu JR, Seibenhener ML, Wooten MW. Evidence for p62 aggregate formation: Role in cell survival. *The Federation of European Biochemical Societies Letters*. 2005;**579**(22):5029-5034
- [115] Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Øvervatn A, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *The Journal of Cell Biology*. 2005;**171**(4):603-614
- [116] Komatsu M, Waguri S, Koike M, Sou Y shin, Ueno T, Hara T, et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell*. 2007;**131**(6):1149-1163

- [117] Kwok CT, Morris A, de Belleruche JS. Sequestosome-1 (SQSTM1) sequence variants in ALS cases in the UK: Prevalence and coexistence of SQSTM1 mutations in ALS kindred with PDB. *European Journal of Human Genetics*. 2014;**22**(4):492-496
- [118] Teyssou E, Takeda T, Lebon V, Boillée S, Doukouré B, Bataillon G, et al. Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: Genetics and neuropathology. *Acta Neuropathologica*. 2013;**125**(4):511-522
- [119] Rubino E, Chio A, Rogaeva E, Galimberti D, Bruni AC, St PH. SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Neurology*. 2012;**79**(15):1556-62
- [120] Haack TB, Ignatius E, Calvo-Garrido J, et al. Absence of the Autophagy Adaptor SQSTM1/p62 Causes Childhood-Onset Neurodegeneration with Ataxia, Dystonia, and Gaze Palsy. *American Journal of Human Genetics*. 2016;**99**(3):735-743.
- [121] Babu JR, Geetha T, Wooten MW. Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. *Journal of Neurochemistry*. 2005;**94**(1):192-203
- [122] Zhang Y-J, Gendron TF, Xu Y-F, Ko L-W, Yen S-H, Petrucelli L. Phosphorylation regulates proteasomal-mediated degradation and solubility of TAR DNA binding protein-43 C-terminal fragments. *Molecular Neurodegeneration*. 2010;**5**:33
- [123] Ko HS, Uehara T, Tsuruma K, Nomura Y. Ubiquitin interacts with ubiquitylated proteins and proteasome through its ubiquitin-associated and ubiquitin-like domains. *The Federation of European Biochemical Societies Letters*. 2004;**566**(1-3):110-114
- [124] N'Diaye E-N, Kajihara KK, Hsieh I, Morisaki H, Debnath J, Brown EJ. PLIC proteins or ubiquilins regulate autophagy-dependent cell survival during nutrient starvation. *The European Molecular Biology Organization Reports*. 2009;**10**(2):173-179
- [125] Rothenberg C, Srinivasan D, Mah L, Kaushik S, Peterhoff CM, Ugolino J, et al. Ubiquitin functions in autophagy and is degraded by chaperone-mediated autophagy. *Human Molecular Genetics*. 2010;**19**(16):3219-3232
- [126] Cassel JA, Reitz AB. Ubiquitin-2 (UBQLN2) binds with high affinity to the C-terminal region of TDP-43 and modulates TDP-43 levels in H4 cells: Characterization of inhibition by nucleic acids and 4-aminoquinolines. *Biochimica et Biophysica Acta - Proteins Proteomics*. 2013;**1834**(6):964-971
- [127] Majcher V, Goode A, James V, Layfield R. Autophagy receptor defects and ALS-FTLD. *Molecular and Cellular Neurosciences*. 2015;**66**(Part A):43-52
- [128] Gilpin KM, Chang L, Monteiro MJ. ALS-linked mutations in ubiquitin-2 or hnRNPA1 reduce interaction between ubiquitin-2 and hnRNPA1. *Human Molecular Genetics*. 2015;**24**(9):2565-2577
- [129] Wu Q, Liu M, Huang C, Liu X, Huang B, Li N, et al. Pathogenic Ubqln2 gains toxic properties to induce neuron death. *Acta Neuropathologica*. 2014;**129**(3):417-428

- [130] Le NTT, Chang L, Kovlyagina I, Georgiou P, Safren N, Braunstein KE, et al. Motor neuron disease, TDP-43 pathology, and memory deficits in mice expressing ALS-FTD-linked UBQLN2 mutations. In: *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(47):E7580-E7589.
- [131] Ceballos-Diaz C, Rosario AM, Park H-J, Chakrabarty P, Sacino A, Cruz PE, et al. Viral expression of ALS-linked ubiquilin-2 mutants causes inclusion pathology and behavioral deficits in mice. *Molecular Neurodegeneration*. 2015;**10**(1):25
- [132] Huang, B., Wu, Q., Zhou, H., Huang, C. and Xia, X.-G. Increased Ubqln2 expression causes neuron death in transgenic rats. *J. Neurochem.*, 2016;**139**: 285-293.
- [133] Walters KJ, Kleijnen MF, Goh AM, Wagner G, Howley PM. Structural studies of the interaction between ubiquitin family proteins and proteasome subunit S5a. *Biochemistry*. 2002;**41**(6):1767-1777
- [134] Hjerpe R, Bett JS, Keuss MJ, Solovyova A, McWilliams TG, Johnson C, et al. UBQLN2 mediates Autophagy-Independent protein aggregate clearance by the proteasome. *Cell*. 2016;**166**(4):935-949
- [135] Osaka M, Ito D, Suzuki N. Disturbance of proteasomal and autophagic protein degradation pathways by amyotrophic lateral sclerosis-linked mutations in ubiquilin 2. *Biochemical and Biophysical Research Communications*. 2016;**472**(2):324-331
- [136] Xia Y, Yan LH, Huang B, Liu M, Liu X, Huang C. Pathogenic mutation of UBQLN2 impairs its interaction with UBXD8 and disrupts endoplasmic reticulum-associated protein degradation. *Journal of Neurochemistry*. 2014;**129**(1):99-106
- [137] Iguchi Y, Katsuno M, Ikenaka K, Ishigaki S, Sobue G. Amyotrophic lateral sclerosis: An update on recent genetic insights. *Journal of Neurology*. 2013;**260**(11):2917-2927
- [138] Wild P, Farhan H, McEwan DG, Wagner S, Rogov V V., Brady NR, et al. Phosphorylation of the autophagy. *Science* (80-). 2011;**333**(July):228-233
- [139] Wong YC, Holzbaur ELF. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;**111**(42):E4439-4448
- [140] Korac J, Schaeffer V, Kovacevic I, Clement AM, Jungblut B, Behl C, et al. Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. *Journal of Cell Science*. 2013;**126**(2):580-592
- [141] Liu Z, Chen P, Gao H, Gu Y, Yang J, Peng H, et al. Ubiquitylation of autophagy receptor optineurin by HACE1 activates selective autophagy for tumor suppression. *Cancer Cell*. 2014;**26**(1):106-120
- [142] Morton S, Hesson L, Pegg M, Cohen P. Enhanced binding of TBK1 by an optineurin mutant that causes a familial form of primary open angle glaucoma. *The Federation of European Biochemical Societies Letters*. 2008;**582**(6):997-1002

- [143] Chalasan MLS, Kumari A, Radha V, Swarup G. E50K-OPTN-Induced Retinal Cell Death Involves the Rab GTPase-Activating Protein, TBC1D17 Mediated Block in Autophagy. *PLoS ONE*. 2014;**9**(4):e95758.
- [144] Borghero G, Pugliatti M, Marrosu F, Marrosu MG, Murru MR, Floris G, et al. TBK1 is associated with ALS and ALS-FTD in Sardinian patients. *Neurobiology of Aging*. 2016;**43**:1-5
- [145] Gijssels I, Van Mossevelde S, van der Zee J, Sieben A, Philtjens S, Heeman B, et al. Loss of *TBK1* is a frequent cause of frontotemporal dementia in a Belgian cohort. *Neurology*. 2015;**85**(24):2116-2125
- [146] Le Ber I, De Septenville A, Millecamps S, Camuzat A, Caroppo P, Couratier P, et al. TBK1 mutation frequencies in French frontotemporal dementia and amyotrophic lateral sclerosis cohorts. *Neurobiology of Aging*. 2015;**36**(11):3116.e5-3116.e8
- [147] Tsai PC, Liu YC, Lin KP, Liu YT, Liao YC, Hsiao CT, et al. Mutational analysis of TBK1 in Taiwanese patients with amyotrophic lateral sclerosis. *Neurobiology of Aging*. 2016;**40**:191.e11-6
- [148] Oakes JA, Davies MC, Collins MO. TBK1: a new player in ALS linking autophagy and neuroinflammation. *Molecular Brain*. 2017;**10**:5.
- [149] Minegishi Y, Nakayama M, Iejima D, Kawase K, Iwata T. Significance of optineurin mutations in glaucoma and other diseases. *Progress in Retinal and Eye Research*. 2016;**55**:149-181
- [150] Moore AS, Holzbaur ELF. Dynamic recruitment and activation of ALS-associated TBK1 with its target optineurin are required for efficient mitophagy. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;**113**(24):E3349–E3358.
- [151] DeJesus-hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Supplemental information expanded GGGGCC hexanucleotide repeat in non-coding region of C9ORF72 causes chromosome 9p-Linked FTD and ALS. *Neuron*. 2011;**72**:245-256
- [152] Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*. 2011;**72**:257-268
- [153] Gijssels I, Van Langenhove T, van der Zee J, Sleegers K, Philtjens S, Kleinberger G, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: A gene identification study. *The Lancet Neurology*. 2012;**11**:54-65
- [154] Majounie E, Renton AE, Mok K, Doppler EGP, Waite A, Rollinson S, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: A cross-sectional study. *The Lancet Neurology*. 2012;**11**(4):323-330

- [155] van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S, et al. A Pan-European study of the C9orf72 repeat associated with FTLN: Geographic prevalence, genomic instability, and intermediate repeats. *Human Mutation*. 2013;**34**(2): 363-373
- [156] Al-Sarraj S, King A, Troakes C, Smith B, Maekawa S, Bodi I, et al. P62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLN and MND/ALS. *Acta Neuropathologica*. 2011;**122**(6):691-702
- [157] Brettschneider J, Van Deerlin VM, Robinson JL, Kwong L, Lee EB, Ali YO, et al. Pattern of ubiquilin pathology in ALS and FTLN indicates presence of C9ORF72 hexanucleotide expansion. *Acta Neuropathologica*. 2012;**123**(6):825-839
- [158] King A, Maekawa S, Bodi I, Troakes C, Al-Sarraj S. Ubiquitinated, p62 immunopositive cerebellar cortical neuronal inclusions are evident across the spectrum of TDP-43 proteinopathies but are only rarely additionally immunopositive for phosphorylation-dependent TDP-43. *Neuropathology*. 2011;**31**(3):239-249
- [159] Mahoney CJ, Beck J, Rohrer JD, Lashley T, Mok K, Shakespeare T, et al. Frontotemporal dementia with the C9ORF72 hexanucleotide repeat expansion: Clinical, neuroanatomical and neuropathological features. *Brain*. 2012;**135**(3):736-750
- [160] Mori K, Weng S-M, Arzberger T, May S, Rentzsch K, Kremmer E, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLN/ALS. *Science*. 2013;**339**:1335-1338
- [161] Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li H-R, et al. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**(47):E4530-4539
- [162] Mizielinska S, Lashley T, Norona FE, Clayton EL, Ridler CE, Fratta P, et al. C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathologica*. 2013;**126**(6):845-857
- [163] Gendron TF, Bieniek KF, Zhang YJ, Jansen-West K, Ash PEA, Caulfield T, et al. Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathologica*. 2013;**126**(6):829-844
- [164] Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee K-H, et al. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature*. 2015;**525**(7567):129-133
- [165] Gendron TF, Belzil V V, Zhang Y-J, Petrucelli L. Mechanisms of toxicity in C9FTLN/ALS. *Acta Neuropathologica*. 2014;**127**(3):359-376
- [166] Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature*. 2015;**525**(7567):56-61

- [167] Khosravi B, Hartmann H, May S, Möhl C, Ederle H, Michaelson M, et al. Cytoplasmic poly-GA aggregates impair nuclear import of TDP-43 in *C9orf72* ALS/FTLD. *Human Molecular Genetics*. 2017;**26**(4):790-800
- [168] Zhang Y-J, Gendron TF, Grima JC, Sasaguri H, Jansen-West K, Xu Y-F, et al. C9ORF72 poly(GA) aggregates sequester and impair HR23 and nucleocytoplasmic transport proteins. *Nature Neuroscience*. 2016;**19**(5):668-677
- [169] Lee KH, Zhang P, Kim HJ, Mitrea DM, Sarkar M, Freibaum BD, et al. C9orf72 dipeptide repeats impair the assembly, dynamics, and function of Membrane-Less organelles. *Cell*. 2016;**167**(3):774-788.e17
- [170] Fratta P, Poulter M, Lashley T, Rohrer JD, Polke JM, Beck J, et al. Homozygosity for the C9orf72 GGGGCC repeat expansion in frontotemporal dementia. *Acta Neuropathologica*. 2013;**126**(3):401-409
- [171] van Blitterswijk M, Gendron TF, Baker MC, DeJesus-Hernandez M, Finch NCA, Brown PH, et al. Novel clinical associations with specific C9ORF72 transcripts in patients with repeat expansions in C9ORF72. *Acta Neuropathologica*. 2015;**130**(6):863-876
- [172] Waite AJ, Bäumer D, East S, et al. Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion. *Neurobiology of Aging*. 2014;**35**(7):1779.e5-1779.e13.
- [173] Therrien M, Rouleau GA, Dion PA, Parker JA. Deletion of C9ORF72 Results in Motor Neuron Degeneration and Stress Sensitivity in *C. elegans*. *PLoS ONE*. 2013;**8**(12):e83450.
- [174] Ciura S, Lattante S, Le Ber I, Latouche M, Tostivint H, Brice A, et al. Loss of function of C9orf72 causes motor deficits in a zebrafish model of amyotrophic lateral sclerosis. *Annals of Neurology*. 2013;**74**:180-187
- [175] Koppers M, Blokhuis AM, Westeneng HJ, Terpstra ML, Zundel CAC, Vieira De Sá R, et al. C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. *Annals of Neurology*. 2015;**78**(3):426-438
- [176] Burberry A, Suzuki N, Wang JY, Moccia R, Mordes DA, Stewart MH, et al. Loss-of-function mutations in the C9ORF72 mouse ortholog cause fatal autoimmune disease. *Science Translational Medicine*. 2016;**8**(347):347ra93
- [177] ORourke JG, Bogdanik L, Yanez A, Lall D, Wolf AJ, Muhammad AKMG, et al. C9orf72 is required for proper macrophage and microglial function in mice. *Science* (80-). 2016;**351**(6279):1324-1329
- [178] Atanasio A, Decman V, White D, Ramos M, Ikiz B, Lee H-C, et al. C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production, and glomerulonephropathy in mice. *Scientific Reports*. 2016;**6**(November 2015):23204
- [179] Sullivan PM, Zhou X, Robins AM, Paushter DH, Kim D, Smolka MB, et al. The ALS/FTLD associated protein C9orf72 associates with SMCR8 and WDR41 to regulate the autophagy-lysosome pathway. *Acta Neuropathologica Communications*. 2016;**4**(1):51

- [180] Sudria-Lopez E, Koppers M, de Wit M, van der Meer C, Westeneng HJ, Zundel CAC, et al. Full ablation of C9orf72 in mice causes immune system-related pathology and neoplastic events but no motor neuron defects. *Acta Neuropathologica*. 2016;**132**(1):145-147
- [181] Jiang J, Zhu Q, Gendron TF, Saberi S, McAlonis-Downes M, Seelman A, et al. Gain of toxicity from ALS/FTD-Linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-Containing RNAs. *Neuron*. 2016;**90**(3):535-550
- [182] Farg MA, Sundaramoorthy V, Sultana JM, Yang S, Atkinson RAK, Levina V, et al. C9ORF72, implicated in amyotrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking. *Human Molecular Genetics*. 2014;**23**(13):3579-3595
- [183] Sellier C, Campanari M-L, Julie Corbier C, Gaucherot A, Kolb-Cheynel I, Oulad-Abdelghani M, et al. Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. *The European Molecular Biology Organization Journal*. 2016;**35**(Icm):1-22
- [184] Yang M, Liang C, Swaminathan K, Herrlinger S, Lai F, Shiekhatter R, et al. A C9ORF72/SMCR8-containing complex regulates ULK1 and plays a dual role in autophagy. *Science Advances*. 2016;**2**(9):e1601167-e1601167
- [185] Webster CP, Smith EF, Bauer CS, Moller A, Guillaume M, Ferraiuolo L, et al. The C9orf72 protein interacts with Rab 1 a and the ULK 1 complex to regulate initiation of autophagy. *The European Molecular Biology Organization Journal*. 2016;**35**(15):1-21
- [186] Ugolino J, Ji YJ, Conchina K, Chu J, Nirujogi RS, Pandey A, et al. Loss of C9orf72 Enhances Autophagic Activity via Deregulated mTOR and TFEB Signaling. *PLoS Genetics*. 2016;**12**(11):e1006443. doi:10.1371/journal.pgen.1006443.
- [187] Amick J, Roczniak-Ferguson A, Ferguson SM. C9orf72 binds SMCR8, localizes to lysosomes, and regulates mTORC1 signaling. *Molecular Biology of the Cell*. 2016;**27**(20):3040-3051.
- [188] Maharjan N, Kunzli C, Buthey K SS. C9ORF72 Regulates stress granule formation and its deficiency impairs stress granule assembly, hypersensitizing cells to stress. *Human Molecular Genetics*. 2016;**25**(15):3341-3360
- [189] Sivadasan R, Hornburg D, Drepper C, Frank N, Jablonka S, Hansel A, et al. C9ORF72 interaction with cofilin modulates actin dynamics in motor neurons. *Nature Neuroscience*. 2016;**19**(12):1610-1618
- [190] Snowden JS, Hu Q, Rollinson S, Halliwell N, Robinson A, Davidson YS, et al. The most common type of FTLD-FUS (aFTLD-U) is associated with a distinct clinical form of frontotemporal dementia but is not related to mutations in the FUS gene. *Acta Neuropathologica*. 2011;**122**(1):99-110
- [191] Lagier-Tourenne C, Polymenidou M, Cleveland DW. TDP-43 and FUS/TLS: Emerging roles in RNA processing and neurodegeneration. *Human Molecular Genetics*. 2010;**19**(R1):46-64

- [192] Bosco DA, Lemay N, Ko HK, Zhou H, Burke C, Kwiatkowski TJ, et al. Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules. *Human Molecular Genetics*. 2010;**19**(21):4160-4175
- [193] Thomas M, Alegre-Abarrategui J, Wade-Martins R. RNA dysfunction and aggregopathy at the centre of an amyotrophic lateral sclerosis/frontotemporal dementia disease continuum. *Brain*. 2013;**136**(5):1345-1360
- [194] Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. *Human Mutation*. 2012;**33**(9):1340-1344
- [195] Kabashi E, Valdmanis P, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, Bouchard JP, Lacomblez L, Pochigaeva K, Salachas F, Pradat PF, Camu W, Meininger V, Dupre N, Rouleau GA. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nature Genetics*. 2008;**40**(5):572-574
- [196] Huang C-C, Bose JK, Majumder P, Lee K-H, Huang J-TJ, Huang JK, et al. Metabolism and mis-metabolism of the neuropathological signature protein TDP-43. *Journal of Cell Science*. 2014;**127**(Pt 14):3024-3038
- [197] Xia Q, Wang H, Hao Z, Fu C, Hu Q, Gao F, et al. TDP-43 loss of function increases TFEB activity and blocks autophagosome-lysosome fusion. *The European Molecular Biology Organization Journal*. 2016;**35**(2):121-142
- [198] Monahan Z, Shewmaker F, Pandey UB. Stress granules at the intersection of autophagy and ALS. *Brain Research*. 2016;**1649**:189-200
- [199] Bose JK, Huang CC, Shen CKJ. Regulation of autophagy by neuropathological protein TDP-43. *The Journal of Biological Chemistry*. 2011;**286**(52):44441-44448
- [200] Molliex A, Temirov J, Lee J, Coughlin M, Kanagaraj AP, Kim HJ, et al. Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell*. 2015;**163**(1):123-133
- [201] Murakami T, Qamar S, Lin JQ, Schierle GSK, Rees E, Miyashita A, et al. ALS/FTD Mutation-Induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron*. 2015;**88**(4):678-690
- [202] Li YR, King OD, Shorter J, Gitler AD. Stress granules as crucibles of ALS pathogenesis. *The Journal of Cell Biology*. 2013;**201**(3):361-372
- [203] Taylor JP, Brown RH, Cleveland DW. Decoding ALS: From genes to mechanism. *Nature*. 2016;**539**(7628):197-206
- [204] Zhou Y, Liu S, Liu G, Öztürk A, Hicks GG. ALS-Associated FUS Mutations Result in Compromised FUS Alternative Splicing and Autoregulation. *PLoS Genetics*. 2013;**9**(10):e1003895.
- [205] Ryu HH, Jun MH, Min KJ, Jang DJ, Lee YS, Kim HK, et al. Autophagy regulates amyotrophic lateral sclerosis-linked fused in sarcoma-positive stress granules in neurons. *Neurobiology of Aging*. 2014;**35**(12):2822-2831

- [206] Gal J, Zhang J, Kwinter DM, Zhai J, Jia H, Jia J, et al. Nuclear localization sequence of FUS and induction of stress granules by ALS mutants. *Neurobiology of Aging*. 2011;**32**(12):2323.e27-2323.e40
- [207] Ling S-C, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis. *Neuron*. 2013;**79**(3):416-438
- [208] Dormann D, Haass C. TDP-43 and FUS: A nuclear affair. *Trends in Neurosciences*. 2011; **34**(7):339-348
- [209] Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, et al. A Liquid-to-Solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell*. 2015;**162**(5):1066-1077
- [210] Soo KY, Sultana J, King AE, Atkinson R, Warraich ST, Sundaramoorthy V, et al. ALS-associated mutant FUS inhibits macroautophagy which is restored by overexpression of Rab1. *Cell Death Discovery*. 2015;**1**(July):15030
- [211] Van Swieten J, Spillantini MG. Hereditary frontotemporal dementia caused by Tau gene mutations. *Brain Pathology*. 2007;**17**(1):63-73
- [212] Kuusisto E, Salminen A, Alafuzoff I. Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. *Neuroreport*. 2001;**12**(10):2085-2090
- [213] Ramesh Babu J, Lamar Seibenhener M, Peng J, Strom AL, Kemppainen R, Cox N, et al. Genetic inactivation of p62 leads to accumulation of hyperphosphorylated tau and neurodegeneration. *Journal of Neurochemistry*. 2008;**106**(1):107-120
- [214] Arendt T, Stieler JT, holzer M. Tau and tauopathies. *Brain Research Bulletin*. 2016;**126**(3):238-292
- [215] David DC, Layfield R, Serpell L, Narain Y, Goedert M, Spillantini MG. Proteasomal degradation of tau protein. *Journal of Neurochemistry*. 2002;**83**(1):176-185
- [216] Hatakeyama S, Matsumoto M, Kamura T, Murayama M, Chui DH, Planel E, et al. U-box protein carboxyl terminus of Hsc70-interacting protein (CHIP) mediates poly-ubiquitylation preferentially on four-repeat Tau and is involved in neurodegeneration of tauopathy. *Journal of Neurochemistry*. 2004;**91**(2):299-307
- [217] Dolan PJ, Johnson GVW. A caspase cleaved form of tau is preferentially degraded through the autophagy pathway. *The Journal of Biological Chemistry*. 2010;**285**(29):21978-21987
- [218] Hamano T, Gendron TF, Causevic E, Yen SH, Lin WL, Isidoro C, et al. Autophagic-lysosomal perturbation enhances tau aggregation in transfectants with induced wild-type tau expression. *The European Journal of Neuroscience*. 2008;**27**(5):1119-1130
- [219] Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, et al. Rapamycin alleviates toxicity of different aggregate-prone proteins. *Human Molecular Genetics*. 2006;**15**(3):433-442

- [220] Krüger U, Wang Y, Kumar S, Mandelkow EM. Autophagic degradation of tau in primary neurons and its enhancement by trehalose. *Neurobiology of Aging*. 2012;**33**(10):2291-2305
- [221] Schaeffer V, Goedert M. Stimulation of autophagy is neuroprotective in a mouse model of human tauopathy. *Autophagy*. 2012;**8**(11):1686-1687
- [222] Polito VA, Li H, Martini-Stoica H, Wang B, Yang L, Xu Y, et al. Selective clearance of aberrant tau proteins and rescue of neurotoxicity by transcription factor EB. *EMBO Molecular Medicine*. 2014;**6**(9):1142-1160
- [223] Witman GB, Cleveland DW, Weingarten MD, Kirschner MW. Tubulin requires tau for growth onto microtubule initiating sites (flagella/in vitro assembly/electron microscopy). *Cell Biology*. 1976;**73**(11):4070-4074
- [224] Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proceedings of the National Academy of Sciences of the United States of America*. 1975;**72**(5):1858-1862
- [225] Adams SJ, Crook RJP, Deture M, Randle SJ, Innes AE, Yu XZ, et al. Overexpression of wild-type murine tau results in progressive tauopathy and neurodegeneration. *American Journal of Pathology*. 2009;**175**(4):1598-1609
- [226] Santacruz K, Lewis J, Spire S, Paulson J, Kotilinek L, Ingelsson M, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science*. 2005;**309**(5733):476-481
- [227] Love S, Bridges LR, Case CP. Neurofibrillary tangles in Niemann-Pick disease type C. *Brain*. 1995;**118**:119-129
- [228] Auer I, Schmidt M, Lee V, et al. Paired-Helical-Filament-Tau (PHFTAU) IN Niemann-Pick Type-C disease is similar to phftau in Alzheimers-Disease. *Acta Neuropathologica*. 1995;**90**:547-551
- [229] Perlson E, Maday S, Fu M meng, Moughamian AJ, Holzbaur ELF. Retrograde axonal transport: Pathways to cell death? *Trends in Neurosciences*. 2010;**33**(7):335-344