

## Review Article

# The role of autophagy-lysosomal pathway in motor neuron diseases

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Motor neuron diseases (MNDs) include a broad group of diseases in which neurodegeneration mainly affects upper and/or lower motor neurons (MNs). Although the involvement of specific MNs, symptoms, age of onset, and progression differ in MNDs, the main pathogenic mechanism common to most MNDs is represented by proteostasis alteration and proteotoxicity. This pathomechanism may be directly related to mutations in genes encoding proteins involved in the protein quality control system, particularly the autophagy-lysosomal pathway (ALP). Alternatively, proteostasis alteration can be caused by aberrant proteins that tend to misfold and to aggregate, two related processes that, over time, cannot be properly handled by the ALP. Here, we summarize the main ALP features, focusing on different routes utilized to deliver substrates to the lysosome and how the various ALP pathways intersect with the intracellular trafficking of membranes and vesicles. Next, we provide an overview of the mutated genes that have been found associated with MNDs, how these gene products are involved in different steps of ALP and related processes. Finally, we discuss how autophagy can be considered a valid therapeutic target for MNDs treatment focusing on traditional autophagy modulators and on emerging approaches to overcome their limitations.

## Introduction

Motor neuron diseases (MNDs) are a large class of neurodegenerative diseases (NDs) primarily affecting neurons that control the skeletal muscle fibers. These neurons comprise upper motor neurons (MNs) localized in the motor cortex and lower MNs localized in the bulbar regions or in the anterior horns of the spinal cord. Other cell types surrounding (e.g. glial and microglial cells) or connecting to (e.g. sensory neurons, interneurons, muscle cells, etc.) MNs may contribute to disease onset and progression, and ultimately to paralysis and death of the patients. These neural systems are governed by a complex architecture, thus various MN types are differentially and selectively involved in these disorders, leading to multifaceted clinical manifestations that considerably vary among affected individuals.

To simplify, MNDs can be roughly classified into three major categories that reflect both the clinical signs and the type of MNs affected: (i) with lower MNs involvement (e.g. spinal muscular atrophy (SMA), spinobulbar muscular atrophy (SBMA or Kennedy's disease), progressive muscular atrophy (PMA), pseudobulbar palsy (in the bulbar region), monomelic amyotrophy (MMA), lethal congenital contracture syndrome (LCCS), etc.), (ii) with upper MNs involvement (e.g. primary lateral sclerosis (PLS) and hereditary spastic paraplegias (HSP), etc.), and (iii) with combined upper and lower MNs involvement (e.g. amyotrophic lateral sclerosis (ALS), etc.) [1–3]. Overall, motor manifestations depend on which MNs are affected: lower MN loss results in muscle weakness, and upper MN loss in spasticity; mixed clinical signs associate initially to upper and lower MN loss, but when the disease

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progresses weakness and flaccid paralysis prevail over spasticity. Noteworthy, this basic classification does not fully reflect the existence of mixed forms, as, for example, ALS with frontotemporal dementia (FTD) [4].

No cure is available for most of these MNDs. Only for specific forms of SMA some highly innovative genetic approaches have recently proved to be therapeutically efficient [5–7].

The molecular bases of these MNDs may considerably differ and, while specific inherited (familial) forms are characterized by mutations in genes encoding proteins involved in a variety of intracellular and extracellular biological functions, some MNDs occur in a sporadic manner, making it very difficult to determine the causes of MN loss. Nevertheless, sporadic forms often display a dysregulation of the same proteins that have been identified mutated in familial forms of MNDs (see the case of sporadic and familial ALS) [8]. Intriguingly, many of these proteins and their mutated counterparts act in the neuronal response to proteotoxic stresses, which includes the protein quality control (PQC) system and particularly the autophagy-lysosomal pathway (ALP). ALP is essential to degrade damaged organelles or toxic protein aggregates and inclusions capable to alter cellular homeostasis causing MN death. This review will thus focus on the role of ALP in MNDs.

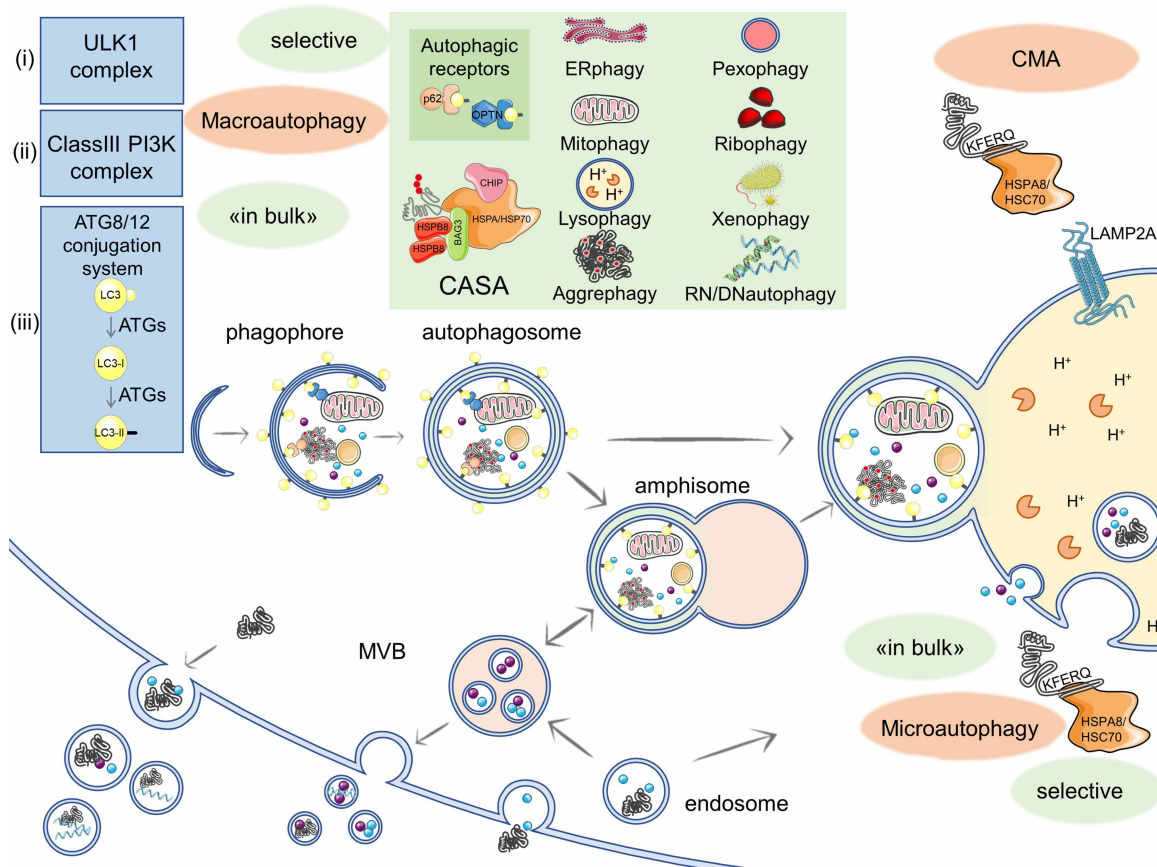
## The autophagy-lysosomal pathway

The term ‘autophagy’ refers to various cellular processes involved in proteins or organelles clearance via the lysosomal system. Autophagy eliminates unused, excessive, and/or defective macromolecules (either endogenous or exogenous) and recycles their components for other biosynthetic pathways (Figure 1). Autophagy takes place in the cell cytoplasm, and it is an essential arm of the PQC and RNA quality control system, which works together with the proteasome in a finely tuned equilibrium. The fate of a given substrate is regulated by a plethora of chaperone proteins, that define how substrates will be cleared from cells. Autophagy dysregulations cause several acute and chronic human diseases and promote the aging process; these findings have stimulated the search for autophagy modulators as potential therapeutic tools to efficiently counteract them [9–11].

Autophagy is a complex system, in which the delivery of cargoes to lysosomes for degradation follows different routes named (i) macroautophagy (usually referred to as autophagy), (ii) microautophagy, and (iii) chaperone-mediated autophagy (CMA).

Macroautophagy implies that cargoes are sequestered by ‘omegasomes’ released by the endoplasmic reticulum (ER) or other membranous compartments (e.g. late endosomes, Golgi apparatus, or plasma membrane). Omegasomes release double-membrane structures to enclose substrates generating the autophagosomes. This process is tightly regulated and controlled by the autophagy-related proteins (named ATGs) which orchestrate autophagosome assembly, trafficking, and fusion to lysosomes [10,12]. The type of cargo determines whether macroautophagy occurs ‘in bulk’ (i.e. cytoplasm portions engulfed into autophagosomes), or ‘selectively’, thanks to specific autophagy receptors (e.g. sequestosome 1 (SQSTM1/p62), optineurin (OPTN), etc.) that bridge cargoes to the autophagosome membranes. This is enabled by the presence of an LC3-interacting region (LIR) in most of the autophagy receptors. The LIR allows them to interact with members of the LC3/GABA Type A Receptor-Associated Protein (GABARAP) family (e.g. the microtubule-associated protein light chain 3 (MAP-LC3 or LC3)) anchored in their lipidated form (LC3-II) on the autophagosomes surface [13]. Through this mechanism, damaged organelles or other substrates are selectively removed from the cytoplasm [14]. For instance, terms such as ERphagy, mitophagy, lysophagy, ribophagy, pexophagy, RN/DNAutophagy, xenophagy, aggrephagy describe the selective removal of damaged structures of ER, mitochondria, lysosomes, ribosomes, peroxisomes, nucleic acids, pathogens, and protein aggregates, respectively (Figure 1). Once substrates have been engulfed into autophagosomes, macroautophagy ends with the fusion of autophagosomes with lysosomes for cargo degradation.

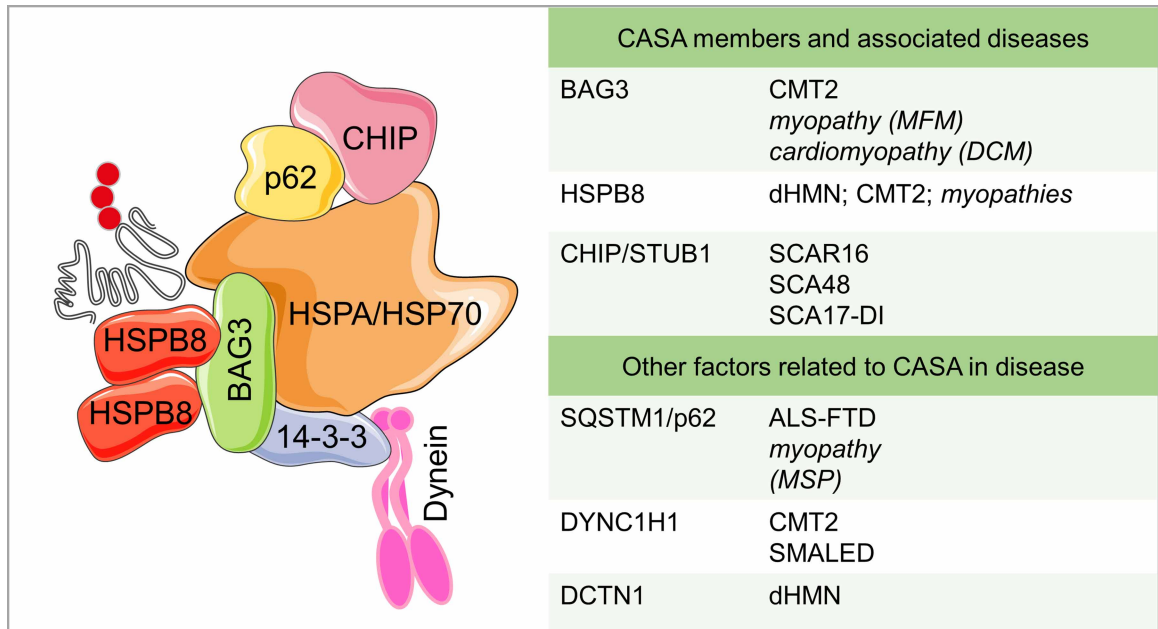
In diseases characterized by the presence of misfolded proteins prone to aggregate (including MNDs), aggrephagy may utilize a specific form of cargo delivery to the microtubule organization center (MTOC), where aggresomes are assembled [15–19]. This route, known as chaperone-assisted selective autophagy (CASA) [20,21], is based on misfolded protein recognition by a heteromeric complex of the small heat shock protein B8 (HSPB8) and its obligatory partner Bcl-2-associated athanogene 3 (BAG3) [22,23]. Once formed, HSPB8/BAG3 associate to HSAs/HSP70s bound to the carboxyl terminus of HSC70-interacting protein (CHIP or STUB1), a U-box-containing E3 ubiquitin-protein ligase that tags misfolded proteins with a polyubiquitin chain for autophagy receptor recognition (SQSTM1/p62 for CASA). This protein complex, named CASA complex, is then transported along microtubules to the MTOC by the dynein machinery bound to BAG3 [22,23]. Next, the autophagy receptors allow the LC3-II-mediated insertion into autophagosomes. The relevance of the CASA in cell homeostasis is underlined by the fact that, when mutated, most of its members cause neuronal or muscular disorders (see Figure 2 for details).



**Figure 1. Overview of the autophagy pathway.**

Macroautophagy is finely regulated by ATGs and other proteins forming multimeric complexes responsible for (i) initiation (ULK1 complex, activated by various signals), (ii) phagophore formation (Class III PI3K complex), and (iii) elongation (ATG8/12 conjugation systems). In macroautophagy, cargoes are engulfed «in bulk» or selectively (green box), with the involvement of autophagic receptors (e.g. SQSTM1/p62, OPTN), into membranous structures (phagophore) which close forming a double membrane vesicle decorated with LC3-lipidated proteins (autophagosome), which fuses with the lysosome. In microautophagy, a direct invagination of the lysosomal membrane («in bulk») sequesters the substrates to be degraded or, alternatively, substrates carrying a KFERQ-like motif are recognized and directed to endosomes by the HSPA8/HSC70; in CMA, substrates carrying a KFERQ-like motif are bound by HSPA8/HSC70 and internalized into the lysosome in a LAMP2A-dependent manner. Autophagy pathway cross-talks with the endosomal system: substrates can be internalized into endosomes. Endosomes can be directly targeted to lysosomes or mature in multivesicular bodies (MVBs) that fuse with autophagosomes forming amphisomes, which are addressed to lysosomal degradation. Substrates can be also cleared from cells through exocytosis by MVBs fusion with cell membrane or other vesicles budding. This figure was created using Servier Medical Art templates, licensed under a Creative Commons Attribution 3.0 Unported License; <https://smart.servier.com> [23].

Regarding mammalian microautophagy, cargoes are specifically engulfed into lysosomes via the intervention of late endosomes. Protein recognition can be achieved either ‘in bulk’ or (like CMA, see below) selectively thanks to a KFERQ-like motif in the target protein which is recognized by the constitutive form of HSAs/HSP70s, the heat shock cognate 70 (HSPA8/HSC70) [24]. In microautophagy, HSAs/HSP70s activities are mediated by an invagination of the late endosome membrane with the involvement of the endosomal sorting complex required for transport (ESCRT) machinery. Entrapped proteins and organelles can be then directly processed in late endosomes or after their fusion with lysosomes [9,25–27]. HSPA8/HSC70 plays a pivotal role also in CMA. In CMA, the HSPA8/HSC70-bound substrate is not subjected to endosomal engulfment. Instead, the HSPA8/HSC70 recognizes and unfolds the cargo that is directly routed to lysosomes where it is released to the CMA receptor LAMP2A, thus escaping the late endosomal engulfment [28]. In CMA, only the proteins



**Figure 2. CASA involvement in MNDs and other neuromuscular conditions.**

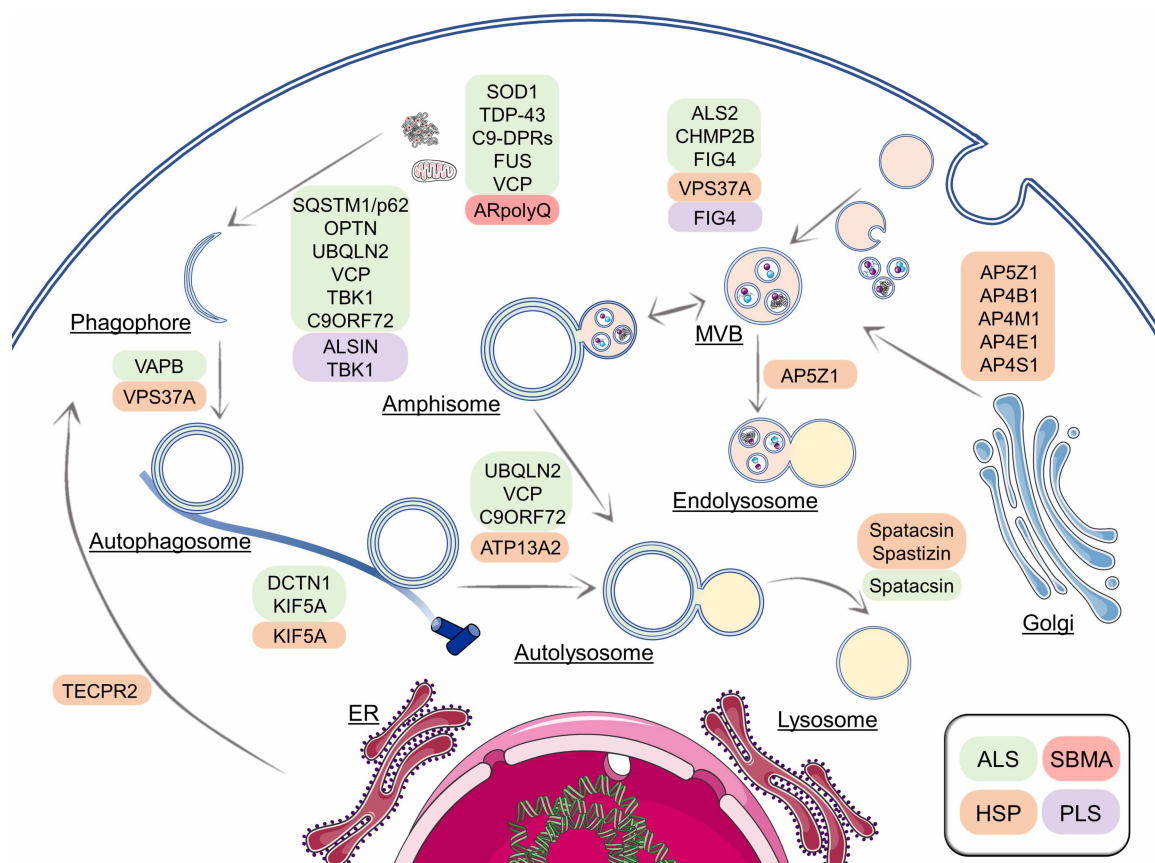
The list reports factors of the CASA pathway of which mutations have been found in diseases affecting the neuromuscular system. In particular, BAG3 and HSPB8 mutations are described in neuropathies (CMT2 and dHMN) and myopathies. BAG3 mutations are also causative of dilated cardiomyopathy (DCM). CHIP/STUB1 mutations are associated with different forms of SCA. Other factors that participate in CASA pathway are SQSTM1/p62, mutated in ALS-FTD and MSP, the dynein machinery components DYNC1H1 and DCTN1, mutated in CMT2 and SMALED or dHMN, respectively. CMT2 = Charcot-Marie-Tooth type 2; dHMN = distal hereditary motor neuropathy; DCM = dilated cardiomyopathy; SCA = spinocerebellar ataxia; (SCA)R = recessive; (SCA)-DI = Digenic; MSP = multisystem proteinopathy; SMALED = spinal muscular atrophy with lower extremity predominance. This figure was created using Servier Medical Art templates, licensed under a Creative Commons Attribution 3.0 Unported License; <https://smart.servier.com> [23].

possessing the KFERQ-like motif can be cleared [29], even if this motif is relatively frequent in mammalian proteins and there is the intriguing possibility to activate it via post-translational modifications (phosphorylation, acetylation) acting as an off/on switch to regulate protein degradation [24]. When the cargo is released from HSPA8/HSC70 it is associated to LAMP2A, which oligomerizes into the lysosomal membrane. Oligomerization promotes the translocation of the cargo to the lumen, where another HSPA8/HSC70 extracts the cargo allowing its degradation (Figure 1).

## Mutant genes encoding autophagy-related proteins are linked to MNDs

As mentioned above, in the past two decades, it emerged that many mutant genes causative of specific MND forms encode proteins differentially involved in the regulatory steps of autophagy.

Indeed, by merely looking at the many different HSP forms, several mutated genes, called Spastic Paraplegia (SPG) genes, encode proteins that are key regulators of the endolysosomal system and autophagy. An autosomal dominant form of HSP is caused by a mutation of the *SPG42* gene encoding a protein involved in ER membrane transport, bone morphogenetic protein (BMP) signaling and autophagy [30–32]; other autosomal recessive forms are caused by mutant genes encoding proteins involved in autophagy as well as in lysosome shaping (e.g. *SPG11*, encoding spatacsin), in endosomal trafficking (e.g. *SPG15*, encoding spastizin), in vesicle formation and trafficking (e.g. *SPG47*, *SPG50*, *SPG51*, *SPG52*, *SPG53*, encoding AP4B1, AP4M1, AP4E1, AP4S1, VPS37A, respectively), in Golgi apparatus-multivesicular bodies (MVB) dynamics (e.g. *SPG48*, encoding AP5Z1), and lysosomal targeting to autophagosomes (e.g. *SPG49*, encoding TECPR2), or in membrane trafficking and mitochondrial function (e.g. *SPG78*, encoding ATP13A2) [30–34] (Figure 3 and Table 1). Mutations of these genes



**Figure 3. Schematic representation of the main genes involved in autophagy and endolysosome pathway associated to MNDs.**

In MNDs, various mutations have been identified in genes encoding for proteins involved in different steps of autophagy and endolysosome pathways which result impaired and dysfunctional. In parallel, mutant MNDs-related genes encode for proteins prone to misfold and aggregate which are substrates of autophagy. ALS = amyotrophic lateral sclerosis; HSP = hereditary spastic paraplegia; PLS = primary lateral sclerosis; SBMA = spinobulbar muscular atrophy. This figure was created using Servier Medical Art templates, licensed under a Creative Common Attribution 3.0 Unported License; <https://smart.servier.com>.

result through different mechanisms in MNs death and involve other neuronal populations, giving rise to the complex and mixed clinical manifestations which coexist with motor dysfunctions.

Even more complex is the autophagy involvement in ALS [4,11,84]. As for HSP, several familial ALS forms are caused by mutations in genes encoding proteins regulating the endolysosomal and autophagic pathways (as extensively reviewed in [85]). Examples are the *C9ORF72* (encoding the C9orf72-SMCR8 complex subunit, guanine nucleotide exchange factor C9orf72, recently involved in autophagy), the *DCTN1* and *KIF5A* (encoding, respectively, dynein subunit 1 (a component of dynein motor complex) and kinesin family member 5A, involved in the autophago-lysosome fusion process and, for dynein/dynactin, in the routing of the CASA complex to the MTOC), *VCP* (encoding the valosin containing protein, a hexameric complex involved in misfolded protein extraction for their degradation via different pathways, including autophagy), *SQSTM1/p62* and *OPTN* (encoding the Sequestosome 1 and the optineurin, two autophagy receptors), *TBK1* (encoding the TANK binding kinase 1, involved in the innate immune response, inflammation, cell proliferation responses, and autophagy), *FIG4* (encoding the factor-induced gene 4, a magnesium-dependent phosphatase converting phosphatidylinositol-3,5-bisphosphate (PI(3,5)P<sub>2</sub>) into phosphatidylinositol-3-phosphate (PI(3)P) on endosomal membranes, a process relevant for endosomal transport and autophagy), and others.

Moreover, several mutant genes encode proteins not directly involved in autophagy, but related to mechanisms that facilitate the process (e.g. genes encoding proteins involved in vesicle trafficking (and apoptosis,

**Table 1. Genes cited in this review involved in ALP**

Gene	Protein	Disease	References
ALS2	Alsin	ALS/PLS	(35, 36)
ANXA11	Annexin A11 (ANXA11)	ALS	(37)
AR	Androgen Receptor (AR)	SBMA	(38)
ATXN2	Ataxin 2 (ATXN2)	ALS	(39, 40)
C9orf72	Chromosome 9 opening reading frame 72 (C9ORF72)	ALS	(41, 42)
CCNF	Cyclin F (CCNF)	ALS	(43)
CHMP2B	Charged multivesicular body protein 2B (CHMP2B)	ALS	(44)
DCTN1	Dynactin subunit 1 (DCTN1)	ALS	(45, 46)
ERLIN2	Erlin 2	HSP/PLS	(47, 48)
FIG4	Factor-induced gene 4 (FIG4)	ALS/PLS	(49)
FUS	Fused in Sarcoma (FUS)	ALS	(50, 51)
KIF5A	Kinesin family member 5A (KIF5A)	ALS/HSP	(52, 53)
OPTN	Optineurin (OPTN)	ALS	(54)
SMN1	Survival Motor Neuron protein 1 (SMN1)	SMA	(55, 56)
SOD1	Superoxide Dismutase 1 (SOD1)	ALS	(57)
SPG11	Spatacsin	HSP/ALS	(58-60)
SPG15 (ZFVVE26)	Spastizin	HSP	(61-63)
SPG42 (SLC33A1)	SLC33A1	HSP	(64)
SPG47 (AP4B1)	AP4B1	HSP	(65)
SPG48 (AP5Z1)	AP5Z1	HSP	(66)
SPG49 (TECPR2)	TECPR2	HSP	(67)
SPG50 (AP4M1)	AP4M1	HSP	(68)
SPG51 (AP4E1)	AP4E1	HSP	(65, 69)
SPG52 (AP4S1)	AP4S1	HSP	(65)
SPG53 (VPS37A)	VPS37A	HSP	(70)
SPG7	SPG7	PLS/HSP	(71)
SPG78 (ATP13A2)	ATP13A2	HSP	(72, 73)
SQSTM1	Sequestosome 1 (SQSTM1/p62)	ALS	(74)
TARDBP-43	TAR DNA Binding Protein 43 (TDP-43)	ALS	(75-78)
TBK1	TANK binding kinase 1 (TBK1)	ALS/PLS	(79, 80)
UBQLN2	Ubiquilin 2 (UBQLN2)	ALS	(81)
VAPB	VAMP associated protein B/C (VAPB)	ALS	(82)
VCP	Valosin-Containing Protein (VCP)	ALS/HSP	(83)

In blue, genes encoding proteins whose functions have been related to ALP. In yellow, genes that, when mutated, encodes protein products prone to misfold and aggregate, impairing proteostasis. Genes with both features are in yellow with blue lines.

exocytosis, and cytokinesis), like *ANXA11*, encoding annexin A11; in endocytosis (and RNA metabolism), like *ATXN2*, encoding ataxin 2; in sorting of endosomal cargo proteins, like *CHMP2B*, encoding the charged MVB protein 2B, a component of the ESCRT complex III; in vesicle trafficking from the ER-membrane, like *VAPB*, encoding VAMP associated protein B/C (Figure 3). Also, the molecular re-routing system from autophagy to proteasome and *vice versa* may indirectly cause autophagy alterations. In fact, this system maintains the fine-tuned equilibrium between these two degradative pathways. The best example is the routing system based on the activity of HSPAs/HSP70s-CHIP, which is differentially regulated by their selective association to HSPB8/BAG3 (CASA for autophagic degradation) or to BAG1 (for proteasome degradation) [22]. Autophagy could be required as an alternative mechanism, in case of mutations occurring in *UBQLN2* or *CCNF* (encoding, respectively, Ubiquilin 2 and cyclin F, the first physically associated with both proteasome and ubiquitin ligases and involved in protein degradation via proteasome, the second catalyzing ubiquitin transfer to substrates for proteasomal degradation), even if it is still unclear whether the autophagic response may compensate the altered proteasome pathway.

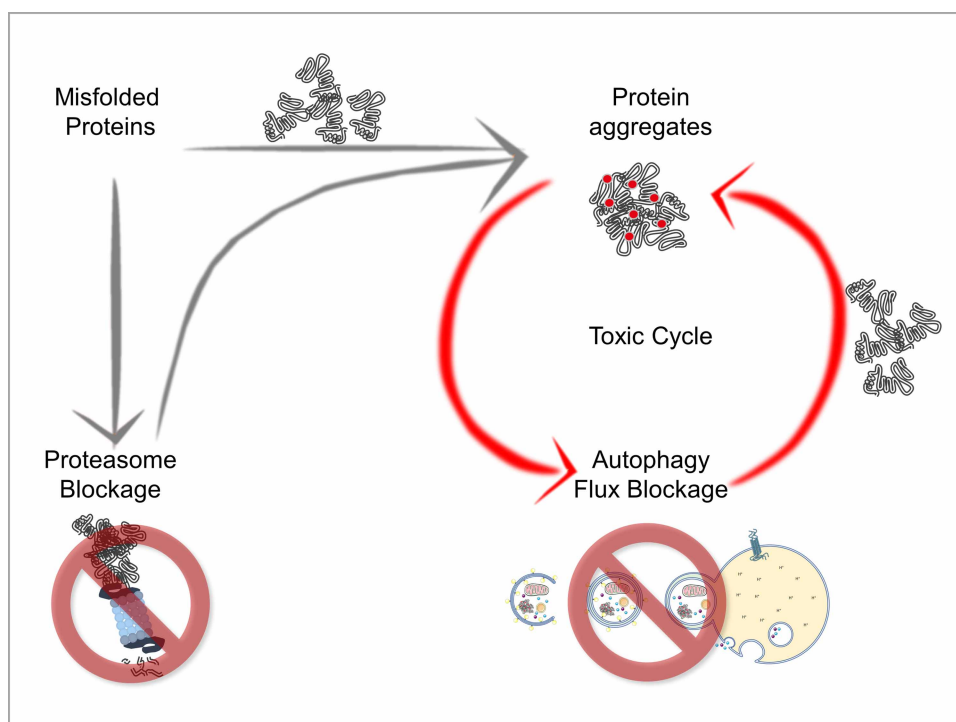
The impact of some of the mutations/variants reported above on ALS onset and progression is debated. A recent database (ALSod (<https://alsod.ac.uk/>)) has tried to correlate how the variants are linked to ALS, even when evidence is weak.

It should be underlined that mutations in some of the genes associated to HSP and ALS may also be responsible for the appearance of PLS (e.g. *SPG7*, *TBK1*, *ALS2*, *ERLIN2*, *FIG4*) [86]. Similarly, a FTD phenotype may be present at different degrees in ALS cases (even as pure FTD with some ALS-related mutant genes). Therefore, it is difficult to draw clear correlations between genotype and phenotype in these diseases.

Even in the case of SMA, the causative survival MN (SMN1) protein (a protein implicated in spliceosome assembly and ribonucleoproteins biogenesis, mRNA trafficking, local translation, and cytoskeletal dynamics) has been found involved in endocytosis and autophagy (see [87] for review), although the mechanism by which SMN controls or is regulated by autophagy is still poorly understood [88–90].

## Mutant genes encoding proteins prone to misfold and aggregate cause MNDs

While some mutated genes causative of MNDs directly modulate ALP, many others indirectly impact on autophagy as a consequence of the aberrant biochemical properties acquired by the mutant protein. This occurs when a mutation modifies the native protein (i) altering its capability to properly fold, (ii) enhancing its hydrophobicity, (iii) affecting highly disordered domains, or (iv) altering its interaction with partners, etc. These mechanisms are not mutually exclusive and often increase the propensity of the mutant proteins to clump together, forming aggregate structures of diverse nature: liquid–liquid droplets or fluid condensates, solid-like aggregates or densely packed insoluble protein inclusions, etc., based on their mechanism of formation and/or their stage of maturation. As a function of their biochemical state, aggregates can be dissolved or further processed to be cleared from cells via autophagy. However, aggregated proteins may impair the autophagic flux, causing severe consequences to the PQC system, with the degradative capability of affected neurons (or surrounding cells affected in MNDs) being overwhelmed. Indeed, this is the case of SBMA, caused by the transcription factor androgen receptor (AR) carrying an elongated polyglutamine tract (ARpolyQ) in its N-terminus. Soon after its translation, the polyQ is masked by specific chaperones (HSPAs/HSP70s, HSP90, etc.), that are released upon AR-binding with its natural androgenic ligands. These molecules activate the AR, allowing conformational changes that unmask the polyQ, causing ARpolyQ aggregation and impairment of the autophagic process [91–98]. Despite a deep investigation, it is still not totally clear whether aggregates cause autophagic flux blockage, or if a defective autophagy results in poor protein clearance and thus excessive



**Figure 4. Toxic cycle nourishing proteostasis imbalance.**

An increased load in misfolded substrates may result in protein aggregates formation, which, in turn, hampers the autophagic flux. Impaired autophagy is not able to clear cells from misfolded substrates, which accumulate increasing the burden of misfolded and aggregating substrates. Proteasome saturation feeds this toxic cycle, worsening proteostasis imbalance.

protein aggregation. Likely, the two processes equally contribute to generate a toxic cycle in which defective autophagy allows protein accumulation, that in turn, perturbs the autophagic pathway, potentiating the deleterious effects of ARpolyQ in MNs (Figure 4). This toxic cycle could also involve the proteasome system, which has a low degradative capacity and only processes monomeric unfolded proteins. Thus, when the proteasome is saturated by an excessive amount of substrates to be degraded, these accumulate worsening the autophagic flux blockage previously mentioned [99–107]. Similar processes take place also in the case of various mutant proteins causative of familial ALS or HSP. In fact, like the ARpolyQ in SBMA, most of these mutated proteins tend to misfold and aggregate (e.g.: Superoxide Dismutase 1 (SOD1), TAR DNA-Binding Protein 43 (TDP-43, and its fragment of 35 and 25 kDa), fused in sarcoma (FUS), VCP, the dipeptide repeats (DPRs) products of the C9ORF72 mRNA, etc.) [100,108–129], possibly impairing the degradative systems including autophagy. Notably, the exogenous expression or a pharmacological transcriptional induction of HSPB8 [22,116,117,130], both robustly enhance mutant misfolded and aggregated protein clearance. Since HSPB8 is not an autophagy inducer, its enhancement facilitates the delivery of misfolded proteins along microtubules for their autophagic clearance [104,131–134], improving or restoring a normal autophagic flux.

## Autophagy modulation as therapeutic tool in MNDs

Based on what reported above, autophagy activation may represent a valuable therapeutic approach for MNDs, but this approach has to be carefully evaluated considering the specific alteration present in each clinical form studied.

For example, in case of insufficient autophagy (e.g. lack of crucial factors linked to haploinsufficiency in MNDs associated with loss-of-function mutations; recessive forms involving proteins which can be replaced by the activity of other factors, etc.), it might be useful to stimulate the remaining functional wild type allele, or ‘redundant’ factors to restore autophagy. Unfortunately, autophagy and its regulation may considerably differ in the various cell types affected in MNDs (neurons, MNs, glial cells, microglia, muscle cells) making it difficult to identify the proper therapeutic agent for each MND form [11].

In general, drugs stimulating autophagy are relatively poorly specific. This is the case of the mammalian target of rapamycin (mTOR) inhibitor rapamycin, tested in MND animal models [135] and under investigation in ND patients [136,137], that acts not only on autophagy, but on a plethora of other intracellular pathways. Lithium has also been tested in some forms of MNDs, even if its activity as an autophagy inducer is rather controversial [138–141].

Other autophagy inducers, like trehalose and the more stable derivatives melibiose and lactulose, appear to be more promising, since their molecular mechanism of action is more selective and impacts directly on the autophagy master regulator transcription factor EB (TFEB) [104,142–146]. Trehalose has already been successfully tested in several animal models of NDs [147–159]. No double-blind clinical trial has been started yet, even if a single-arm, open-label pilot study has been recently reported on Niemann–Pick disease types A and B patients to assess its potential efficacy after intravenous delivery for 3 months. Despite the size of the study was small, a positive (not statistically relevant) trend was reported [160], suggesting that larger studies are needed to verify trehalose efficacy.

In the case of some MND forms in which autophagy is already enhanced in response to the overload of aberrant aggregation-prone proteins, generic autophagy activators may not be effective treatments. Instead, the pharmacological targeting of factors specifically acting on misfolded protein recognition and delivery to autophagy will represent a valid therapeutic strategy. The drug colchicine has these properties since it has been identified as a potent activator of the human HSPB8 promoter in MNs. Its capability to enhance HSPB8 expression was correlated with an increased CASA-mediated removal of ALS-associated proteins (TDP-43 and its disease associated fragments) in ALS cell and fly models [161]. A double-blind clinical trial with colchicine is presently ongoing on a group of ALS patients [133].

## Conclusions

In conclusion, autophagy is an essential cellular process that maintains proteostasis in neurons and other cell types typically affected in MNDs. Its role in MNDs is extremely complex as both its dysregulation and its insufficient or excessive activity play multiple roles in the appearance and/or progression of different forms of MNDs and other NDs. Moreover, it is still unclear why this relatively conserved mechanism among the various cell types of the human body associates to so many different pathological conditions. Since these diseases are characterized by the selective vulnerability of different neuronal populations, other modifier factors might differentially contribute to exacerbate or attenuate, respectively, the deleterious or protective activities of



autophagy in each neuronal type. Therefore, the identification of a proper target to be tackled by drugs capable of modulating the autophagic pathways is still challenging. However, it is emerging that approaches aimed to facilitate an already activated autophagy (e.g. those acting on the selective delivery of misfolded proteins to the autophago-lysosomes for their clearance) will provide more tailored treatments to counteract the neurotoxicity of aggregation-prone proteins in a wide variety of MNDs linked to defective response to proteotoxic stimuli.

## Perspectives

- MNDs comprise several untreatable neurodegenerative diseases characterized by the loss of neurons controlling voluntary movements. Several MND forms are associated to alteration of the main degradative systems, particularly the ALP.
- Several genes found causative of MNDs are involved in the regulation of the various forms of autophagy. Other genes code proteins that perturb proteostasis in neurons indirectly altering autophagy.
- Different compounds have been developed to stimulate and/or facilitate the ALP, some have already been successfully tested in animal models of MNDs and may be utilized soon for advanced clinical trials in MND patients.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Abbreviations

ALP, autophagy-lysosomal pathway; ALS, amyotrophic lateral sclerosis; ANXA11, annexin A11; AR, androgen receptor; ATGs, autophagy-related proteins; ATXN2, ataxin 2; BAG3, Bcl-2-associated athanogene 3; BMP, bone morphogenetic protein; CASA, chaperone-assisted selective autophagy; CCNF, cyclin F; CHIP/STUB1, carboxyl terminus of Hsc70-interacting protein; CHMP2B, charged multivesicular body protein 2B; CMA, chaperone-mediated autophagy; DCTN1, dynactin subunit 1; DPRs, dipeptide repeats; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; FIG4, factor-induced gene 4; FTD, frontotemporal dementia; FUS, fused in sarcoma; GABARAP, GABA Type A Receptor-Associated Protein; HSP, hereditary spastic paraplegia; HSPA8/HSC70, heat shock cognate 70; HSAs/HSP70s, heat shock protein 70; HSPB8, heat shock protein B8; KIF5A, kinesin family member 5A; LAMP2A, lysosome-associated membrane protein type 2A; LCCS, lethal congenital contracture syndrome; LIR, LC3-interacting region; MAP-LC3, microtubule-associated protein light chain 3; MMA, monomelic amyotrophy; MNDs, motor neuron diseases; MNs, motor neurons; MTOC, microtubule organization center; mTOR, mammalian target of rapamycin; MVBs, multivesicular bodies; NDs, neurodegenerative diseases; OPTN, optineurin; PI(3)P, phosphatidylinositol-3-phosphate; PI(3,5)P2, phosphatidylinositol-3,5-bisphosphate; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; polyQ, polyglutamine; PQC, protein quality control; SBMA,

spinobulbar muscular atrophy; SMA, spinal muscular atrophy; SMN, survival MN; SOD1, superoxide dismutase 1; SPG, spastic paraplegia [gene]; SQSTM1/p62, sequestosome 1; TBK1, TANK binding kinase 1; TDP-43, TAR DNA-binding protein 43; TFEB, transcription factor EB; UBQLN2, ubiquilin 2; VAPB, VAMP associated protein B/C; VCP, valosin containing protein.

## References

- 1 Figlewicz, D.A. and Orrell, R.W. (2003) The genetics of motor neuron diseases. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* **4**, 225–231 <https://doi.org/10.1080/1466082031001128>
- 2 James, P.A. and Talbot, K. (2006) The molecular genetics of non-ALS motor neuron diseases. *Biochim. Biophys. Acta* **1762**, 986–1000 <https://doi.org/10.1016/j.bbadis.2006.04.003>
- 3 Dion, P.A., Daoud, H. and Rouleau, G.A. (2009) Genetics of motor neuron disorders: new insights into pathogenic mechanisms. *Nat. Rev. Genet.* **10**, 769–782 <https://doi.org/10.1038/nrg2680>
- 4 Casterton, R.L., Hunt, R.J. and Fanto, M. (2020) Pathomechanism heterogeneity in the amyotrophic lateral sclerosis and frontotemporal dementia disease spectrum: providing focus through the lens of autophagy. *J. Mol. Biol.* **432**, 2692–2713 <https://doi.org/10.1016/j.jmb.2020.02.018>
- 5 Aslesh, T. and Yokota, T. (2022) Restoring SMN expression: an overview of the therapeutic developments for the treatment of spinal muscular atrophy. *Cells* **11**, 417–434 <https://doi.org/10.3390/cells11030417>
- 6 Chilcott, E.M., Muiruri, E.W., Hirst, T.C. and Yanez-Munoz, R.J. (2021) Systematic review and meta-analysis determining the benefits of in vivo genetic therapy in spinal muscular atrophy rodent models. *Gene Ther.* Advance online publication. <https://doi.org/10.1038/s41434-021-00292-4>
- 7 Poletti, A. and Fischbeck, K.H. (2020) Combinatorial treatment for spinal muscular atrophy: an editorial for ‘Combined treatment with the histone deacetylase inhibitor LBH589 and a splice-switch antisense oligonucleotide enhances SMN2 splicing and SMN expression in Spinal Muscular Atrophy cells’ on page 264. *J. Neurochem.* **153**, 146–149 <https://doi.org/10.1111/jnc.14974>
- 8 Robberecht, W. and Philips, T. (2013) The changing scene of amyotrophic lateral sclerosis. *Nat. Rev. Neurosci.* **14**, 248–264 <https://doi.org/10.1038/nrn3430>
- 9 Fleming, A., Bourdenx, M., Fujimaki, M., Karabiyik, C., Krause, G.J., Lopez, A. et al. (2022) The different autophagy degradation pathways and neurodegeneration. *Neuron* **110**, 935–966 <https://doi.org/10.1016/j.neuron.2022.01.017>
- 10 Kaushik, S., Tasset, I., Arias, E., Pampliega, O., Wong, E., Martinez-Vicente, M. et al. (2021) Autophagy and the hallmarks of aging. *Ageing Res. Rev.* **72**, 101468 <https://doi.org/10.1016/j.arr.2021.101468>
- 11 Chua, J.P., De Calbiac, H., Kabashi, E. and Barmada, S.J. (2022) Autophagy and ALS: mechanistic insights and therapeutic implications. *Autophagy* **18**, 254–282 <https://doi.org/10.1080/15548627.2021.1926656>
- 12 Mizushima, N. (2020) The ATG conjugation systems in autophagy. *Curr. Opin. Cell Biol.* **63**, 1–10 <https://doi.org/10.1016/jceb.2019.12.001>
- 13 Stolz, A., Ernst, A. and Dikic, I. (2014) Cargo recognition and trafficking in selective autophagy. *Nat. Cell Biol.* **16**, 495–501 <https://doi.org/10.1038/ncb2979>
- 14 Lamark, T. and Johansen, T. (2021) Mechanisms of selective autophagy. *Annu. Rev. Cell Dev. Biol.* **37**, 143–169 <https://doi.org/10.1146/annurev-cellbio-120219-035530>
- 15 Bence, N.F., Sampat, R.M. and Kopito, R.R. (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. *Science* **292**, 1552–1555 <https://doi.org/10.1126/science.292.5521.1552>
- 16 Bennett, E.J., Bence, N.F., Jayakumar, R. and Kopito, R.R. (2005) Global impairment of the ubiquitin-proteasome system by nuclear or cytoplasmic protein aggregates precedes inclusion body formation. *Mol. Cell* **17**, 351–365 <https://doi.org/10.1016/j.molcel.2004.12.021>
- 17 Johnston, J.A., Illing, M.E. and Kopito, R.R. (2002) Cytoplasmic dynein/dynactin mediates the assembly of aggresomes. *Cell Motil. Cytoskelet.* **53**, 26–38 <https://doi.org/10.1002/cm.10057>
- 18 Johnston, J.A., Ward, C.L. and Kopito, R.R. (1998) Aggresomes: a cellular response to misfolded proteins. *J. Cell Biol.* **143**, 1883–1898 <https://doi.org/10.1083/jcb.143.7.1883>
- 19 Kopito, R.R. (2000) Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol.* **10**, 524–530 [https://doi.org/10.1016/S0962-8924\(00\)01852-3](https://doi.org/10.1016/S0962-8924(00)01852-3)
- 20 Arndt, V., Dick, N., Tawo, R., Dreiseidler, M., Wenzel, D., Hesse, M. et al. (2010) Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr. Biol.* **20**, 143–148 <https://doi.org/10.1016/j.cub.2009.11.022>
- 21 Carra, S., Seguin, S.J. and Landry, J. (2008) Hspb8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. *Autophagy* **4**, 237–239 <https://doi.org/10.4161/auto.5407>
- 22 Cristofani, R., Crippa, V., Rusmini, P., Cicardi, M.E., Meroni, M., Licata, N.V. et al. (2017) Inhibition of retrograde transport modulates misfolded protein accumulation and clearance in motoneuron diseases. *Autophagy* **13**, 1280–1303 <https://doi.org/10.1080/15548627.2017.1308985>
- 23 Cristofani, R., Piccolella, M., Crippa, V., Tedesco, B., Montagnani Marelli, M., Poletti, A. et al. (2021) The role of HSPB8, a component of the chaperone-assisted selective autophagy machinery, in cancer. *Cells* **10**, 335–358 <https://doi.org/10.3390/cells10020335>
- 24 Kaushik, S. and Cuervo, A.M. (2018) The coming of age of chaperone-mediated autophagy. *Nat. Rev. Mol. Cell Biol.* **19**, 365–381 <https://doi.org/10.1038/s41580-018-0001-6>
- 25 Kunz, J.B., Schwarz, H. and Mayer, A. (2004) Determination of four sequential stages during microautophagy in vitro. *J. Biol. Chem.* **279**, 9987–9996 <https://doi.org/10.1074/jbc.M307905200>
- 26 Sahu, R., Kaushik, S., Clement, C.C., Cannizzo, E.S., Scharf, B., Follenzi, A. et al. (2011) Microautophagy of cytosolic proteins by late endosomes. *Dev. Cell* **20**, 131–139 <https://doi.org/10.1016/j.devcel.2010.12.003>
- 27 Park, J.S., Kim, D.H. and Yoon, S.Y. (2016) Regulation of amyloid precursor protein processing by its KFERQ motif. *BMB Rep.* **49**, 337–342 <https://doi.org/10.5483/BMBRep.2016.49.6.212>
- 28 Chiang, H.L. and Dice, J.F. (1988) Peptide sequences that target proteins for enhanced degradation during serum withdrawal. *J. Biol. Chem.* **263**, 6797–6805 [https://doi.org/10.1016/S0021-9258\(18\)68713-7](https://doi.org/10.1016/S0021-9258(18)68713-7)

- 29 Cuervo, A.M. and Dice, J.F. (1996) A receptor for the selective uptake and degradation of proteins by lysosomes. *Science*. **273**, 501–503 <https://doi.org/10.1126/science.273.5274.501>
- 30 Marchesi L, T., Leblanc, M. and Stevanin, G. (2021) Current knowledge of endolysosomal and autophagy defects in hereditary spastic paraplegia. *Cells* **10**, 1678–1702 <https://doi.org/10.3390/cells10071678>
- 31 Boutry, M., Morais, S. and Stevanin, G. (2019) Update on the genetics of spastic paraplegias. *Curr. Neurol. Neurosci. Rep.* **19**, 18 <https://doi.org/10.1007/s11910-019-0930-2>
- 32 Teinert, J., Behne, R., Wimmer, M. and Ebrahimi-Fakhari, D. (2020) Novel insights into the clinical and molecular spectrum of congenital disorders of autophagy. *J. Inher. Metab. Dis.* **43**, 51–62 <https://doi.org/10.1002/jimd.12084>
- 33 Fraiberg, M., Tamim-Yecheskel, B.C., Kokabi, K., Subic, N., Heimer, G., Eck, F. et al. (2021) Lysosomal targeting of autophagosomes by the TECPR domain of TECPR2. *Autophagy* **17**, 3096–3108 <https://doi.org/10.1080/15548627.2020.1852727>
- 34 Tamim-Yecheskel, B.C., Fraiberg, M., Kokabi, K., Freud, S., Shatz, O., Marvaldi, L. et al. (2021) A tecpr2 knockout mouse exhibits age-dependent neuroaxonal dystrophy associated with autophagosome accumulation. *Autophagy* **17**, 3082–3095 <https://doi.org/10.1080/15548627.2020.1852724>
- 35 Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S. 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>
- 36 Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M. 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>
- 37 Smith, B.N., Topp, S.D., Fallini, C., Shibata, H., Chen, H.J., Troakes, C. et al. (2017) Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis. *Sci. Transl. Med.* **9**, eaad9157 <https://doi.org/10.1126/scitranslmed.aad9157>
- 38 La Spada, A.R., Wilson, E.M., Lubahn, D.B., Harding, A.E. and Fischbeck, K.H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79 <https://doi.org/10.1038/352077a0>
- 39 Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X. et al. (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* **466**, 1069–1075 <https://doi.org/10.1038/nature09320>
- 40 Tazen, S., Figueroa, K., Kwan, J.Y., Goldman, J., Hunt, A., Sampson, J. et al. (2013) Amyotrophic lateral sclerosis and spinocerebellar ataxia type 2 in a family with full CAG repeat expansions of ATXN2. *JAMA Neurol.* **70**, 1302–1304 <https://doi.org/10.1001/jamaneurol.2013.443>
- 41 Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R. et al. (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**, 257–268 <https://doi.org/10.1016/j.neuron.2011.09.010>
- 42 DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J. et al. (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**, 245–256 <https://doi.org/10.1016/j.neuron.2011.09.011>
- 43 Williams, K.L., Topp, S., Yang, S., Smith, B., Fifita, J.A., Warraich, S.T. et al. (2016) CCNF mutations in amyotrophic lateral sclerosis and frontotemporal dementia. *Nat. Commun.* **7**, 11253 <https://doi.org/10.1038/ncomms11253>
- 44 Parkinson, N., Ince, P.G., Smith, M.O., Highley, R., Skibinski, G., Andersen, P.M. et al. (2006) ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* **67**, 1074–1077 <https://doi.org/10.1212/01.wnl.0000231510.89311.8b>
- 45 Munch, C., Sedlmeier, R., Meyer, T., Homberg, V., Sperfeld, A.D., Kurt, A. et al. (2004) Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology* **63**, 724–726 <https://doi.org/10.1212/01.WNL.0000134608.83927.B1>
- 46 Puls, I., Jonnakuty, C., LaMonte, B.H., Holzbaur, E.L., Tokito, M., Mann, E. et al. (2003) Mutant dynactin in motor neuron disease. *Nat. Genet.* **33**, 455–456 <https://doi.org/10.1038/ng1123>
- 47 Alazami, A.M., Adly, N., Al Dhalaan, H. and Alkuraya, F.S. (2011) A nullimorphic ERLIN2 mutation defines a complicated hereditary spastic paraplegia locus (SPG18). *Neurogenetics* **12**, 333–336 <https://doi.org/10.1007/s10048-011-0291-8>
- 48 Al-Saif, A., Bohlega, S. and Al-Mohanna, F. (2012) Loss of ERLIN2 function leads to juvenile primary lateral sclerosis. *Ann. Neurol.* **72**, 510–516 <https://doi.org/10.1002/ana.23641>
- 49 Chow, C.Y., Landers, J.E., Bergren, S.K., Sapp, P.C., Grant, A.E., Jones, J.M. et al. (2009) Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. *Am. J. Hum. Genet.* **84**, 85–88 <https://doi.org/10.1016/j.ajhg.2008.12.010>
- 50 Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J. et al. (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323**, 1208–1211 <https://doi.org/10.1126/science.1165942>
- 51 Kwiatkowski TJ, J., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C. et al. (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323**, 1205–1208 <https://doi.org/10.1126/science.1166066>
- 52 Nicolas, A., Kenna, K.P., Renton, A.E., Ticozzi, N., Faghri, F., Chia, R. et al. (2018) Genome-wide analyses identify KIF5A as a novel ALS gene. *Neuron* **97**, 1268–1283.e6 <https://doi.org/10.1016/j.neuron.2018.02.027>
- 53 Brenner, D., Yilmaz, R., Muller, K., Grehl, T., Petri, S., Meyer, T. et al. (2018) Hot-spot KIF5A mutations cause familial ALS. *Brain* **141**, 688–697 <https://doi.org/10.1093/brain/awx370>
- 54 Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y. et al. (2010) Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* **465**, 223–226 <https://doi.org/10.1038/nature08971>
- 55 Rodrigues, N.R., Owen, N., Talbot, K., Ignatius, J., Dubowitz, V. and Davies, K.E. (1995) Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Hum. Mol. Genet.* **4**, 631–634 <https://doi.org/10.1093/hmg/4.4.631>
- 56 Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L. et al. (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165 [https://doi.org/10.1016/0092-8674\(95\)90460-3](https://doi.org/10.1016/0092-8674(95)90460-3)
- 57 Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A. et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62 <https://doi.org/10.1038/362059a0>
- 58 Stevanin, G., Santorelli, F.M., Azzedine, H., Coutinho, P., Chomilier, J., Denora, P.S. et al. (2007) Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. *Nat. Genet.* **39**, 366–372 <https://doi.org/10.1038/ng1980>
- 59 Del Bo, R., Di Fonzo, A., Ghezzi, S., Locatelli, F., Stevanin, G., Costa, A. et al. (2007) SPG11: a consistent clinical phenotype in a family with homozygous spatacsin truncating mutation. *Neurogenetics* **8**, 301–305 <https://doi.org/10.1007/s10048-007-0095-z>
- 60 Orlicchio, A., Babalini, C., Borreca, A., Patrono, C., Massa, R., Basaran, S. et al. (2010) SPATACIN mutations cause autosomal recessive juvenile amyotrophic lateral sclerosis. *Brain* **133**, 591–598 <https://doi.org/10.1093/brain/awp325>

- 61 Hanein, S., Martin, E., Boukhris, A., Byrne, P., Goizet, C., Hamri, A. et al. (2008) Identification of the SPG15 gene, encoding spastizin, as a frequent cause of complicated autosomal-recessive spastic paraplegia, including Kjellin syndrome. *Am. J. Hum. Genet.* **82**, 992–1002 <https://doi.org/10.1016/j.ajhg.2008.03.004>
- 62 Hughes, C.A., Byrne, P.C., Webb, S., McMonagle, P., Patterson, V., Hutchinson, M. et al. (2001) SPG15, a new locus for autosomal recessive complicated HSP on chromosome 14q. *Neurology* **56**, 1230–1233 <https://doi.org/10.1212/WNL.56.9.1230>
- 63 Elleuch, N., Bouslam, N., Hanein, S., Lossos, A., Hamri, A., Klebe, S. et al. (2007) Refinement of the SPG15 candidate interval and phenotypic heterogeneity in three large Arab families. *Neurogenetics* **8**, 307–315 <https://doi.org/10.1007/s10048-007-0097-x>
- 64 Lin, P., Li, J., Liu, Q., Mao, F., Li, J., Qiu, R. et al. (2008) A missense mutation in SLC33A1, which encodes the acetyl-CoA transporter, causes autosomal-dominant spastic paraplegia (SPG42). *Am. J. Hum. Genet.* **83**, 752–759 <https://doi.org/10.1016/j.ajhg.2008.11.003>
- 65 Jamra R, A., Philippe, O., Raas-Rothschild, A., Eck, S.H., Graf, E., Buchert, R. et al. (2011) Adaptor protein complex 4 deficiency causes severe autosomal-recessive intellectual disability, progressive spastic paraplegia, shy character, and short stature. *Am. J. Hum. Genet.* **88**, 788–795 <https://doi.org/10.1016/j.ajhg.2011.04.019>
- 66 Slabicki, M., Theis, M., Krastev, D.B., Samsonov, S., Mundwiller, E., Junqueira, M. et al. (2010) A genome-scale DNA repair RNAi screen identifies SPG48 as a novel gene associated with hereditary spastic paraplegia. *PLoS Biol.* **8**, e1000408 <https://doi.org/10.1371/journal.pbio.1000408>
- 67 Oz-Levi, D., Ben-Zeev, B., Ruzzo, E.K., Hitomi, Y., Gelman, A., Pelak, K. et al. (2012) Mutation in TECPR2 reveals a role for autophagy in hereditary spastic paraparesis. *Am. J. Hum. Genet.* **91**, 1065–1072 <https://doi.org/10.1016/j.ajhg.2012.09.015>
- 68 Verkerk, A.J., Schot, R., Dumeé, B., Schellekens, K., Swagemakers, S., Bertoli-Avella, A.M. et al. (2009) Mutation in the AP4M1 gene provides a model for neuroaxonal injury in cerebral palsy. *Am. J. Hum. Genet.* **85**, 40–52 <https://doi.org/10.1016/j.ajhg.2009.06.004>
- 69 Moreno-De-Luca, A., Helmers, S.L., Mao, H., Burns, T.G., Melton, A.M., Schmidt, K.R. et al. (2011) Adaptor protein complex-4 (AP-4) deficiency causes a novel autosomal recessive cerebral palsy syndrome with microcephaly and intellectual disability. *J. Med. Genet.* **48**, 141–144 <https://doi.org/10.1136/jmg.2010.082263>
- 70 Zivony-Elboun, Y., Westbroek, W., Kfir, N., Savitzki, D., Shoval, Y., Bloom, A. et al. (2012) A founder mutation in Vps37A causes autosomal recessive complex hereditary spastic paraparesis. *J. Med. Genet.* **49**, 462–472 <https://doi.org/10.1136/jmedgenet-2012-100742>
- 71 Yang, Y., Zhang, L., Lynch, D.R., Lukas, T., Ahmeti, K., Sleiman, P.M. et al. (2016) Compound heterozygote mutations in SPG7 in a family with adult-onset primary lateral sclerosis. *Neurol. Genet.* **2**, e60 <https://doi.org/10.1212/NXG.000000000000060>
- 72 Kara, E., Tucci, A., Manzoni, C., Lynch, D.S., Elpidorou, M., Bettencourt, C. et al. (2016) Genetic and phenotypic characterization of complex hereditary spastic paraplegia. *Brain* **139**, 1904–1918 <https://doi.org/10.1093/brain/aww111>
- 73 Estrada-Cuzcano, A., Martin, S., Chamova, T., Synofzik, M., Timmann, D., Hølemans, T. et al. (2017) Loss-of-function mutations in the ATP13A2/PARK9 gene cause complicated hereditary spastic paraplegia (SPG78). *Brain* **140**, 287–305 <https://doi.org/10.1093/brain/aww307>
- 74 Fecto, F., Yan, J., Vemula, S.P., Liu, E., Yang, Y., Chen, W. et al. (2011) SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* **68**, 1440–1446 <https://doi.org/10.1001/archneurol.2011.250>
- 75 Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B. et al. (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* **319**, 1668–1672 <https://doi.org/10.1126/science.1154584>
- 76 Van Deerlin, V.M., Leverenz, J.B., Bekris, L.M., Bird, T.D., Yuan, W., Elman, L.B. et al. (2008) TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. *Lancet Neurol.* **7**, 409–416 [https://doi.org/10.1016/S1474-4422\(08\)70071-1](https://doi.org/10.1016/S1474-4422(08)70071-1)
- 77 Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande Velde, C. et al. (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat. Genet.* **40**, 572–574 <https://doi.org/10.1038/ng.132>
- 78 Gitcho, M.A., Balogh, R.H., Chakraverty, S., Mayo, K., Norton, J.B., Levitch, D. et al. (2008) TDP-43 A315T mutation in familial motor neuron disease. *Ann. Neurol.* **63**, 535–538 <https://doi.org/10.1002/ana.21344>
- 79 Cirulli, E.T., Lasseigne, B.N., Petrovski, S., Sapp, P.C., Dion, P.A., LeBlond, C.S. et al. (2015) Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* **347**, 1436–1441 <https://doi.org/10.1126/science.aaa3650>
- 80 Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K. et al. (2015) Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat. Neurosci.* **18**, 631–636 <https://doi.org/10.1038/nn.4000>
- 81 Deng, H.X., Chen, W., Hong, S.T., Boycott, K.M., Gorrie, G.H., Siddique, N. et al. (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477**, 211–215 <https://doi.org/10.1038/nature10353>
- 82 Nishimura, A.L., Mitne-Neto, M., Silva, H.C., Richieri-Costa, A., Middleton, S., Cascio, D. et al. (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **75**, 822–831 <https://doi.org/10.1086/425287>
- 83 Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q. et al. (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* **68**, 857–864 <https://doi.org/10.1016/j.neuron.2010.11.036>
- 84 Vegeto, E., Villa, A., Della Torre, S., Crippa, V., Rusmini, P., Cristofani, R. et al. (2020) The role of sex and sex hormones in neurodegenerative diseases. *Endocr. Rev.* **41**, 273–319 <https://doi.org/10.1210/endo/bnz005>
- 85 Cozzi, M. and Ferrari, V. (2022 Jul) Autophagy dysfunction in ALS: from transport to protein degradation. *J. Mol. Neurosci.* **72**, 1456–1481 <https://doi.org/10.1007/s12031-022-02029-3>
- 86 Liu, Y., Xu, J., Tao, W., Fu, C., Liu, J., Yu, R. et al. (2019) Exome sequencing identifies a mutation (Y740C) in spastic paraplegia 7 gene associated with adult-onset primary lateral sclerosis in a Chinese family. *Eur. Neurol.* **81**, 87–93 <https://doi.org/10.1159/000500672>
- 87 Chaytow, H., Huang, Y.T., Gillingwater, T.H. and Faller, K.M.E. (2018) The role of survival motor neuron protein (SMN) in protein homeostasis. *Cell. Mol. Life Sci.* **75**, 3877–3894 <https://doi.org/10.1007/s00018-018-2849-1>
- 88 Periyakarupiah, A., de la Fuente, S., Arumugam, S., Bahi, N., Garcera, A. and Soler, R.M. (2016) Autophagy modulators regulate survival motor neuron protein stability in motoneurons. *Exp. Neurol.* **283**, 287–297 <https://doi.org/10.1016/j.expneurol.2016.06.032>
- 89 Sansa, A., Hidalgo, I., Miralles, M.P., de la Fuente, S., Perez-Garcia, M.J., Munell, F. et al. (2021) Spinal muscular atrophy autophagy profile is tissue-dependent: differential regulation between muscle and motoneurons. *Acta Neuropathol. Commun.* **9**, 122 <https://doi.org/10.1186/s40478-021-01223-5>
- 90 Rodriguez-Muela, N., Parkhitko, A., Grass, T., Gibbs, R.M., Norabuena, E.M., Perrimon, N. et al. (2018) Blocking p62-dependent SMN degradation ameliorates spinal muscular atrophy disease phenotypes. *J. Clin. Investig.* **128**, 3008–3023 <https://doi.org/10.1172/JCI95231>

- 91 Li, M., Miwa, S., Kobayashi, Y., Merry, D., Yamamoto, M., Tanaka, F. et al. (1998) Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol.* **44**, 249–254 <https://doi.org/10.1002/ana.410440216>
- 92 Li, M., Nakagomi, Y., Kobayashi, Y., Merry, D.E., Tanaka, F., Doyu, M. et al. (1998) Nonneural nuclear inclusions of androgen receptor protein in spinal and bulbar muscular atrophy. *Am. J. Pathol.* **153**, 695–701 [https://doi.org/10.1016/S0002-9440\(10\)65612-X](https://doi.org/10.1016/S0002-9440(10)65612-X)
- 93 Simeoni, S., Mancini, M.A., Stenoien, D.L., Marcelli, M., Weigel, N.L., Zanisi, M. et al. (2000) Motoneuronal cell death is not correlated with aggregate formation of androgen receptors containing an elongated polyglutamine tract. *Hum. Mol. Genet.* **9**, 133–144 <https://doi.org/10.1093/hmg/9.1.133>
- 94 Stenoien, D.L., Cummings, C.J., Adams, H.P., Mancini, M.G., Patel, K., DeMartino, G.N. et al. (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum. Mol. Genet.* **8**, 731–741 <https://doi.org/10.1093/hmg/8.5.731>
- 95 Piccioni, F., Pinton, P., Simeoni, S., Pozzi, P., Fascio, U., Vismara, G. et al. (2002) Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. *FASEB J.* **16**, 1418–1420 <https://doi.org/10.1096/fj.01-1035fje>
- 96 Poletti, A. (2004) The polyglutamine tract of androgen receptor: from functions to dysfunctions in motor neurons. *Front. Neuroendocrinol.* **25**, 1–26 <https://doi.org/10.1016/j.yfrme.2004.03.001>
- 97 Adachi, H., Katsuno, M., Minamiyama, M., Waza, M., Sang, C., Nakagomi, Y. et al. (2005) Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. *Brain* **128**, 659–670 <https://doi.org/10.1093/brain/awh381>
- 98 Adachi, H., Waza, M., Katsuno, M., Tanaka, F., Doyu, M. and Sobue, G. (2007) Pathogenesis and molecular targeted therapy of spinal and bulbar muscular atrophy. *Neuropathol. Appl. Neurobiol.* **33**, 135–151 <https://doi.org/10.1111/j.1365-2990.2007.00830.x>
- 99 Chua, J.P., Reddy, S.L., Merry, D.E., Adachi, H., Katsuno, M., Sobue, G. et al. (2014) Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. *Hum. Mol. Genet.* **23**, 1376–1386 <https://doi.org/10.1093/hmg/ddt527>
- 100 Cicardi, M.E., Cristofani, R., Crippa, V., Ferrari, V., Tedesco, B., Casarotto, E. et al. (2019) Autophagic and proteasomal mediated removal of mutant androgen receptor in muscle models of spinal and bulbar muscular atrophy. *Front. Endocrinol.* **10**, 569 <https://doi.org/10.3389/fendo.2019.00569>
- 101 Cortes, C.J., Miranda, H.C., Frankowski, H., Batlevi, Y., Young, J.E., Le, A. et al. (2014) Polyglutamine-expanded androgen receptor interferes with TFEB to elicit autophagy defects in SBMA. *Nat. Neurosci.* **17**, 1180–1189 <https://doi.org/10.1038/nn.3787>
- 102 Giorgetti, E., Rusmini, P., Crippa, V., Cristofani, R., Boncoraglio, A., Cicardi, M.E. et al. (2015) Synergic prodegradative activity of bicalutamide and trehalose on the mutant androgen receptor responsible for spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* **24**, 64–75 <https://doi.org/10.1093/hmg/ddu419>
- 103 Montie, H.L., Cho, M.S., Holder, L., Liu, Y., Tsvetkov, A.S., Finkbeiner, S. et al. (2009) Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* **18**, 1937–1950 <https://doi.org/10.1093/hmg/ddp115>
- 104 Rusmini, P., Cortese, K., Crippa, V., Cristofani, R., Cicardi, M.E., Ferrari, V. et al. (2019) Trehalose induces autophagy via lysosomal-mediated TFEB activation in models of motoneuron degeneration. *Autophagy* **15**, 631–651 <https://doi.org/10.1080/15548627.2018.1535292>
- 105 Rusmini, P., Crippa, V., Giorgetti, E., Boncoraglio, A., Cristofani, R., Carra, S. et al. (2013) Clearance of the mutant androgen receptor in motoneuronal models of spinal and bulbar muscular atrophy. *Neurobiol. Aging* **34**, 2585–2603 <https://doi.org/10.1016/j.neurobiolaging.2013.05.026>
- 106 Rusmini, P., Polanco, M.J., Cristofani, R., Cicardi, M.E., Meroni, M., Galbiati, M. et al. (2015) Aberrant autophagic response in the muscle of A knock-in mouse model of spinal and bulbar muscular atrophy. *Sci. Rep.* **5**, 15174 <https://doi.org/10.1038/srep15174>
- 107 Yu, Z., Wang, A.M., Adachi, H., Katsuno, M., Sobue, G., Yue, Z. et al. (2011) Macroautophagy is regulated by the UPR-mediator CHOP and accentuates the phenotype of SBMA mice. *PLoS Genet.* **7**, e1002321 <https://doi.org/10.1371/journal.pgen.1002321>
- 108 Crippa, V., Carra, S., Rusmini, P., Sau, D., Bolzoni, E., Bendotti, C. et al. (2010) A role of small heat shock protein B8 (HspB8) in the autophagic removal of misfolded proteins responsible for neurodegenerative diseases. *Autophagy* **6**, 958–960 <https://doi.org/10.4161/auto.6.7.13042>
- 109 Crippa, V., Galbiati, M., Boncoraglio, A., Rusmini, P., Onesto, E., Giorgetti, E. et al. (2013) Motoneuronal and muscle-selective removal of ALS-related misfolded proteins. *Biochem. Soc. Trans.* **41**, 1598–1604 <https://doi.org/10.1042/BST20130118>
- 110 Kabuta, T., Suzuki, Y. and Wada, K. (2006) Degradation of amyotrophic lateral sclerosis-linked mutant Cu,Zn-superoxide dismutase proteins by macroautophagy and the proteasome. *J. Biol. Chem.* **281**, 30524–30533 <https://doi.org/10.1074/jbc.M603337200>
- 111 Kim, J., Kim, T.Y., Cho, K.S., Kim, H.N. and Koh, J.Y. (2013) Autophagy activation and neuroprotection by progesterone in the G93A-SOD1 transgenic mouse model of amyotrophic lateral sclerosis. *Neurobiol. Dis.* **59**, 80–85 <https://doi.org/10.1016/j.nbd.2013.07.011>
- 112 Li, L., Zhang, X. and Le, W. (2008) Altered macroautophagy in the spinal cord of SOD1 mutant mice. *Autophagy* **4**, 290–293 <https://doi.org/10.4161/auto.5524>
- 113 Morimoto, N., Nagai, M., Ohta, Y., Miyazaki, K., Kurata, T., Morimoto, M. et al. (2007) Increased autophagy in transgenic mice with a G93A mutant SOD1 gene. *Brain Res.* **1167**, 112–117 <https://doi.org/10.1016/j.brainres.2007.06.045>
- 114 Brady, O.A., Meng, P., Zheng, Y., Mao, Y. and Hu, F. (2011) Regulation of TDP-43 aggregation by phosphorylation and p62/SQSTM1. *J. Neurochem.* **116**, 248–259 <https://doi.org/10.1111/j.1471-4159.2010.07098.x>
- 115 Cicardi, M.E., Cristofani, R., Rusmini, P., Meroni, M., Ferrari, V., Vezzoli, G. et al. (2018) Tdp-25 routing to autophagy and proteasome ameliorates its aggregation in amyotrophic lateral sclerosis target cells. *Sci. Rep.* **8**, 12390 <https://doi.org/10.1038/s41598-018-29658-2>
- 116 Crippa, V., Sau, D., Rusmini, P., Boncoraglio, A., Onesto, E., Bolzoni, E. et al. (2010) The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). *Hum. Mol. Genet.* **19**, 3440–3456 <https://doi.org/10.1093/hmg/ddq257>
- 117 Cristofani, R., Crippa, V., Vezzoli, G., Rusmini, P., Galbiati, M., Cicardi, M.E. et al. (2018) The small heat shock protein B8 (HSPB8) efficiently removes aggregating species of dipeptides produced in C9ORF72-related neurodegenerative diseases. *Cell Stress Chaperones* **23**, 1–12 <https://doi.org/10.1007/s12192-017-0806-9>
- 118 Iguchi, Y., Eid, L., Parent, M., Soucy, G., Bareil, C., Riku, Y. et al. (2016) Exosome secretion is a key pathway for clearance of pathological TDP-43. *Brain* **139**, 3187–3201 <https://doi.org/10.1093/brain/aww237>
- 119 Ju, J.S., Fuentealba, R.A., Miller, S.E., Jackson, E., Piwnicka-Worms, D., Baloh, R.H. et al. (2009) Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J. Cell Biol.* **187**, 875–888 <https://doi.org/10.1083/jcb.200908115>
- 120 Ormeno, F., Hormazabal, J., Moreno, J., Riquelme, F., Rios, J., Criollo, A. et al. (2020) Chaperone mediated autophagy degrades TDP-43 protein and is affected by TDP-43 aggregation. *Front. Mol. Neurosci.* **13**, 19 <https://doi.org/10.3389/fnmol.2020.00019>

- 121 Urushitani, M., Sato, T., Bamba, H., Hisa, Y. and Tooyama, I. (2010) Synergistic effect between proteasome and autophagosome in the clearance of polyubiquitinated TDP-43. *J. Neurosci. Res.* **88**, 784–797 <https://doi.org/10.1002/jnr.22243>
- 122 Wang, I.F., Guo, B.S., Liu, Y.C., Wu, C.C., Yang, C.H., Tsai, K.J. et al. (2012) Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43. *Proc. Natl Acad. Sci. U.S.A.* **109**, 15024–15029 <https://doi.org/10.1073/pnas.1206362109>
- 123 Wang, X., Fan, H., Ying, Z., Li, B., Wang, H. and Wang, G. (2010) Degradation of TDP-43 and its pathogenic form by autophagy and the ubiquitin-proteasome system. *Neurosci. Lett.* **469**, 112–116 <https://doi.org/10.1016/j.neulet.2009.11.055>
- 124 Wang, Y., Liu, F.T., Wang, Y.X., Guan, R.Y., Chen, C., Li, D.K. et al. (2018) Autophagic modulation by trehalose reduces accumulation of TDP-43 in a cell model of amyotrophic lateral sclerosis via TFEB activation. *Neurotox Res.* **34**, 109–120 <https://doi.org/10.1007/s12640-018-9865-7>
- 125 Alberti, S., Mateju, D., Mediani, L. and Carra, S. (2017) Granulostasis: protein quality control of RNP granules. *Front. Mol. Neurosci.* **10**, 84 <https://doi.org/10.3389/fnmol.2017.00084>
- 126 Seguin, S.J., Morelli, F.F., Vinet, J., Amore, D., De Biasi, S., Poletti, A. et al. (2014) Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly. *Cell Death Diff.* **21**, 1838–1851 <https://doi.org/10.1038/cdd.2014.103>
- 127 Tresse, E., Salomons, F.A., Vesa, J., Bott, L.C., Kimonis, V., Yao, T.P. et al. (2010) VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. *Autophagy* **6**, 217–227 <https://doi.org/10.4161/autophagy.6.2.11014>
- 128 Kirola, L., Mukherjee, A. and Mutsuddi, M. (2022) Recent updates on the genetics of amyotrophic lateral sclerosis and frontotemporal dementia. *Mol. Neurobiol.* **59**, 5673–5694 <https://doi.org/10.1007/s12035-022-02934-z>
- 129 Liu, M., Pi, H., Xi, Y., Wang, L., Tian, L., Chen, M. et al. (2021) KIF5A-dependent axonal transport deficiency disrupts autophagic flux in trimethyltin chloride-induced neurotoxicity. *Autophagy* **17**, 903–924 <https://doi.org/10.1080/15548627.2020.1739444>
- 130 Carra, S. (2009) The stress-inducible HspB8-Bag3 complex induces the eIF2alpha kinase pathway: implications for protein quality control and viral factory degradation? *Autophagy* **5**, 428–429 <https://doi.org/10.4161/autophagy.5.3.7894>
- 131 Crippa, V., D'Agostino, V.G., Cristofani, R., Rusmini, P., Cicardi, M.E., Messi, E. et al. (2016) Transcriptional induction of the heat shock protein B8 mediates the clearance of misfolded proteins responsible for motor neuron diseases. *Sci. Rep.* **6**, 22827 <https://doi.org/10.1038/srep22827>
- 132 Cristofani, R., Rusmini, P., Galbiati, M., Cicardi, M.E., Ferrari, V., Tedesco, B. et al. (2019) The regulation of the small heat shock protein B8 in misfolding protein diseases causing motoneuronal and muscle cell death. *Front. Neurosci.* **13**, 796 <https://doi.org/10.3389/fnins.2019.00796>
- 133 Mandrioli, J., Crippa, V., Cereda, C., Bonetto, V., Zucchi, E., Gessani, A. et al. (2019) Proteostasis and ALS: protocol for a phase II, randomised, double-blind, placebo-controlled, multicentre clinical trial for colchicine in ALS (Co-ALS). *BMJ Open* **9**, e028486 <https://doi.org/10.1136/bmjopen-2018-028486>
- 134 Adriaenssens, E., Tedesco, B., Mediani, L., Asselbergh, B., Crippa, V., Antoniani, F. et al. (2020) BAG3 pro209 mutants associated with myopathy and neuropathy relocate chaperones of the CASA-complex to aggresomes. *Sci. Rep.* **10**, 8755 <https://doi.org/10.1038/s41598-020-65664-z>
- 135 Zhang, X., Li, L., Chen, S., Yang, D., Wang, Y., Zhang, X. et al. (2011) Rapamycin treatment augments motor neuron degeneration in SOD1(G93A) mouse model of amyotrophic lateral sclerosis. *Autophagy* **7**, 412–425 <https://doi.org/10.4161/autophagy.7.4.14541>
- 136 Mandrioli, J., D'Amico, R., Zucchi, E., Gessani, A., Fini, N., Fasano, A. et al. (2018) Rapamycin treatment for amyotrophic lateral sclerosis: protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). *Medicine* **97**, e11119 <https://doi.org/10.1097/MD.0000000000001119>
- 137 Palma, J.A., Martinez, J., Millar Vernetti, P., Ma, T., Perez, M.A., Zhong, J. et al. (2022) mTOR inhibition with sirolimus in multiple system atrophy: a randomized, double-blind, placebo-controlled futility trial and 1-year biomarker longitudinal analysis. *Mov. Disord.* **37**, 778–789 <https://doi.org/10.1002/mds.28923>
- 138 Damri, O., Shemesh, N. and Agam, G. (2020) Is there justification to treat neurodegenerative disorders by repurposing drugs? The case of Alzheimer's disease, lithium, and autophagy. *Int. J. Mol. Sci.* **22**, 189–206 <https://doi.org/10.3390/ijms22010189>
- 139 Costa, A.J., Erustes, A.G., Sinigaglia, R., Girardi, C.E.N., Pereira, G., Ureshino, R.P. et al. (2021) Lack of autophagy induction by lithium decreases neuroprotective effects in the striatum of aged rats. *Pharmaceutics* **13**, 135–148 <https://doi.org/10.3390/pharmaceutics13020135>
- 140 Pasquali, L., Longone, P., Isidoro, C., Ruggieri, S., Paparelli, A. and Fornai, F. (2009) Autophagy, lithium, and amyotrophic lateral sclerosis. *Muscle Nerve* **40**, 173–194 <https://doi.org/10.1002/mus.21423>
- 141 Motoi, Y., Shimada, K., Ishiguro, K. and Hattori, N. (2014) Lithium and autophagy. *ACS Chem. Neurosci.* **5**, 434–442 <https://doi.org/10.1021/cn500056q>
- 142 Medina, D.L., Fraldi, A., Bouche, V., Annunziata, F., Mansueto, G., Spampinato, C. et al. (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev. Cell* **21**, 421–430 <https://doi.org/10.1016/j.devcel.2011.07.016>
- 143 Palmieri, M., Impey, S., Kang, H., di Ronza, A., Pelz, C., Sardiello, M. et al. (2011) Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Hum. Mol. Genet.* **20**, 3852–3866 <https://doi.org/10.1093/hmg/ddr306>
- 144 Settembre, C. and Ballabio, A. (2011) TFEB regulates autophagy: an integrated coordination of cellular degradation and recycling processes. *Autophagy* **7**, 1379–1381 <https://doi.org/10.4161/autophagy.7.11.17166>
- 145 Medina, D.L. and Ballabio, A. (2015) Lysosomal calcium regulates autophagy. *Autophagy* **11**, 970–971 <https://doi.org/10.1080/15548627.2015.1047130>
- 146 Medina, D.L., Di Paola, S., Peluso, I., Armani, A., De Stefani, D., Venditti, R. et al. (2015) Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* **17**, 288–299 <https://doi.org/10.1038/ncb3114>
- 147 Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N.R., Doi, H. et al. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.* **10**, 148–154 <https://doi.org/10.1038/nm985>
- 148 Liu, R., Barkhordarian, H., Emadi, S., Park, C.B. and Sierks, M.R. (2005) Trehalose differentially inhibits aggregation and neurotoxicity of beta-amyloid 40 and 42. *Neurobiol. Dis.* **20**, 74–81 <https://doi.org/10.1016/j.nbd.2005.02.003>
- 149 Davies, J.E., Sarkar, S. and Rubinsztein, D.C. (2006) Trehalose reduces aggregate formation and delays pathology in a transgenic mouse model of oculopharyngeal muscular dystrophy. *Hum. Mol. Genet.* **15**, 23–31 <https://doi.org/10.1093/hmg/ddi422>
- 150 Sarkar, S., Davies, J.E., Huang, Z., Tunnacliffe, A. and Rubinsztein, D.C. (2007) Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J. Biol. Chem.* **282**, 5641–5652 <https://doi.org/10.1074/jbc.M609532200>
- 151 Rodriguez-Navarro, J.A., Rodriguez, L., Casarejos, M.J., Solano, R.M., Gomez, A., Perucho, J. et al. (2010) Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. *Neurobiol. Dis.* **39**, 423–438 <https://doi.org/10.1016/j.nbd.2010.05.014>

- 152 Perucho, J., Casarejos, M.J., Gomez, A., Solano, R.M., de Yébenes, J.G. and Mena, M.A. (2012) Trehalose protects from aggravation of amyloid pathology induced by isoflurane anesthesia in APP(swe) mutant mice. *Curr. Alzheimer Res.* **9**, 334–343 <https://doi.org/10.2174/156720512800107573>
- 153 Schaeffer, V. and Goedert, M. (2012) Stimulation of autophagy is neuroprotective in a mouse model of human tauopathy. *Autophagy* **8**, 1686–1687 <https://doi.org/10.4161/auto.21488>
- 154 Schaeffer, V., Lavenir, I., Ozcelik, S., Tolnay, M., Winkler, D.T. and Goedert, M. (2012) Stimulation of autophagy reduces neurodegeneration in a mouse model of human tauopathy. *Brain* **135**, 2169–2177 <https://doi.org/10.1093/brain/aws143>
- 155 Castillo, K., Nassif, M., Valenzuela, V., Rojas, F., Matus, S., Mercado, G. et al. (2013) Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. *Autophagy* **9**, 1308–1320 <https://doi.org/10.4161/auto.25188>
- 156 Sarkar, S., Chigurupati, S., Raymick, J., Mann, D., Bowyer, J.F., Schmitt, T. et al. (2014) Neuroprotective effect of the chemical chaperone, trehalose in a chronic MPTP-induced Parkinson's disease mouse model. *Neurotoxicology* **44**, 250–262 <https://doi.org/10.1016/j.neuro.2014.07.006>
- 157 Li, Y., Guo, Y., Wang, X., Yu, X., Duan, W., Hong, K. et al. (2015) Trehalose decreases mutant SOD1 expression and alleviates motor deficiency in early but not end-stage amyotrophic lateral sclerosis in a SOD1-G93A mouse model. *Neuroscience* **298**, 12–25 <https://doi.org/10.1016/j.neuroscience.2015.03.061>
- 158 He, Q., Koprach, J.B., Wang, Y., Yu, W.B., Xiao, B.G., Brotchie, J.M. et al. (2016) Treatment with trehalose prevents behavioral and neurochemical deficits produced in an AAV alpha-synuclein rat model of Parkinson's disease. *Mol. Neurobiol.* **53**, 2258–2268 <https://doi.org/10.1007/s12035-015-9173-7>
- 159 Debnath, K., Pradhan, N., Singh, B.K., Jana, N.R. and Jana, N.R. (2017) Poly(trehalose) nanoparticles prevent amyloid aggregation and suppress polyglutamine aggregation in a Huntington's disease model mouse. *ACS Appl. Mater. Interfaces* **9**, 24126–24139 <https://doi.org/10.1021/acsami.7b06510>
- 160 Mobini, M., Radbakhsh, S., Kubaski, F., Eshraghi, P., Vakili, S., Vakili, R. et al. (2022) Impact of intravenous trehalose administration in patients with Niemann-Pick disease types A and B. *J. Clin. Med.* **11**, 247–258 <https://doi.org/10.3390/jcm11010247>
- 161 Crippa, V., Cicardi, M.E., Ramesh, N., Seguin, S.J., Ganassi, M., Bigi, I. et al. (2016) The chaperone HSPB8 reduces the accumulation of truncated TDP-43 species in cells and protects against TDP-43-mediated toxicity. *Hum. Mol. Genet.* **25**, 3908–3924 <https://doi.org/10.1093/hmg/ddw232>