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Macroautophagy in CNS health and disease

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Abstract

Macroautophagy is an evolutionarily conserved process that delivers diverse cellular contents to lysosomes for degradation. As our understanding of this pathway grows, so does our appreciation for its importance in disorders of the CNS. Once implicated primarily in neurodegenerative events owing to acute injury and ageing, macroautophagy is now also linked to disorders of neurodevelopment, indicating that it is essential for both the formation and maintenance of a healthy CNS. In parallel to understanding the significance of macroautophagy across contexts, we have gained a greater mechanistic insight into its physiological regulation and the breadth of cargoes it can degrade. Macroautophagy is a broadly used homeostatic process, giving rise to questions surrounding how defects in this single pathway could cause diseases with distinct clinical and pathological signatures. To address this complexity, we herein review macroautophagy in the mammalian CNS by examining three key features of the process and its relationship to disease: how it functions at a basal level in the discrete cell types of the brain and spinal cord; which cargoes are being degraded in physiological and pathological settings; and how the different stages of the macroautophagy pathway intersect with diseases of neurodevelopment and adult-onset neurodegeneration.

Cells of the mammalian brain and spinal cord rely on robust catabolism to maintain homeostasis and carry out their highly specialized functions. With significant metabolic and catabolic demands, these cells must efficiently recycle macromolecules (for example, proteins and lipids) and organelles (for example, mitochondria and endoplasmic reticulum (ER))[1]. These needs can be met by macroautophagy, a versatile and conserved pathway in which substrates are captured in double-membrane vesicles and trafficked to lysosomes for degradation. Neurons of the PNS and the CNS were among the first cell types in which macroautophagy was examined[2,3]. Whereas acute and prolonged starvation did not trigger the CNS to capture cytosol into double-membrane vesicles, which would later be named autophagosomes by De Duve[4], profound stressors, such as nerve crush and axon transection, evoked a marked macroautophagic response. Since then, work first in primary neurons

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and then in vivo has shown that a broad range of stressors, including hypoxia and ER stress[5–7], can increase autophagosome biogenesis and subsequent degradation, emphasizing the long-held identity of macroautophagy as a stress response pathway in neurons.

Catabolism

The breakdown of complex molecules and structures into smaller units.

A significant shift in how macroautophagy is viewed in the CNS arrived with seminal papers from the groups of Mizushima and Tanaka, which, building upon the work of Ohsumi's group[8,9] and others, demonstrated that broad disruption of autophagy-related (ATG) proteins involved in autophagosome formation (FIG. 1) in neural and glial precursors leads to neurodegeneration, motor dysfunction and death[10,11]. These studies not only cemented macroautophagy dysfunction as convergent with degenerative events and potentially neurodegenerative disease but also indicated that macroautophagy has a basal role in maintaining CNS health. An often-overlooked aspect of these studies is that, although neurodegeneration is a consequence of macroautophagy disruption, the degeneration is not widespread. Instead, preferential vulnerability of neurons to macroautophagy loss — particularly Purkinje cells and cerebral cortical and hippocampal pyramidal neurons — points to neuronal subtype-specific reliance on catabolism to maintain cellular health[12]. Experiments using Cre-based approaches in mice added resolution to this idea (TABLE 1); for example, deletion of the macroautophagy-initiating gene *Atg7* in Purkinje cells leads to neurodegeneration at 2 months[13], while its deletion in dopaminergic neurons results in only modest neurodegeneration even after 1.5 years[14–17]. The reason for these differences is still unknown.

With advances in genetics and cell biology, investigation of the molecules involved in macroautophagy and the relevance to human disease has continued to grow[18]. This research effort has revealed substantial involvement of macroautophagy in CNS development, cellular homeostasis and diseases of both development and adult-onset neurodegeneration. In this Review, we provide an overview of macroautophagy in the mammalian CNS and discuss how this process is modulated in health and disease, including an emerging focus on selective macroautophagic pathways. We also explore how macroautophagy may be differentially adapted by the discrete and highly specialized cell types of the CNS, and the questions that are still outstanding to allow full comprehension of how its 'dysfunction' may or may not contribute to disease.

Molecular overview of macroautophagy

Macroautophagy is one of the three pathways described in eukaryotic cells that are collectively known as autophagy ('self-eating')[19]. Each pathway is distinguished by how cargoes are delivered to the lysosomal lumen. In macroautophagy, cargo is sequestered in a nascent double-membrane autophagosome, which matures and fuses with the lysosome for degradation (FIG. 1). In microautophagy, materials are directly engulfed by the lysosome by invaginations or protrusions of the lysosomal membrane[20]. In chaperone-mediated autophagy, proteins are trafficked to the lysosome by the chaperone heat

shock cognate 71 kDa protein (HSC70) and co-chaperone proteins, then internalized via the lysosomal-associated membrane protein 2A (LAMP2A) receptor on the lysosomal membrane[21]. Although microautophagy has not been extensively examined in mammalian cells, chaperone-mediated autophagy dysregulation in neurodegenerative disease[22] and how to harness its therapeutic potential remain active areas of investigation[23]. As the focus of this Review, however, macroautophagy is hereafter termed autophagy.

The de novo synthesis of autophagosomes, which can range in diameter from hundreds of nanometres to tens of microns, and their subsequent fusion to the lysosome, makes autophagy the most versatile degradative pathway in the cell. As previously described[24,25], the pathway can be separated into three phases: autophagosome biogenesis coupled to cargo capture; membrane growth and closure; and autophagosome maturation via fusion to endocytic and, ultimately, lysosomal structures for degradation (FIG. 1). Autophagy can sequester degradative cargo in two main ways: via the non-selective capture of cytoplasmic components at sites of autophagosome biogenesis, or via the selective capture of particular structures or organelles that are recognized by autophagy adaptor proteins and actively engulfed by an autophagosome (BOX 1).

Endocytic

Pertaining to the internalization of substances into the cell. These membrane-bound structures include endosomes and multivesicular bodies.

Autophagosome biogenesis begins with the activation of the Unc51-like autophagy activating kinase 1 (ULK1) complex, which acts to identify the membrane at which autophagosome biogenesis will occur. For non-selective cargo capture, the kinase complex can be induced by a broad range of cellular stressors, including nutrient shortage, oxidative stress and hypoxia, as it is regulated by the nutrient and metabolic kinases mammalian target of rapamycin (mTOR) and 5′ AMP-activated protein kinase (AMPK)[26,27]. During selective cargo capture, which can occur in the absence of mTOR regulation[28,29], the activation of the ULK1 kinase complex is coordinated with cargo consolidation, in part by the adaptor proteins involved in cargo capture itself[30,31]. Once activated, ULK1 phosphorylates ATG9 and beclin 1 (also known as ATG6), which in complex with ATG14L and the class III phosphatidylinositol 3-kinase (PI3K) vacuolar protein sorting 34 (VPS34)–VPS15 catalytic heterodimer (BOX 2) enriches the nucleation site with phosphatidylinositol 3-phosphate (PI3P), an important component of the autophagosome membrane (FIG. 1). With the recruitment of PI3P-binding proteins and de novo synthesis of phospholipids[32], the autophagosome membrane elongates to envelop cargo.

Membrane elongation is accomplished by ATG2 and ATG9, which together act to transfer lipids and re-equilibrate the membrane[33], along with two ubiquitin-like conjugation systems that lipidate ATG8 homologues through separate reactions. Lipidation of ATG8 homologues is necessary for their incorporation into the growing membrane, and components of these conjugation systems have been exploited for genetic abrogation of autophagy (TABLE 1). Autophagosome formation is then completed upon its closure, which may rely on members

of the endosomal sorting complexes required for transport (ESCRT) machinery such as charged multivesicular body protein 2A (CHMP2A)[34,35].

Once formed, autophagosomes first fuse with endocytic structures to create amphisomes or directly with lysosomes, ultimately forming an autolysosome, in which the decreased pH activates lysosomal hydrolases to degrade cargo (FIG. 1). Studies have implicated the SNaRe protein syntaxin 17 in the fusion of the amphisome and autophagosome to the lysosome[36], but the precise molecular requirements for fusion throughout this pathway remain poorly resolved.

SNARE protein

SNaP receptor, or SNaRe, proteins form a large family of proteins that mediate vesicle fusion.

An early indication that autophagy plays an important role in the CNS is reflected in how autophagy machinery responsible for creation of the autophagosome (reviewed extensively elsewhere[24–26]) is highly expressed across different cell types of the brain. The most recognized, yet most mysterious, autophagy protein may be MAP1 light chain 3 (LC3), which together with $GABA_A$ receptor-associated protein (GABARAP) represent the two families of mammalian homologues of yeast ATG8. LC3 has become synonymous with the autophagosome, as it was the first mammalian homologue identified[37,38]. This led to the development of tools to allow monitoring of these structures in vivo — from the biochemical evaluation of its membrane-bound form to assess autophagic activity[38], to the development of fluorescently tagged LC3 (REF.[39]). It is important to note that of the six known mammalian homologues, three for LC3 (LC3A, LC3B and LC3C) and three for GABARAP (GABARAP, GABARAP-like 1 (GABARAPL1) and GABARAPL2), LC3 is less similar to yeast ATG8 than the GABARAP members and binds lipid least efficiently in vitro[40]. Studies suggest that GABARAPs may be critical for fusion of autophagosomes to lysosomes, whereas LC3 is not[41,42]. Additionally, GABARAP was so-named as it was previously shown to be essential for anterograde trafficking of $GABA_A$ receptors[43]. How its trafficking function and its role in autophagy might interrelate remains unclear.

Selective autophagy and adaptor proteins

As the importance of autophagy under basal conditions emerged, the concept that it could be a purely bulk degradation process was dismissed, leading to new questions about how autophagic cargoes were targeted for degradation. These studies have revealed that exogenous cargoes, such as invading pathogens, disease-associated structures, such as proteinaceous inclusions, and virtually all organelles rely on regulated degradation by this pathway, which can occur independently of the nutrient status of the cell (BOX 1). These findings have led to a notable conceptual shift in that the selective capture of autophagic cargoes governed by adaptor proteins may be the dominant basal mode of autophagy, whereas bulk, non-selective degradation might be relegated to emergent needs.

Echoing the yeast pathway described by Klionsky et al. as cytoplasm-to-vacuole targeting (Cvt)[44], specific cargoes can be targeted to the autophagosome by adaptor proteins, also known as autophagy receptors and selectivity adaptor proteins. In mammals, more than 20 adaptor proteins have thus far been identified[25,45–47] (BOX 1). Adaptor proteins contain domains that bind cargo (ubiquitylated or non-ubiquitylated), a domain to attach to ATG8 homologues, and other elements for oligomerization and signalling, thereby scaffolding substrates to the growing autophagosomal membrane[48,49]. For a given substrate, many adaptor proteins can potentially be involved and, inversely, a given adaptor may link multiple cargo types. For example, adaptor proteins BCL-2 interacting protein 3 like (BNIP3L; also known as NIX), p62 (also known as SQSTM1) and optineurin (OPTN) have been implicated in mitochondrial turnover, whereas the adaptor p62 is involved in most types of selective autophagy and can itself be an autophagic substrate upon self-oligomerization[50,51]. When and how different adaptor proteins are used is currently under investigation, with studies suggesting that cell, cargo and stressor type play important roles[52–54].

In the CNS, different autophagy cargoes are selectively eliminated (BOX 1). Little is known about the regulation of these pathways in the brain but examining the role of adaptor proteins using mouse genetics has led to modest results. For example, loss of function of p62 or OPTN leads to mild changes[55–57], suggesting that these adaptor proteins may have redundant functions. Nonetheless, the adaptor Alfy (also known as WDFY3) was recently shown to be required for the turnover of protein aggregates in the adult brain[58]. Notably, as suggested by the conservation observed with Cvt, the importance of Alfy was consistent with conclusions drawn from HeLa cells[59], indicating that mechanisms of selective autophagy are highly conserved across cell types. However, given the vast differences with which distinct neuronal cell types depend on autophagy, similar discrete differences would also be expected for selective autophagy.

One of the unexpected outcomes of the study of selective autophagy has been the identification of mutations in select adaptor proteins and other molecular players as contributors to diseases of both the developing and ageing CNS (TABLE 2). It is notable that, although deletion of the genes encoding p62 or OPTN in mice leads to little or no phenotype, potential loss-of-function mutations in the orthologous genes in humans cause disease. This may reflect how these adaptor proteins work, coupled with species-specific differences: both adaptor proteins directly interact with the LC3 isoforms and, whereas mice express two isoforms (LC3A and LC3B), humans also express a third isoform (LC3C) that is differentially modified[60]. The prevalence of neurological diseases due to loss of function of these selective autophagy proteins (TABLE 2) gives rise to the hypothesis that changes in homeostasis of discrete cargo, rather than changes in autophagy overall, may underlie the preferential vulnerability associated with specific diseases.

Autophagy modulation in the CNS

The regulation of autophagy is most frequently associated with inhibition of the central nutrient sensing kinase mTOR but AMPK and other proteins also have roles in such regulation[26,27]. In peripheral cells, nutrient deprivation inhibits mTOR to stimulate bulk

autophagy. Whether organismal starvation induces neuronal autophagy and whether mTOR inhibition activates neuronal autophagy remain controversial[39,61–64], especially in primary neurons[7,65,66]. In vivo studies have shown a role for mTOR-mediated autophagy regulation in neurons independently of nutrient sensing[67–70], indicating that cells in the intact brain can dissociate this type of autophagy regulation from its classical signal transduction (that is, nutrient sensing) and may not be influenced by metabolic kinases in the same way as cells in peripheral tissues[71]. Differences between primary cells and neurons in situ may reflect the importance of the cellular milieu on autophagy regulation, and how the intact environment might be necessary to capture all facets of this pathway. An important consideration lacking in the field is how strongly starvation and mTOR inhibition might regulate autophagy in the nutrient-sensing cells of the CNS, including glia and cells of the vasculature (FIG. 2). Given the tight interconnectedness between these different cell types, there is the possibility that non-neuronal cells play a fundamental role from an organismal and tissue-wide perspective. Thus, to understand autophagy and how it may influence CNS health, it is necessary to improve our understanding of how autophagy is used by non-neuronal cells. Moreover, as noted above, if selective autophagy is an important component of CNS physiology and can occur independently of mTOR regulation, relying only on mTOR-mediated autophagosome formation as the readout of autophagic function may not fully consider selective mechanisms (that is, the functional contribution of cargo and related adaptor proteins), thus clouding our understanding of the true relationship between nutrient and energy sensing and autophagy activation in the CNS.

Given the importance of membrane trafficking in all cells of the CNS, special emphasis needs to be placed on understanding the subtle regulation of autophagy achieved through the balance of PI3P. Depending on the binding partners of the class III PI3K complex (BOX 2), PI3P can be allocated for autophagy, endosomal sorting, phagocytosis or other processes, including events that require autophagy proteins such as LC3 (REFS[32,72,73]) (BOX 3). Exogenously increasing PI3P levels in rapidly dividing cells can augment autophagy[28], and therefore changing the balance or localization of ATG14 and its binding partners could potentially enhance or diminish autophagy versus other membrane trafficking events[72,74,75]. These considerations may help delineate the relative contribution of the many membrane trafficking events to the health of CNS cells.

Autophagy in cells of the CNS

Neuronal autophagy.

Among the studies of autophagy in cells of the CNS, studies on the role of autophagy in neurons are the most prevalent. Although neurons are perhaps the least responsive cell type to physiological starvation, early studies demonstrating the marked response of autophagy to degenerative events, along with early genetic studies in mice, likely drove this momentum. Subsequently, the potential for autophagy modulation as a therapeutic approach to multiple neurodegenerative diseases has driven significant interest in how neurons require autophagy to maintain their health.

Neurons are long-lived, terminally differentiated cells with complex morphologies and highly specialized functions that must be supported by appropriate catabolism. Inactivating

autophagy within neuronal subtypes has revealed myriad roles for this pathway in protein homeostasis, organelle turnover, synaptic transmission and, ultimately, cellular integrity (TABLE 1). To contextualize these observations, two key aspects of neuronal autophagy must be considered: the intracellular location of this process and which cargoes are being degraded. To approach the former, a rich body of cell biological work has examined autophagy dynamics in cultured dorsal root ganglion neurons of the PNS, utilizing live cell imaging to track the behaviour of green fluorescent protein-tagged LC3. These studies describe autophagosome biogenesis exclusively at the distal axon, with structures fusing to lysosomes as they retrogradely traffic to the soma[62,66,76–78]. Applying similar approaches to the CNS, primary mouse hippocampal neurons show autophagosome formation predominantly in the distal axon but also in the cell body and dendrites[62,66,67,77,79] (FIG. 2). Although studies in the dendritic compartment are limited, growing evidence suggests that autophagy is essential for establishing spine morphology[68], and studies have shown that autophagosome biogenesis in the postsynaptic somatodendritic compartment can be activity dependent[67,79]. It has been speculated that neurons compartmentalize autophagosome biogenesis distally and lysosomal degradation proximally to efficiently meet the high trafficking demands of axonal transport. In the CNS, this compartmentalization appears to be unique to neurons, with autophagosome biogenesis and lysosomal fusion occurring throughout the entirety of astrocytes[80] and oligodendrocytes[81] (FIG. 2).

Activity dependent

Affected by synaptic transmission.

As we have learned how discrete neuronal compartments rely on autophagy, we are starting to understand the molecular identity of the cargoes being degraded. Nonetheless, we are only beginning to understand how this versatile pathway is being used by the cell. As with all eukaryotic cells, the outright loss of autophagy in neurons leads to a profound disruption of protein homeostasis, causing an accumulation of inclusions of ubiquitinated proteins and p62 throughout the cell[10,11]. Although these inclusions were considered the causative event leading to observed behavioural dysfunction and neurodegeneration, it was soon shown that, despite resolving inclusion body formation, there was neither an improvement nor an exacerbation of the neurodegenerative phenotype[56], reinvigorating the question of how neuronal homeostasis was maintained by autophagy. Autophagy has since been implicated in the regulation of synaptic morphology and function by turning over species in both the presynaptic and postsynaptic compartments, from synaptic vesicle components in dopaminergic and hippocampal neurons to postsynaptic density components (for example, postsynaptic density protein 95 (PSD95), ARC, and glutamate and GABA receptors) in hippocampal and cortical neurons[64,67,68,82–86].

Consistent with these observations, pharmacological manipulation of autophagy in hippocampal neurons indicates involvement of this pathway in memory formation, potentially by increasing dendritic spine density and promoting long-term potentiation[87]. Autophagy has also been shown to be involved in NMDA receptor-dependent long-term depression (NMDAR-LTD) in a complex manner; whereas autophagy inhibition may be involved in NMDAR-LTD induction[88], accumulating evidence suggests that autophagy

is required for early phase NMDAR-LTD by degrading PSD95, which reorganizes the synaptic surface for AMPA receptor mobilization and changes in synaptic strength[89]. Subsequently, the transcription of autophagy genes induced by neuronal activity has been shown to maintain late-phase NMDAR-LTD[90]. In addition to autophagic degradation of synaptic contents, proteins that are enriched in these compartments can also regulate autophagy[84,86,91]. For example, the presynaptic proteins Bassoon and Piccolo have been shown to negatively regulate autophagy in the presynaptic bouton of hippocampal neurons via interaction with ATG5 (REFS[82,83]). Autophagosome formation in the presynapse has also been shown to involve the endocytic protein endophilin A, which is activated by the Parkinson disease (PD)-associated leucine-rich repeat kinase 2 (LRRK2)[92]. The PD-associated lipid phosphatase synaptojanin (FIG. 3) has been shown to be similarly important for presynaptic autophagy[93]. Although synaptic function is fundamental to all neuronal subtypes, observations in medium spiny neurons of the striatum have revealed that a subtype-specific reliance on autophagy can still be observed: medium spiny neurons in the direct and indirect pathway respectively require autophagy for degradation of presynaptic components to establish dendritic structure and degradation of ion channels to establish intrinsic excitability[94]. In addition to synaptic proteins, the autophagic turnover of mitochondria and ER can broadly influence neuronal function through, for example, the critical role of these substrates in calcium buffering[95]. In fact, a recent study using cultured neurons elucidated a role for presynaptic autophagy of ER in controlling ER calcium stores, which in turn regulate neurotransmission[96].

As mentioned above, neuronal subtypes show a differential vulnerability to autophagy dysfunction, with Purkinje cells manifesting early degenerative changes[10,11], while dopaminergic neurons[16,67] and spinal motor neurons[97] showing diminished vulnerability to outright degeneration, although morphological and electrophysiological abnormalities are present (TABLE 1). In Purkinje cells, autophagy inhibition by either *Atg5* or *Atg7* disruption produces an accumulation of membranous structures, axonopathy and dystrophic neurons[13,98]; however, rather than wholly considering autophagy inhibition detrimental and its activation beneficial, autophagy hyperactivation in Purkinje cells has been shown to be toxic and its inhibition can be beneficial in settings of excitotoxic stress[6,12]. Taken together, these results illustrate the complexity of the roles of autophagy in the CNS and that heterogeneous neuronal populations rely on the process for proper function but with discrete differences.

Glial autophagy.

We are just beginning to understand how diverse populations of glia use autophagy under basal and stress conditions. Autophagosomes constitutively form in glia[39] (FIG. 2) and can do so in mTOR-dependent and mTOR-independent manners[99–101]. In response to proteotoxic stress, autophagic turnover of aggregated proteins has been demonstrated in astrocytes[99,102], oligodendrocytes[103] and microglia[104,105].

As the cells responsible for metabolic buffering and modulation of synaptic transmission, astrocytes and their homeostatic signalling have received much attention. Similar to the early studies in neurons, astrocyte autophagy has mostly been studied as a stress reactivity

pathway. In response to acute mechanical injury to the cortex, neuroinflammation induces *Atg7*-dependent mitophagy in astrocytes to maintain mitochondrial network integrity, reduce reactive oxygen species concentrations and prevent astrocyte death[106]. In the setting of hypoxia and ischaemia–reperfusion injury, there is evidence for both adaptive[107] and detrimental[108] roles for astrocyte autophagy for glial and neuronal survival, the precise balance of which remains to be established. The basal role of astrocyte autophagy is not well understood.

Ischaemia–reperfusion injury

Exacerbation of cellular dysfunction and death after the return of blood flow (reperfusion) to tissues that previously had an inadequate blood supply (ischaemia).

Oligodendrocytes appear to use autophagy as a similar prosurvival adaptation to stress, using it to promote neurological recovery after traumatic spinal cord injury[109]. A recent study showed that proper timing and location of myelination by oligodendrocytes is controlled by a lysosomal–apoptotic signalling axis[110]; although autophagy per se was not examined in this study, the findings suggest that myelin components can be degradative cargo in these cells. Although several studies have proposed a role for autophagy in myelin formation and degradation, most have relied on indirect evidence by using pleiotropic autophagy inducers such as mTOR inhibition and starvation paradigms[111,112]. Therefore, the direct role of autophagy in supporting the myelinating function of oligodendrocytes remains to be established.

Microglia are essential for phagocytic activity in the developing and adult brain, and autophagy machinery has been more closely associated with these dynamic membrane functions than those in other glial subtypes. With implications for autism spectrum disorder, microglial loss of *Atg7* results in increased dendritic spine density at glutamatergic synapses and abnormal social interaction and repetitive behaviours, suggesting that microglial autophagy, or possibly non-canonical pathways such as LC3-associated phagocytosis (LAP) and LC3-associated endocytosis (LANDO) (BOX 3), are critical for proper synaptic pruning during development[113]. Microglial autophagy may also enhance the clearance of amyloid-β[104,114–116], with the important caveat that amyloid-β is a cargo for LANDO in these cells[117] (BOX 3). However, a recent study has found that microglia can internalize neuron-released α-synuclein independently from phagocytosis or endocytosis (that is, LAP or LANDO), and require autophagy via interaction with p62 for degradation[105].

Neurovascular autophagy.

The intimate relationship between CNS cells and their blood vessels has long been observed, especially in cerebrovascular disease, but only recently have we appreciated the multidimensional roles of the neurovascular unit (NVU) — comprising vascular cells (pericytes, endothelial cells), glia and neurons — broadly in health and disease[118]. Investigations of autophagy in the NVU are rare, with studies in astrocytes being the most prevalent. As is common for early exploratory studies, the role of autophagy in response to acute stressors has been evaluated, and little is known about its basal role

in the health and survival of these cells as a functional unit. NVU autophagy is often studied in neurons and glia as a response to vascular insult in the setting of ischaemic stroke. Relatively few have examined autophagy specifically in endothelial cells of the NVU but the picture that has emerged is one of prosurvival adaptation to nutrient deprivation, hypoxia and ischaemia–reperfusion injury[119–121]. Neurovascular dysfunction, as indicated by microvascular damage, alterations in blood–brain barrier permeability and cerebral perfusion abnormalities, has been observed across many neurodegenerative diseases, including forms of PD, Alzheimer disease (AD) and amyotrophic lateral sclerosis (ALS)[118,122–124]. How this dysfunction may involve NVU autophagy requires further study.

Neurodevelopmental disorders

Many autophagy machinery components are highly expressed in the developing brain and are subsequently downregulated, suggesting an important role for autophagy during this period of rapid cellular remodelling. Mutations in genes encoding autophagy machinery components as well as upstream regulators, including mTOR, can affect the developing CNS, leading to a range of deficits, from widespread dysfunction to discrete neurological changes[125–129]. In model systems, loss-of-function studies indicate that autophagy machinery and regulators are important for fate specification[130,131], cellular migration[132,133], axon guidance[131,132,134,135] and synaptic remodelling[68,136,137] (TABLE 1).

The described cell biological effects can be due to machinery dysfunction at different stages of the autophagy pathway. For example, axon guidance defects occur by disrupting proteins involved in autophagy induction (for example, ULK1 and ULK2)[131], autophagosome biogenesis (for example, ATG7)[134] and cargo capture (for example, Alfy)[132]. Particular players can also cause a wide breadth of phenotypes: mutations in *WDFY3* are associated with autism spectrum disorder and microcephaly[138], and chronic suppression of mTOR-dependent autophagy is implicated in both the neuropsychiatric manifestation of tuberous sclerosis I and the synaptic defects in fragile X syndrome[139]. Human genetic studies have shown that mutations in *ATG5* (REF.[125]) and, recently, *ATG7* (REF.[129]) can cause syndromes involving ataxia and developmental delay, adding clinical insight into the consequence of global autophagy dysfunction and its convergence on the nervous and musculoskeletal systems. Given the role of autophagy in essential cellular processes in neurodevelopment, it is perhaps unsurprising that defects in core autophagy machinery components can lead to a wide range of disorders, often sharing developmental delay as a main feature.

In addition, a diverse group of developmental disorders that affect the CNS are caused by mutations in single genes that have roles in autophagy and general membrane trafficking, supporting the idea that neurodevelopment is especially sensitive to changes in membrane dynamics[138]. Vici syndrome, a multisystem disorder with defective autophagy caused by mutations in the gene encoding ectopic P granules protein 5 (EPG5)[140], features an absent corpus callosum and profound developmental delay[141–143]. β-Propeller protein-associated neurodegeneration, also known as static encephalopathy of childhood with neurodegeneration, is caused by de novo mutations in the X-linked gene *WDR45* (also known as *WIPI4*; encoding WD repeat domain phosphoinositide-interacting protein

4)[144,145] and manifests with seizures, infantile-onset developmental delay, intellectual disability and ataxia. With age, these patients exhibit cognitive decline, movement disorders and neurodegeneration. The yeast homologue of *WDR45* is *atg18*, which associates with PI3P for autophagic vacuole formation[146]. Sorting nexin 14 (SNX14)-associated autosomal recessive cerebellar ataxia and intellectual disability syndrome[147] has been linked to impaired autophagosome clearance and lysosomal function in model systems and patient-derived cells[148]. Three forms of hereditary spastic paraplegia (SPG11, SPG15 and SPG49) are caused by genes that are involved in early autophagosome formation[149,150]. In humans, mutations in *VPS15*, encoding a component of the VPS34 complex (BOX 2), cause developmental cortical atrophy and epilepsy. In mice, *Vps15* hypomorphism disrupts endosomal–lysosomal trafficking and its ablation causes the accumulation of autophagic structures and, ultimately, cortical atrophy[151]. Although these mutations can be linked to autophagy changes, their effects can also be attributed to overall membrane trafficking alterations, which may explain the breadth of features observed.

Adult-onset neurodegenerative diseases

Perhaps the most widely studied area of CNS autophagy is its role in neurodegenerative disease. Genetic and pathological evidence has connected autophagy directly or indirectly to all commonly studied adult-onset neurodegenerative disorders, including AD, ALS, frontotemporal dementia (FTD)/frontotemporal lobar dementia (FTLD), Huntington disease (HD), PD and other synucleinopathies such as dementia with Lewy bodies (DLB) and multiple systems atrophy (MSA) (FIG. 3). As discussed above, loss-of-function studies have shown that autophagy is essential for neural health[10,11] (TABLE 1), but the direct links between autophagy and neurodegenerative disease pathogenesis remain to be established. Across these diseases, autophagy dysfunction may be the primary insult, contribute to or modify underlying mechanisms, or be epiphenomena alongside degenerative events. Given that complete loss of autophagy in neural cells causes degeneration in the early postnatal period in animal studies and that severe dysfunction can be incompatible with life or cause paediatric neurological deficits, the adult-onset and cell type-specific nature of these diseases suggests that subtle autophagy alterations can cause pathology to accumulate over time, that ageing shifts how the CNS relies on autophagy mechanisms or that a combination of the two occurs.

When we organize disease-associated genes by their putative sites of involvement along the autophagy pathway (FIG. 3a), models of autophagy dysfunction emerge. Cargo capture and autophagosome formation are more represented by ALS/FTD-causative genes (FIG. 3b); PD-associated mutations are found either at cargo capture or degradation (FIG. 3c); and late-onset AD (LOAD) susceptibility loci map onto later stages of the pathway involving autophagosome maturation and fusion with the lysosome (FIG. 3d). Additionally, genetic disruptions in the cargoes themselves (for example, huntingtin in HD; TAR DNA-binding protein 43 (TDP43), fused in sarcoma (FUS) and superoxide dismutase 1 (SOD1) in ALS and FTD/FTLD; α-synuclein in PD, DLB and MSA) can lead to pathology[152,153] as can mutations in autophagy adaptors (reviewed in REF.[154]) (TABLE 2) and other selective autophagy machinery.

At the level of cargo capture, mutations in autophagy-related proteins have been identified in patients with ALS, a disease characterized by degeneration of upper and lower motor neurons and subsequent muscle weakness, disability and death. Mutations in genes encoding p62 (REFS[155,156]), the mitophagy adaptor OPTN[157] or its partner TANK-binding kinase 1 (TBK1)[158] can cause rare, familial forms of ALS. The phosphorylation of OPTN and p62 by TBK1 has been shown to be required for the targeting of these adaptors to the autophagosome[159] (FIG. 3b). TBK1 is also a component of the innate immune response by regulating type I interferon signalling[160], connecting autophagy and innate immunity at the mitochondrion[161]. Recent studies have shown that motor neuron autophagy loss[97], *Tbk1* heterozygosity[162] or ALS-linked *TBK1* mutations[163] modify pathogenesis and progression of the SOD1-G93A mouse model of ALS such that disease onset is accelerated but lifespan is extended. These suggest that autophagy is important for maintenance of the neuromuscular junction but, later in disease course, could contribute to degeneration via aberrant autophagy and inflammation in motor neurons, interneurons and glia. These studies further emphasize the need to understand how autophagy functions across the discrete cell types of the CNS in physiological and pathological states.

Upper and lower motor neurons

Upper motor neurons project from the motor cortex of the cerebrum and brainstem to form the corticospinal, corticobulbar and other tracts. lower motor neurons project from the spinal cord to effector muscles to carry out a movement.

It is increasingly clear that ALS exists on an aetiological and clinicopathological spectrum with FTD, as the hexanucleotide (G_4C_2) repeat expansion at the *C9ORF72* locus is the most common cause of sporadic and familial ALS and FTD[164,165]. Studies of the C9ORF72 protein strongly suggest that it plays a regulatory role in autophagosome biogenesis and membrane trafficking events[166,167] (FIG. 3b). With its expansion creating dipeptide repeat protein products that can aggregate, the hexanucleotide repeat may serve as a second hit with proteotoxic stress, further exacerbating the impact of diminished autophagosome biogenesis[168,169]. Mutations in *TARDBP*, the gene encoding TDP43, can cause ALS/FTD[153,170–172], and TDP43-positive aggregates are observed in patients with and without *TARDBP* mutations[173,174]. Not only can these TDP43-positive inclusions be substrates for autophagy[175,176] but TDP43 itself has been implicated in the transcriptional regulation of autophagy-associated genes through its function as a DNA/RNA-binding protein and splicing regulator[177–179]. Together, both *C9ORF72*-associated and *TARDBP*-associated ALS/FTD show potential feedforward loops related to autophagic substrate burden and regulation. Examining the many disease-causing mutations in ALS, a model for autophagy dysfunction as a key pathogenic player can be drawn (FIG. 3b).

In PD, progressive loss of dopaminergic neurons in the substantia nigra pars compacta leads to the clinical features of tremor, rigidity, bradykinesia and postural instability. Hypotheses of autophagy dysfunction in PD have been supported by human pathology and genetic studies, which implicate presynaptic autophagy and the autophagic turnover of certain cargoes (α-synuclein and mitochondria) in pathophysiology[180] (FIG. 3c). The first gene implicated in PD was *SNCA*, which encodes α-synuclein[181,182]. α-Synuclein is

not only a cargo for autophagy but has been associated with multiple vesicular trafficking events, including autophagy and lysosomal function[183–185]. Additionally, α-synuclein often accumulates in Lewy bodies in brain tissue from patients with idiopathic PD as well as other synucleinopathies[186,187]. Other autophagy-related proteins implicated in disease are PTEN-induced kinase 1 (PINK1) and parkin (encoded by *PRKN*) (BOX 1; FIG. 3c). Mutations in either *PINK1* or *PRKN* can cause rare, autosomal recessive, early-onset PD[188,189], and have been connected to mitophagy by cell-based and invertebrate studies[190–192]. Notably, loss of these PD-causative genes in mice or rats does not result in defective mitophagy or recapitulate disease[193,194] (TABLE 1), raising the question of if and how PINK1-dependent or parkin-dependent mitophagy contributes to PD in humans. In mice, combined burdens of *Pink1* or *Prkn* deletion with either peripheral inflammation[195] or mitochondrial DNA mutational stress[196] produce PD-like dopaminergic neuron loss and motor dysfunction, suggesting complex interactions between autophagy machinery, mitochondrial quality control and inflammation. The role of these interactions in human neurodegenerative disease remains to be fully explored.

As described above, the PD-associated LRRK2 and synaptojanin (encoded by *SYNJ1*) (FIG. 3c) have been shown to regulate presynaptic autophagy[92,93]. Induced pluripotent stem cell-derived neurons from patients with mutated *SYNJ1* exhibit accumulation of new autophagic structures that cannot mature[93]. These studies suggest potential convergent mechanisms of disease in LRRK2-associated and synaptojanin-associated PD[84,91]. How the proposed mechanisms of mitophagy dysfunction and presynaptic autophagy dysfunction may interrelate, and their importance in idiopathic or other familial forms of PD, remain to be determined.

Later in the autophagy pathway, defects in autophagosome–lysosome fusion and acidification are also associated with disease, particularly in PD (FIG. 3c) and AD (FIG. 3d). Genes encoding various lysosomal components can cause PD and AD in addition to lysosomal storage disorders that have neurological manifestations[197]. The genetic contributors to AD are complex but some insight is provided when stratifying by age of onset. In contrast to the genetic risk of early-onset AD, characterized by a small number of highly penetrant mutations, LOAD appears to result from multiple low penetrance mutations that may interact with one another and environmental factors. Interestingly, LOAD risk variants cluster around the endo-lysosomal system; for example, bridging integrator 1 (BIN1) and CD2-associated protein (CD2AP) seem to have roles in endosomal transport, while progranulin (GRN) and phospholipase D3 (PLD3) are localized to the lysosome[198] (FIG. 3d). Studies in AD suggest that impaired lysosome-mediated clearance is pathogenic, while autophagosome formation appears adequate[199–201]. The AD brain is characterized by pathological accumulation of extracellular amyloid-β plaques and intraneuronal neurofibrillary tau-containing tangles[202]. Although amyloid-β[203] and tau[204] are cargo for autophagy (FIG. 3d), with the former relying on autophagy machinery for secretion into the extracellular space and the latter using autophagy for degradation, the recently identified role of microglial LANDO in AD pathology[117] must be considered when investigating early autophagic events in AD.

In addition to its dysfunction being central to disease pathogenesis, another area in which autophagy is often examined is for its ability to eliminate aggregated proteins. From pathological studies in humans, a hallmark of many neurodegenerative diseases is the accumulation of protein aggregates and/or dysfunctional organelles (for example, mitochondria) in vulnerable cell types[205]. Such proteins include, but are not limited to, amyloid-β and tau in AD; mutant huntingtin in HD; α-synuclein in PD, DLB and MSA; tau, TDP43 and FUS in FTD/FTLD; and p62, TDP43, FUS and SOD1 in ALS (FIG. 3). This has led to the pathophysiological designation of these disorders as proteinopathies, with the hypothesis that aggregates disrupt critical cellular processes and that dysfunctional catabolism contributes to disease. In support of this, the aggrephagy adaptor Alfy has been shown to modify HD pathogenesis in model systems and cells from patients with this disease[58]. The identification of Alfy as an adaptor protein for aggrephagy allows for studies segregating the contribution of inclusions, rather than non-aggregated mutant protein, to pathogenesis. Consistent with cell-based studies[59], Alfy has been shown to be essential for aggregate turnover in the cells of the adult brain. Loss of function in a mouse model of HD led to an accelerated appearance of aggregated proteins and an accelerated onset of motor dysfunction. Notably, it did not accelerate cell death, suggesting that, at least in HD, the proteinopathy can modify disease onset but not necessarily impact neurodegeneration[58]. Alternatively, given that this model does not have a neurodegenerative phenotype, increasing aggregation was not sufficient to produce vulnerability to degenerative events. In cell-based studies, Alfy gain of function has been shown to increase aggregate clearance[59]. Whether this outcome will be replicated in vivo and whether it might bring phenotypic benefit remains to be seen, especially given how autophagy dysfunction is a feature associated with all proteinopathies.

Conclusions

Taken together, autophagy is a fundamental pathway for neural cells that is used to maintain homeostasis and augments in response to stress. It is essential in the developing and ageing brain, and the broad use of this pathway has complicated our understanding of how it contributes to disease. Despite the many gains that have been made over the past two decades, there is still much to be learned from further study of CNS autophagy. A prominent question that arises from observations across human genetics and model systems is how the disruption of this single pathway can lead to a wide array of diseases that primarily impact the CNS. We hypothesize that the answer might lie not in the gross disruption of the pathway per se but, rather, on unique combinations of factors: which cell types exhibit autophagy dysfunction, where in the cell the defects are occurring, which cargoes might be especially affected and where in the pathway the dysfunction is observed. Thus far, insights gained from the broad range of diseases that arise from mutations in genes encoding selective autophagy adaptor proteins suggest that the turnover of specific cargoes may contribute to the unique pathological signature of each disease. However, since we still understand very little about how the vast majority of cells in the CNS rely on this fundamental pathway, our insight remains limited.

As our knowledge of this fundamental pathway grows, we will benefit from investigating the areas described here to untangle the complex roles of CNS autophagy in health and

disease. Considering therapeutic approaches aimed at augmenting autophagic degradation, it is increasingly clear that we must better understand the interrelationship between the different selective pathways, if any should exist, and the limiting factors for autophagy, including how different cell types require autophagy, and which cargoes are particularly sensitive to autophagy dysfunction. For example, would the enhanced turnover of aggregated proteins diminish the turnover of mitochondria or ER, or are the pathways physically or mechanistically distinct enough that each selective pathway might be independently manipulated? By integrating the progress made thus far, enhanced resolution will provide a more comprehensive framework for understanding autophagy in basal physiology of the CNS, its contribution to pathology in developmental and adult-onset neurodegenerative disorders, and opportunities for therapeutic intervention.

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References

1. Stavoe AKH & Holzbaur ELF Autophagy in neurons. Annu. Rev. Cell Dev. Biol 35, 477–500 (2019). [PubMed: 31340124]
2. Dixon JS “Phagocytic” lysosomes in chromatolytic neurones. Nature 215, 657–658 (1967). [PubMed: 6050233]
3. Holtzman E & Novikoff AB Lysosomes in the rat sciatic nerve following crush. J. Cell Biol 27, 651–669 (1965). [PubMed: 5885432]
4. De Duve C & Wattiaux R Functions of lysosomes. Annu. Rev. Physiol 28, 435–492 (1966). [PubMed: 5322983] After discovering the lysosome, De Duve created the term ‘autophagy’ and, along with Novikoff and others, laid the foundation for the subsequent decades of study of this process in the CNS.
5. Adhami F et al. Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. Am. J. Pathol 169, 566–583 (2006). [PubMed: 16877357]
6. Koike M et al. Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. Am. J. Pathol 172, 454–469 (2008). [PubMed: 18187572]
7. Young JE, Martinez RA & La Spada AR Nutrient deprivation induces neuronal autophagy and implicates reduced insulin signaling in neuroprotective autophagy activation. J. Biol. Chem 284, 2363–2373 (2009). [PubMed: 19017649]
8. Tsukada M & Ohsumi Y Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett 333, 169–174 (1993). [PubMed: 8224160] By screening mutant yeast for autophagy defects, Tsukada and Ohsumi started the investigation of the molecular machinery underpinning autophagy.
9. Mizushima N et al. A protein conjugation system essential for autophagy. Nature 395, 395–398 (1998). [PubMed: 9759731]
10. Komatsu M et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441, 880–884 (2006). [PubMed: 16625205] This landmark study, together with Hara et al. (2006), established that autophagy is essential for CNS physiology and that its dysfunction leads to degenerative changes.
11. Hara T et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441, 885–889 (2006). [PubMed: 16625204]

12. Yue Z et al. A novel protein complex linking the delta 2 glutamate receptor and autophagy: implications for neurodegeneration in lurcher mice. Neuron 35, 921–933 (2002). [PubMed: 12372286]
13. Komatsu M et al. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc. Natl Acad. Sci. USA 104, 14489–14494 (2007). [PubMed: 17726112]
14. Ahmed I et al. Development and characterization of a new Parkinson's disease model resulting from impaired autophagy. J. Neurosci 32, 16503–16509 (2012). [PubMed: 23152632]
15. Friedman LG et al. Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of α-synuclein and LRRK2 in the brain. J. Neurosci 32, 7585–7593 (2012). [PubMed: 22649237]
16. Inoue K et al. Coordinate regulation of mature dopaminergic axon morphology by macroautophagy and the PTEN Signaling pathway. PLoS Genet 9, e1003845 (2013). [PubMed: 24098148]
17. Sato S et al. Loss of autophagy in dopaminergic neurons causes Lewy pathology and motor dysfunction in aged mice. Sci. Rep 8, 2813 (2018). [PubMed: 29434298]
18. Ohsumi Y Historical landmarks of autophagy research. Cell Res. 24, 9–23 (2014). [PubMed: 24366340]
19. Parzych KR & Klionsky DJ An overview of autophagy: morphology, mechanism, and regulation. Antioxid. Redox Signal 20, 460–473 (2014). [PubMed: 23725295]
20. Schuck S Microautophagy — distinct molecular mechanisms handle cargoes of many sizes. J. Cell Sci 133, jcs246322 (2020). [PubMed: 32907930]
21. Kaushik S & Cuervo AM The coming of age of chaperone-mediated autophagy. Nat. Rev. Mol. Cell Biol 19, 365–381 (2018). [PubMed: 29626215]
22. Bourdenx M et al. Chaperone-mediated autophagy prevents collapse of the neuronal metastable proteome. Cell 184, 2696–2714.e25 (2021). [PubMed: 33891876]
23. Cuervo AM & Wong E Chaperone-mediated autophagy: roles in disease and aging. Cell Res. 24, 92–104 (2014). [PubMed: 24281265]
24. Dikic I & Elazar Z Mechanism and medical implications of mammalian autophagy. Nat. Rev. Mol. Cell Biol 19, 349–364 (2018). [PubMed: 29618831]
25. Morishita H & Mizushima N Diverse cellular roles of autophagy. Annu. Rev. Cell Dev. Biol 35, 453–475 (2019). [PubMed: 31283377]
26. Nakatogawa H Mechanisms governing autophagosome biogenesis. Nat. Rev. Mol. Cell Biol 21, 439–458 (2020). [PubMed: 32372019]
27. He C & Klionsky DJ Regulation mechanisms and signaling pathways of autophagy. Annu. Rev. Genet 43, 67–93 (2010).
28. Yamamoto A, Cremona ML & Rothman JE Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. J. Cell Biol 172, 719–731 (2006). [PubMed: 16505167]
29. Lynch-Day MA & Klionsky DJ The Cvt pathway as a model for selective autophagy. FEBS Lett 584, 1359–1366 (2010). [PubMed: 20146925]
30. Zaffagnini G & Martens S Mechanisms of selective autophagy. J. Mol. Biol 428, 1714–1724 (2016). [PubMed: 26876603]
31. Turco E, Fracchiolla D & Martens S Recruitment and activation of the ULK1/Atg1 kinase complex in selective autophagy. J. Mol. Biol 432, 123–134 (2020). [PubMed: 31351898]
32. Schütter M, Giavalisco P, Brodesser S & Graef M Local fatty acid channeling into phospholipid synthesis drives phagophore expansion during autophagy. Cell 180, 135–149 (2020). [PubMed: 31883797]
33. Ghanbarpour A, Valverde DP, Melia TJ & Reinisch KM A model for a partnership of lipid transfer proteins and scramblases in membrane expansion and organelle biogenesis. Proc. Natl Acad. Sci. USA 118, e2101562118 (2021). [PubMed: 33850023]
34. Zhen Y et al. ESCRT-mediated phagophore sealing during mitophagy. Autophagy 16, 826–841 (2019). [PubMed: 31366282]

35. Takahashi Y et al. An autophagy assay reveals the ESCRT-III component CHMP2A as a regulator of phagophore closure. Nat. Commun 9, 2855 (2018). [PubMed: 30030437]
36. Itakura E, Kishi-Itakura C & Mizushima N The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 151, 1256–1269 (2012). [PubMed: 23217709]
37. Aplin A, Jasionowski T, Tuttle DL, Lenk SE & Dunn WAJ Cytoskeletal elements are required for the formation and maturation of autophagic vacuoles. J. Cell Physiol 152, 458–466 (1992). [PubMed: 1506410]
38. Kabeya Y et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 19, 5720–5728 (2000). [PubMed: 11060023]
39. Mizushima N, Yamamoto A, Matsui M, Yoshimori T & Ohsumi Y In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol. Biol. Cell 15, 1101–1111 (2004). [PubMed: 14699058] This study created a mouse with fluorescently tagged LC3, a critical step in tool development to understand autophagic function in vivo.
40. Nath S et al. Lipidation of the LC3/GABARAP family of autophagy proteins relies on a membrane-curvature-sensing domain in Atg3. Nat. Cell Biol 16, 415–424 (2014). [PubMed: 24747438]
41. Nguyen TN et al. Atg8 family LC3/GABARAP proteins are crucial for autophagosome-lysosome fusion but not autophagosome formation during PINK1/Parkin mitophagy and starvation. J. Cell Biol 215, 857–874 (2016). [PubMed: 27864321]
42. Weidberg H et al. LC3 and GATE-16 N termini mediate membrane fusion processes required for autophagosome biogenesis. Dev. Cell 20, 444–454 (2011). [PubMed: 21497758]
43. Wang H, Bedford FK, Brandon NJ, Moss SJ & Olsen RW GABA(A)-receptor-associated protein links GABA(A) receptors and the cytoskeleton. Nature 397, 69–72 (1999). [PubMed: 9892355]
44. Klionsky DJ, Cueva R & Yaver DS Aminopeptidase I of Saccharomyces cerevisiae is localized to the vacuole independent of the secretory pathway. J. Cell Biol 119, 287–299 (1992). [PubMed: 1400574]
45. Rogov V, Dotsch V, Johansen T & Kirkin V Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. Mol. Cell 53, 167–178 (2014). [PubMed: 24462201]
46. Gubas A & Dikic I A guide to the regulation of selective autophagy receptors. FEBS J 289, 75–89 (2021). [PubMed: 33730405]
47. Kirkin V & Rogov VV A diversity of selective autophagy receptors determines the specificity of the autophagy pathway. Mol. Cell 76, 268–285 (2019). [PubMed: 31585693]
48. Stolz A, Ernst A & Dikic I Cargo recognition and trafficking in selective autophagy. Nat. Cell Biol 16, 495–501 (2014). [PubMed: 24875736]
49. Kim BW, Kwon DH & Song HK Structure biology of selective autophagy receptors. BMB Rep 49, 73–80 (2016). [PubMed: 26698872]
50. Khaminets A, Behl C & Dikic I Ubiquitin-dependent and independent signals in selective autophagy. Trends Cell Biol 26, 6–16 (2016). [PubMed: 26437584]
51. Bjørkøy G et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J. Cell Biol 171, 603–614 (2005). [PubMed: 16286508]
52. Palikaras K, Lionaki E & Tavernarakis N Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat. Cell Biol 20, 1013–1022 (2018). [PubMed: 30154567]
53. Nthiga TM et al. Regulation of Golgi turnover by CALCOCO1-mediated selective autophagy. J. Cell Biol 220, e202006128(2021). [PubMed: 33871553]
54. Yamamoto A Go for the Golgi: eating selectively with Calcoco1. J. Cell Biol 220, e202105005 (2021). [PubMed: 33999115]
55. Narendra D, Kane LA, Hauser DN, Fearnley IM & Youle RJ p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy 6, 1090–1106 (2010). [PubMed: 20890124]

56. Komatsu M et al. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131, 1149–1163 (2007). [PubMed: 18083104] This study separated autophagy disruption from the development of inclusion bodies, indicating that the regulation of neuronal health by autophagy goes beyond proteostasis alone.
57. Kurashige T et al. Optineurin defects cause TDP43-pathology with autophagic vacuolar formation. Neurobiol. Dis 148, 105215 (2021). [PubMed: 33296728]
58. Fox LM et al. Huntington's disease pathogenesis is modified in vivo by Alfy/Wdfy3 and selective macroautophagy. Neuron 105, 813–821 (2020). [PubMed: 31899071] Investigating the role of protein aggregates in neurodegenerative pathogenesis, this study found that the autophagy adaptor Alfy confers resistance to HD-like phenotypes.
59. Filimonenko M et al. The selective macroautophagic degradation of aggregated proteins requires the PI3P-binding protein Alfy. Mol. Cell 38, 265–279 (2010). [PubMed: 20417604]
60. Dhingra A, Alexander D, Reyes-Reveles J, Sharp R & Boesze-Battaglia K Microtubule-associated protein 1 light chain 3 (LC3) isoforms in RPE and retina. Adv. Exp. Med. Biol 1074, 609–616 (2018). [PubMed: 29721994]
61. Komatsu M et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J. Cell Biol 169, 425–434 (2005). [PubMed: 15866887]
62. Maday S & Holzbaur ELF Compartment-specific regulation of autophagy in primary neurons. J. Neurosci 36, 5933–5945 (2016). [PubMed: 27251616]
63. Kaushik S et al. Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance. Cell Metab. 14, 173–183 (2011). [PubMed: 21803288]
64. Nikoletopoulou V, Sidiropoulou K, Kallergi E, Dalezios Y & Tavernarakis N Modulation of autophagy by BDNF underlies synaptic plasticity. Cell Metab. 26, 230–242.e5 (2017). [PubMed: 28683289]
65. Tsvetkov AS et al. A small-molecule scaffold induces autophagy in primary neurons and protects against toxicity in a Huntington disease model. Proc. Natl Acad. Sci. USA 107, 16982–16987 (2010). [PubMed: 20833817]
66. Maday S & Holzbaur ELF Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway. Dev. Cell 30, 71–85 (2014). [PubMed: 25026034] This significant study described neuronal compartmentalization of autophagosome biogenesis, maturation and fusion to the lysosome.
67. Hernandez D et al. Regulation of presynaptic neurotransmission by macroautophagy. Neuron 74, 277–284 (2012). [PubMed: 22542182] This study, focusing on dopaminergic neurons, established that autophagy modulates presynaptic structure and function.
68. Tang G et al. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron 83, 1131–1143 (2014). [PubMed: 25155956]
69. Lieberman OJ et al. mTOR suppresses macroautophagy during striatal postnatal development and is hyperactive in mouse models of autism spectrum disorders. Front. Cell Neurosci 14, 70 (2020). [PubMed: 32296308]
70. Khobrekar NV, Quintremil S, Dantas TJ & Vallee RB The Dynein adaptor RILP controls neuronal autophagosome biogenesis, transport, and clearance. Dev. Cell 53, 141–153 (2020). [PubMed: 32275887]
71. Yamamoto A & Yue Z Autophagy and its normal and pathogenic states in the brain. Annu. Rev. Neurosci 37, 55–78 (2014). [PubMed: 24821313]
72. Zhong Y et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. Nat. Cell Biol 11, 468–476 (2009). [PubMed: 19270693] Together with Matsunaga et al. (2009), this study provided critical insight into the molecular regulation of autophagy versus other membrane fates by upstream lipid kinase complexes.
73. Tooze SA & Yoshimori T The origin of the autophagosomal membrane. Nat. Cell Biol 12, 831–835 (2010). [PubMed: 20811355]
74. Itakura E, Kishi C, Inoue K & Mizushima N Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. Mol. Biol. Cell 19, 5360–5372 (2008). [PubMed: 18843052]

75. Matsunaga K et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat. Cell Biol 11, 385–396 (2009). [PubMed: 19270696]
76. Lee S, Sato Y & Nixon RA Lysosomal proteolysis inhibition selectively disrupts axonal transport of degradative organelles and causes an Alzheimer's-like axonal dystrophy. J. Neurosci 31, 7817–7830 (2011). [PubMed: 21613495]
77. Maday S, Wallace KE & Holzbaur ELF Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. J. Cell Biol 196, 407–417 (2012). [PubMed: 22331844]
78. Cheng XT, Zhou B, Lin MY, Cai Q & Sheng ZH Axonal autophagosomes recruit dynein for retrograde transport through fusion with late endosomes. J. Cell Biol 209, 377–386 (2015). [PubMed: 25940348]
79. Shehata M, Matsumura H, Okubo-Suzuki R, Ohkawa N & Inokuchi K Neuronal stimulation induces autophagy in hippocampal neurons that is involved in AMPA receptor degradation after chemical long-term depression. J. Neurosci 32, 10413–10422 (2012). [PubMed: 22836274]
80. Di Malta C, Fryer JD, Settembre C & Ballabio A Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder. Proc. Natl Acad. Sci. USA 109, 2334–2342 (2012).
81. Bankston AN et al. Autophagy is essential for oligodendrocyte differentiation, survival, and proper myelination. Glia 67, 1745–1759 (2019). [PubMed: 31162728]
82. Okerlund ND et al. Bassoon controls presynaptic autophagy through Atg5. Neuron 93, 897–913 (2017). [PubMed: 28231469]
83. Hoffmann-Conaway S et al. Parkin contributes to synaptic vesicle autophagy in Bassoon-deficient mice. eLife 9, e56590 (2020). [PubMed: 32364493]
84. Vijayan V & Verstreken P Autophagy in the presynaptic compartment in health and disease. J. Cell Biol 216, 1895–1906 (2017). [PubMed: 28515275]
85. Sumitomo A et al. Ulk2 controls cortical excitatory–inhibitory balance via autophagic regulation of p62 and GABAA receptor trafficking in pyramidal neurons. Hum. Mol. Genet 27, 3165–3176 (2018). [PubMed: 29893844]
86. Nikoletopoulou V & Tavernarakis N Regulation and roles of autophagy at synapses. Trends Cell Biol 28, 646–661 (2018). [PubMed: 29731196]
87. Glatigny M et al. Autophagy is required for memory formation and reverses age-related memory decline. Curr. Biol 29, 435–448 (2019). [PubMed: 30661803]
88. Shen H, Zhu H, Panja D, Gu Q & Li Z Autophagy controls the induction and developmental decline of NMDAR-LTD through endocytic recycling. Nat. Commun 11, 2979 (2020). [PubMed: 32532981]
89. Compans B et al. NMDAR-dependent long-term depression is associated with increased short term plasticity through autophagy mediated loss of PSD-95. Nat. Commun 12, 2849 (2021). [PubMed: 33990590]
90. Pan Y et al. Neuronal activity recruits the CRTC1/CREB axis to drive transcription-dependent autophagy for maintaining late-phase LTD. Cell Rep. 36, 109398 (2021). [PubMed: 34289350]
91. Birdsall V & Waites CL Autophagy at the synapse. Neurosci. Lett 697, 24–28 (2019). [PubMed: 29802916]
92. Soukup SF et al. A LRRK2-dependent endophilina phosphoswitch is critical for macroautophagy at presynaptic terminals. Neuron 92, 829–844 (2016). [PubMed: 27720484]
93. Vanhauwaert R et al. The SAC1 domain in synaptojanin is required for autophagosome maturation at presynaptic terminals. EMBO J 36, 1392–1411 (2017). [PubMed: 28331029]
94. Lieberman OJ et al. Cell-type-specific regulation of neuronal intrinsic excitability by macroautophagy. eLife 9, e50843 (2020). [PubMed: 31913125]
95. Hirabayashi Y et al. ER-mitochondria tethering by PDZD8 regulates Ca^{2+} dynamics in mammalian neurons. Science 358, 623–630 (2017). [PubMed: 29097544]
96. Kuijpers M et al. Neuronal autophagy regulates presynaptic neurotransmission by controlling the axonal endoplasmic reticulum. Neuron 109, 299–313.e9 (2021). [PubMed: 33157003] This study linked ER-phagy to neurotransmission through the control of calcium stores in the ER.

97. Rudnick ND et al. Distinct roles for motor neuron autophagy early and late in the SOD1 G93A mouse model of ALS. Proc. Natl Acad. Sci. USA 114, E8294–E8303 (2017). [PubMed: 28904095]

98. Nishiyama J, Miura E, Mizushima N, Watanabe M & Yuzaki M Aberrant membranes and double-membrane structures accumulate in the axons of Atg5-null Purkinje cells before neuronal death. Autophagy 3, 591–596 (2007). [PubMed: 17912025]

99. Tang G, Yue Z, Tallóczy Z & Goldman JE Adaptive autophagy in Alexander disease-affected astrocytes. Autophagy 4, 701–703 (2008).

100. Manzoni C et al. mTOR independent regulation of macroautophagy by leucine rich repeat kinase 2 via Beclin-1. Sci. Rep 6, 35106 (2016). [PubMed: 27731364]

101. Ulland TK et al. TREM2 maintains microglial metabolic fitness in Alzheimer's disease. Cell 170, 649–663 (2017). [PubMed: 28802038]

102. Jänen SB, Chaachouay H & Richter-Landsberg C Autophagy is activated by proteasomal inhibition and involved in aggresome clearance in cultured astrocytes. Glia 58, 1766–1774 (2010). [PubMed: 20645412]

103. Schwarz L, Goldbaum O, Bergmann M, Probst-Cousin S & Richter-Landsberg C Involvement of macroautophagy in multiple system atrophy and protein aggregate formation in oligodendrocytes. J. Mol. Neurosci 47, 256–266 (2012). [PubMed: 22411133]

104. Cho MH et al. Autophagy in microglia degrades extracellular β-amyloid fibrils and regulates the NLRP3 inflammasome. Autophagy 10, 1761–1775 (2014). [PubMed: 25126727]

105. Choi I et al. Microglia clear neuron-released α-synuclein via selective autophagy and prevent neurodegeneration. Nat. Commun 11, 1386 (2020). [PubMed: 32170061]

106. Motori E et al. Inflammation-induced alteration of astrocyte mitochondrial dynamics requires autophagy for mitochondrial network maintenance. Cell Metab. 18, 844–859 (2013). [PubMed: 24315370]

107. Sheng R et al. Autophagy activation is associated with neuroprotection in a rat model of focal cerebral ischemic preconditioning. Autophagy 6, 482–494 (2010). [PubMed: 20400854]

108. Ni Y et al. RIP1K contributes to neuronal and astrocytic cell death in ischemic stroke via activating autophagic-lysosomal pathway. Neuroscience 371, 60–74 (2018). [PubMed: 29102662]

109. Ohri SS et al. Blocking autophagy in oligodendrocytes limits functional recovery after spinal cord injury. J. Neurosci 38, 5900–5912 (2018). [PubMed: 29793971]

110. Sun LO et al. Spatiotemporal control of CNS myelination by oligodendrocyte programmed cell death through the TFEB-PUMA axis. Cell 175, 1811–1826 (2018). [PubMed: 30503207]

111. Smith CM, Mayer JA & Duncan ID Autophagy promotes oligodendrocyte survival and function following dysmyelination in a long-lived myelin mutant. J. Neurosci 33, 8088–8100 (2013). [PubMed: 23637198]

112. Lebrun-Julien F et al. Balanced mTORC1 activity in oligodendrocytes is required for accurate CNS myelination. J. Neurosci 34, 8432–8448 (2014). [PubMed: 24948799]

113. Kim HJ et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. Mol. Psychiatry 22, 1576–1584 (2017). [PubMed: 27400854]

114. Lucin KM et al. Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease. Neuron 79, 873–886 (2013). [PubMed: 24012002]

115. Shibuya Y, Chang CC, Huang LH, Bryleva EY & Chang TY Inhibiting ACAT1/SOAT1 in microglia stimulates autophagy-mediated lysosomal proteolysis and increases Aβ1–42 clearance. J. Neurosci 34, 14484–14501 (2014). [PubMed: 25339759]

116. Wang Y et al. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell 160, 1061–1071 (2015). [PubMed: 25728668]

117. Heckmann BL et al. LC3-associated endocytosis facilitates β-amyloid clearance and mitigates neurodegeneration in murine Alzheimer's disease. Cell 178, 536–551 (2019). [PubMed: 31257024] This work identified an endocytic pathway involving a subset of autophagy machinery (LANDO) in microglia, emphasizing the need to consider non-autophagic functions of such machinery in the study and interpretation of CNS autophagy.

118. Iadecola C The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. Neuron 96, 17–42 (2017). [PubMed: 28957666]
119. Wang X et al. An updated review of autophagy in ischemic stroke: from mechanisms to therapies. Exp. Neurol 340, 113684 (2021). [PubMed: 33676918]
120. Yang Z et al. Autophagy protects the blood-brain barrier through regulating the dynamic of claudin-5 in short-term starvation. Front. Physiol 10, 2 (2019). [PubMed: 30713499]
121. Zhang X et al. Autophagy induced by oxygen-glucose deprivation mediates the injury to the neurovascular unit. Med. Sci. Monit 25, 1373–1382 (2019). [PubMed: 30787267]
122. Kortekaas R et al. Blood–brain barrier dysfunction in parkinsonian midbrain in vivo. Ann. Neurol 57, 176–179 (2005). [PubMed: 15668963]
123. Sweeney MD, Ayyadurai S & Zlokovic BV Pericytes of the neurovascular unit: key functions and signaling pathways. Nat. Neurosci 19, 771–783 (2016). [PubMed: 27227366]
124. Muoio V, Persson PB & Sendeski MM The neurovascular unit–concept review. Acta Physiol. 210, 790–798 (2014).
125. Kim M et al. Mutation in ATG5 reduces autophagy and leads to ataxia with developmental delay. eLife 5, e12245 (2016). [PubMed: 26812546]
126. Xu J et al. A variant of the autophagy-related 5 gene is associated with child cerebral palsy. Front. Cell Neurosci 11, 407 (2017). [PubMed: 29326554]
127. Hentges KE et al. FRAP/mTOR is required for proliferation and patterning during embryonic development in the mouse. Proc. Natl Acad. Sci. USA 98, 13796–13801 (2001). [PubMed: 11707573]
128. Hoeffer CA et al. Removal of FKBP12 enhances mTOR-Raptor interactions, LTP, memory, and perseverative/repetitive behavior. Neuron 60, 832–845 (2008). [PubMed: 19081378]
129. Collier JJ et al. Developmental consequences of defective ATG7-mediated autophagy in humans. N. Engl. J. Med 384, 2406–2417 (2021). [PubMed: 34161705]
130. Lv X et al. The crucial role of Atg5 in cortical neurogenesis during early brain development. Sci. Rep 4, 6010 (2014). [PubMed: 25109817]
131. Wang B et al. The autophagy-inducing kinases, ULK1 and ULK2, regulate axon guidance in the developing mouse forebrain via a noncanonical pathway. Autophagy 14, 796–811 (2014).
132. Dragich JM et al. Autophagy linked FYVE (Alfy/WDFY3) is required for establishing neuronal connectivity in the mammalian brain. eLife 5, e14810 (2016). [PubMed: 27648578]
133. Orosco LA et al. Loss of Wdfy3 in mice alters cerebral cortical neurogenesis reflecting aspects of the autism pathology. Nat. Commun 5, 4692 (2014). [PubMed: 25198012]
134. Coupé B et al. Loss of autophagy in pro-opiomelanocortin neurons perturbs axon growth and causes metabolic dysregulation. Cell Metab. 15, 247–255 (2012). [PubMed: 22285542]
135. Joo JH et al. The noncanonical role of ULK/ATG1 in ER-to-Golgi trafficking is essential for cellular homeostasis. Mol. Cell 62, 491–506 (2016). [PubMed: 27203176]
136. Lieberman OJ, McGuirt AF, Tang G & Sulzer D Roles for neuronal and glial autophagy in synaptic pruning during development. Neurobiol. Dis 122, 49–63 (2019). [PubMed: 29709573]
137. Hill SE & Colón-Ramos DA The journey of the synaptic autophagosome: a cell biological perspective. Neuron 105, 961–973 (2020). [PubMed: 32191859]
138. Marsh D & Dragich JM Autophagy in mammalian neurodevelopment and implications for childhood neurological disorders. Neurosci. Lett 697, 29–33 (2019). [PubMed: 29665429]
139. Yan J, Porch MW, Court-Vazquez B, Bennett MVL & Zukin RS Activation of autophagy rescues synaptic and cognitive deficits in fragile X mice. Proc. Natl Acad. Sci. USA 115, E9707–E9716 (2018). [PubMed: 30242133]
140. Cullup T et al. Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. Nat. Genet 45, 83–87 (2013). [PubMed: 23222957]
141. Byrne S et al. EPG5-related Vici syndrome: a paradigm of neurodevelopmental disorders with defective autophagy. Brain 139, 765–781 (2016). [PubMed: 26917586]
142. Zhao YG, Zhao H, Sun H & Zhang H Role of Epg5 in selective neurodegeneration and Vici syndrome. Autophagy 9, 1258–1262 (2013). [PubMed: 23674064]

143. Wang Z et al. The vici syndrome protein EPG5 Is a Rab7 effector that determines the fusion specificity of autophagosomes with late endosomes/lysosomes. Mol. Cell 63, 781–795 (2016). [PubMed: 27588602]

144. Haack TB et al. Exome sequencing reveals de novo WDR45 mutations causing a phenotypically distinct, X-linked dominant form of NBIA. Am. J. Hum. Genet 91, 1144–1149 (2012). [PubMed: 23176820]

145. Saitsu H et al. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat. Genet 45, 445–459 (2013). [PubMed: 23435086]

146. Polson HE et al. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy 6, 506–522 (2010). [PubMed: 20505359]

147. Thomas AC et al. Mutations in SNX14 cause a distinctive autosomal-recessive cerebellar ataxia and intellectual disability syndrome. Am. J. Hum. Genet 95, 611–621 (2014). [PubMed: 25439728]

148. Akizu N et al. Biallelic mutations in SNX14 cause a syndromic form of cerebellar atrophy and lysosome-autophagosome dysfunction. Nat. Genet 47, 528–534 (2015). [PubMed: 25848753]

149. Ebrahimi-Fakhari D et al. Congenital disorders of autophagy: an emerging novel class of inborn errors of neuro-metabolism. Brain 139, 317–337 (2016). [PubMed: 26715604]

150. Renvoisé B et al. Lysosomal abnormalities in hereditary spastic paraplegia types SPG15 and SPG11. Ann. Clin. Transl. Neurol 1, 379–389 (2014). [PubMed: 24999486]

151. Gstrein T et al. Mutations in Vps15 perturb neuronal migration in mice and are associated with neurodevelopmental disease in humans. Nat. Neurosci 21, 207–217 (2018). [PubMed: 29311744]

152. Taylor JP, Hardy J & Fischbeck KH Toxic proteins in neurodegenerative disease. Science 296, 1991–1995 (2002). [PubMed: 12065827]

153. Lattante S, Rouleau GA & Kabashi E TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. Hum. Mutat 34, 812–826 (2018).

154. Deng Z et al. Autophagy receptors and neurodegenerative diseases. Trends Cell Biol 27, 491–504 (2017). [PubMed: 28169082]

155. Fecto F et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch. Neurol 68, 1440–1446 (2011). [PubMed: 22084127]

156. Teyssou E et al. Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: genetics and neuropathology. Acta Neuropathol 125, 511–522 (2013). [PubMed: 23417734]

157. Maruyama H et al. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 465, 223–226 (2010). [PubMed: 20428114] This work showed that mutations in the adaptor protein OPTN can cause ALS and, in cell-based studies of the mutations, demonstrated defects in proteostasis and inflammation.

158. Cirulli ET et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 347, 1436–1441 (2015). [PubMed: 25700176]

159. Richter B et al. Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc. Natl Acad. Sci. USA 113, 4039–4044 (2016). [PubMed: 27035970]

160. Fitzgerald KA et al. IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. Nat. Immunol 4, 491–496 (2003). [PubMed: 12692549]

161. West AP, Shadel GS & Ghosh S Mitochondria in innate immune responses. Nat. Rev. Immunol 11, 389–402 (2011). [PubMed: 21597473]

162. Brenner D et al. Heterozygous Tbk1 loss has opposing effects in early and late stages of ALS in mice. J. Exp. Med 216, 267–278 (2019). [PubMed: 30635357]

163. Gerbino V et al. The Loss of TBK1 kinase activity in motor neurons or in all cell types differentially impacts ALS disease progression in SOD1 mice. Neuron 106, 789–805 (2020). [PubMed: 32220666]

164. Renton AE et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268 (2011). [PubMed: 21944779]

165. DeJesus-Hernandez M et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256 (2011). [PubMed: 21944778]
166. Webster CP et al. The C9orf72 protein interacts with Rab1a and the ULK1 complex to regulate initiation of autophagy. EMBO J 35, 1656–1676 (2016). [PubMed: 27334615]
167. Farg MA et al. C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking. Hum. Mol. Genet 23, 3579–3595 (2017).
168. Boivin M et al. Reduced autophagy upon C9ORF72 loss synergizes with dipeptide repeat protein toxicity in G4C2 repeat expansion disorders. EMBO J 39, e100574 (2020). [PubMed: 31930538]
169. Zhu Q et al. Reduced C9ORF72 function exacerbates gain of toxicity from ALS/FTD-causing repeat expansion in C9orf72. Nat. Neurosci 23, 615–624 (2020). [PubMed: 32284607]
170. Sreedharan J et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672 (2008). [PubMed: 18309045]
171. Van Deerlin VM et al. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol 7, 409–416 (2008). [PubMed: 18396105]
172. Borroni B et al. Mutation within TARDBP leads to frontotemporal dementia without motor neuron disease. Hum. Mutat 30, E974–E983 (2009). [PubMed: 19655382]
173. Mackenzie IR & Rademakers R The role of transactive response DNA-binding protein-43 in amyotrophic lateral sclerosis and frontotemporal dementia. Curr. Opin. Neurol 21, 693–700 (2008). [PubMed: 18989115]
174. Johnson BS et al. TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. J. Biol. Chem 284, 20329–20339 (2009). [PubMed: 19465477]
175. Scotter EL et al. Differential roles of the ubiquitin proteasome system and autophagy in the clearance of soluble and aggregated TDP-43 species. J. Cell Sci 127, 1263–1278 (2014). [PubMed: 24424030]
176. Barmada SJ et al. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. Nat. Chem. Biol 10, 677–685 (2014). [PubMed: 24974230]
177. Bose JK, Huang CC & Shen CK Regulation of autophagy by neuropathological protein TDP-43. J. Biol. Chem 286, 44441–44448 (2011). [PubMed: 22052911]
178. Xia Q et al. TDP-43 loss of function increases TFEB activity and blocks autophagosome-lysosome fusion. EMBO J 35, 121–142 (2016). [PubMed: 26702100]
179. Ling JP, Pletnikova O, Troncoso JC & Wong PC TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. Science 349, 650–655 (2015). [PubMed: 26250685]
180. Hou X, Watzlawik JO, Fiesel FC & Springer W Autophagy in Parkinson's disease. J. Mol. Biol 432, 2651–2672 (2020). [PubMed: 32061929]
181. Polymeropoulos MH et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276, 2045–2047 (1997). [PubMed: 9197268]
182. Singleton AB et al. alpha-Synuclein locus triplication causes Parkinson's disease. Science 302, 841 (2003). [PubMed: 14593171]
183. Abeliovich A & Gitler AD Defects in trafficking bridge Parkinson's disease pathology and genetics. Nature 539, 207–216 (2016). [PubMed: 27830778]
184. Singh PK & Muqit MMK Parkinson's: a disease of aberrant vesicle trafficking. Annu. Rev. Cell Dev. Biol 36, 237–264 (2020). [PubMed: 32749865]
185. Stojkovska I et al. Rescue of α-synuclein aggregation in Parkinson's patient neurons by synergistic enhancement of ER proteostasis and protein trafficking. Neuron 110, 436–451.e11 (2022). [PubMed: 34793693]
186. Pihlstrøm L et al. A comprehensive analysis of SNCA-related genetic risk in sporadic Parkinson disease. Ann. Neurol 84, 117–129 (2018). [PubMed: 30146727]
187. Spillantini MG et al. Alpha-synuclein in Lewy bodies. Nature 388, 839–840 (1997). [PubMed: 9278044]

188. Kitada T et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392, 605–608 (1998). [PubMed: 9560156]
189. Valente EM et al. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am. J. Hum. Genet 68, 895–900 (2001). [PubMed: 11254447]
190. Geisler S et al. The PINK1/Parkin-mediated mitophagy is compromised by PD-associated mutations. Autophagy 6, 871–878 (2010). [PubMed: 20798600]
191. Lazarou M et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature 524, 309–314 (2015). [PubMed: 26266977] This study mechanistically characterized the role of PD-associated PINK1 in mitophagy.
192. Pickrell AM & Youle RJ The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron 85, 257–273 (2015). [PubMed: 25611507]
193. Goldberg MS et al. Parkin-deficient mice exhibit nigrostriatal deficits but not loss of dopaminergic neurons. J. Biol. Chem 278, 43628–43635 (2003). [PubMed: 12930822]
194. Perez FA & Palmiter RD Parkin-deficient mice are not a robust model of parkinsonism. Proc. Natl Acad. Sci. USA 102, 2174–2179 (2005). [PubMed: 15684050]
195. Matheoud D et al. Intestinal infection triggers Parkinson's disease-like symptoms in Pink1$^{-/-}$ mice. Nature 571, 565–569 (2019). [PubMed: 31316206]
196. Sliter DA et al. Parkin and PINK1 mitigate STING-induced inflammation. Nature 561, 258–262 (2018). [PubMed: 30135585]
197. Lie PPY & Nixon RA Lysosome trafficking and signaling in health and neurodegenerative diseases. Neurobiol. Dis 122, 94–105 (2019). [PubMed: 29859318]
198. Van Acker ZP, Bretou M & Annaert W Endo-lysosomal dysregulations and late-onset Alzheimer's disease: impact of genetic risk factors. Mol. Neurodegener 14, 20 (2019). [PubMed: 31159836]
199. Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA & Nixon RA Properties of the endosomal-lysosomal system in the human central nervous system: disturbances mark most neurons in populations at risk to degenerate in Alzheimer's disease. J. Neurosci 16, 186–199 (1996). [PubMed: 8613784]
200. Nixon RA et al. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J. Neuropathol. Exp. Neurol 64, 113–122 (2005). [PubMed: 15751225] This ultrastructural study in cortices from patients provided key evidence of autophagy dysfunction in AD.
201. Boland B et al. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J. Neurosci 28, 6926–6937 (2008). [PubMed: 18596167]
202. Bloom GS Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. 71, 505–508 (2014). [PubMed: 24493463]
203. Nilsson P et al. Aβ secretion and plaque formation depend on autophagy. Cell Rep. 5, 61–69 (2013). [PubMed: 24095740]
204. Inoue K et al. Macroautophagy deficiency mediates age-dependent neurodegeneration through a phospho-tau pathway. Mol. Neurodegener 7, 48 (2012). [PubMed: 22998728]
205. Croce KR & Yamamoto A Dissolving the complex role aggregation plays in neurodegenerative disease. Mov. Disord 36, 1061–1069 (2021). [PubMed: 33755257]
206. Fimia GM et al. Ambra1 regulates autophagy and development of the nervous system. Nature 447, 1121–1125 (2007). [PubMed: 17589504]
207. Kuma A et al. The role of autophagy during the early neonatal starvation period. Nature 432, 1032–1036 (2004). [PubMed: 15525940]
208. Qu X et al. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 128, 931–946 (2007). [PubMed: 17350577]
209. Yue Z, Jin S, Yang C, Levine AJ & Heintz N Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc. Natl Acad. Sci. USA 100, 15077–15082 (2003). [PubMed: 14657337]

210. Kaushik S et al. Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Rep. 13, 258–265 (2012). [PubMed: 22249165]
211. Liang CC, Wang C, Peng X, Gan B & Guan JL Neural-specific deletion of FIP200 leads to cerebellar degeneration caused by increased neuronal death and axon degeneration. J. Biol. Chem 285, 3499–3509 (2010). [PubMed: 19940130]
212. Kurth I et al. Mutations in FAM134B, encoding a newly identified Golgi protein, cause severe sensory and autonomic neuropathy. Nat. Genet 41, 1179–1181 (2009). [PubMed: 19838196]
213. Khaminets A et al. Regulation of endoplasmic reticulum turnover by selective autophagy. Nature 522, 354–358 (2015). [PubMed: 26040720]
214. Oliveras-Salvá M, Van Rompuy AS, Heeman B, Van den Haute C & Baekelandt V Loss-of-function rodent models for parkin and PINK1. J. Parkinsons Dis 1, 229–251 (2011). [PubMed: 23939304]
215. Matsumoto G, Wada K, Okuno M, Kurosawa M & Nukina N Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. Mol. Cell 44, 279–289 (2011). [PubMed: 22017874]
216. Rea SL, Majcher V, Searle MS & Layfield R SQSTM1 mutations — bridging Paget disease of bone and ALS/FTLD. Exp. Cell Res 325, 27–37 (2014). [PubMed: 24486447]
217. Cuyvers E et al. Genetic variability in SQSTM1 and risk of early-onset Alzheimer dementia: a European early-onset dementia consortium study. Neurobiol. Aging 36, 2005.e15–2005.e22 (2015).
218. Komatsu M, Kageyama S & Ichimura Y p62/SQSTM1/A170: physiology and pathology. Pharmacol. Res 66, 457–462 (2012). [PubMed: 22841931]
219. Moscat J & Diaz-Meco MT p62 at the crossroads of autophagy, apoptosis, and cancer. Cell 137, 1001–1004 (2009). [PubMed: 19524504]
220. Moscat J, Karin M & Diaz-Meco MT p62 in cancer: signaling adaptor beyond autophagy. Cell 167, 606–609 (2016). [PubMed: 27768885]
221. Padman BS et al. LC3/GABARAPs drive ubiquitin-independent recruitment of Optineurin and NDP52 to amplify mitophagy. Nat. Commun 10, 408 (2019). [PubMed: 30679426]
222. Heo JM, Ordureau A, Paulo JA, Rinehart J & Harper JW The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. Mol. Cell 60, 7–20 (2015). [PubMed: 26365381]
223. Schwab C, Yu S, McGeer EG & McGeer PL Optineurin in Huntington's disease intranuclear inclusions. Neurosci. Lett 506, 149–154 (2012). [PubMed: 22085693]
224. Mankouri J et al. Optineurin negatively regulates the induction of IFNbeta in response to RNA virus infection. PLoS Pathog. 6, e1000778 (2010). [PubMed: 20174559]
225. Outlioua A, Pourcelot M & Arnoult D The role of optineurin in antiviral type I interferon production. Front. Immunol 9, 853 (2018). [PubMed: 29755463]
226. Odagiri S et al. Autophagic adapter protein NBR1 is localized in Lewy bodies and glial cytoplasmic inclusions and is involved in aggregate formation in α-synucleinopathy. Acta Neuropathol. 124, 173–186 (2012). [PubMed: 22484440]
227. Rué L et al. Brain region- and age-dependent dysregulation of p62 and NBR1 in a mouse model of Huntington's disease. Neurobiol. Dis 52, 219–228 (2013). [PubMed: 23295856]
228. Stessman HA et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. Nat. Genet 49, 515–526 (2017). [PubMed: 28191889]
229. Iossifov I et al. The contribution of de novo coding mutations to autism spectrum disorder. Nature 515, 216–221 (2014). [PubMed: 25363768]
230. Clausen TH et al. p62/SQSTM1 and ALFY interact to facilitate the formation of p62 bodies/ALIS and their degradation by autophagy. Autophagy 6, 330–344 (2010). [PubMed: 20168092]
231. Kim S et al. NDP52 associates with phosphorylated tau in brains of an Alzheimer disease mouse model. Biochem. Biophys. Res. Commun 454, 196–201 (2014). [PubMed: 25450380]
232. Fan S et al. The role of autophagy and autophagy receptor NDP52 in microbial infections. Int. J. Mol. Sci 21, 2008 (2020).

233. Till A et al. Autophagy receptor CALCOCO2/NDP52 takes center stage in Crohn disease. Autophagy 9, 1256–1257 (2013). [PubMed: 23820297]
234. Jin S & Cui J BST2 inhibits type I IFN (interferon) signaling by accelerating MAVS degradation through CALCOCO2-directed autophagy. Autophagy 14, 171–172 (2018). [PubMed: 29165031]
235. Sabaie H, Ahangar NK, Ghafouri-Fard S, Taheri M & Rezazadeh M Clinical and genetic features of PEHO and PEHO-Like syndromes: a scoping review. Biomed. Pharmacother 131, 110793 (2020). [PubMed: 33152950]
236. Wyant GA et al. NUFIP1 is a ribosome receptor for starvation-induced ribophagy. Science 360, 751–758 (2018). [PubMed: 29700228]
237. Bardoni B et al. NUFIP1 (nuclear FMRP interacting protein 1) is a nucleocytoplasmic shuttling protein associated with active synaptoneurosomes. Exp. Cell Res 289, 95–107 (2003). [PubMed: 12941608]
238. Chen Q et al. ATL3 is a tubular ER-phagy receptor for GABARAP-mediated selective autophagy. Curr. Biol 29, 846–855.e6 (2019). [PubMed: 30773365]
239. Kornak U et al. Sensory neuropathy with bone destruction due to a mutation in the membrane-shaping atlastin GTPase 3. Brain 137, 683–692 (2014). [PubMed: 24459106]
240. Neufeldt CJ et al. ER-shaping atlastin proteins act as central hubs to promote flavivirus replication and virion assembly. Nat. Microbiol 4, 2416–2429 (2019). [PubMed: 31636417]
241. Zou Y et al. Identification of rare RTN3 variants in Alzheimer's disease in Han Chinese. Hum. Genet 137, 141–150 (2018). [PubMed: 29356939]
242. Murayama KS et al. Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. Eur. J. Neurosci 24, 1237–1244 (2006). [PubMed: 16965550]
243. Kuang E et al. ER Ca^{2+} depletion triggers apoptotic signals for endoplasmic reticulum (ER) overload response induced by overexpressed reticulon 3 (RTN3/HAP). J. Cell Physiol 204, 549–559 (2005). [PubMed: 15799019]
244. Tang BL & Liou YC Novel modulators of amyloid-beta precursor protein processing. J. Neurochem 100, 314–323 (2007). [PubMed: 17241154]
245. Mo J, Chen J & Zhang B Critical roles of FAM134B in ER-phagy and diseases. Cell Death Dis. 11, 983 (2020). [PubMed: 33199694]
246. Chiramel AI, Dougherty JD, Nair V, Robertson SJ & Best SM FAM134B, the selective autophagy receptor for endoplasmic reticulum turnover, inhibits replication of ebola virus strains makona and mayinga. J. Infect. Dis 214 (Suppl. 3), S319–S325 (2016). [PubMed: 27511895]
247. Farré JC & Subramani S Mechanistic insights into selective autophagy pathways: lessons from yeast. Nat. Rev. Mol. Cell Biol 17, 537–552 (2016). [PubMed: 27381245]
248. Chu CT et al. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat. Cell Biol 15, 1197–1205 (2013). [PubMed: 24036476]
249. Wilkinson S Emerging principles of selective ER autophagy. J. Mol. Biol 432, 185–205 (2020). [PubMed: 31100386]
250. Lystad AH & Simonsen A Mechanisms and pathophysiological roles of the ATG8 conjugation machinery. Cells 8, 973 (2019).
251. Jean S & Kiger AA Classes of phosphoinositide 3-kinases at a glance. J. Cell Sci 127, 923–928 (2014). [PubMed: 24587488]
252. Simonsen A & Tooze SA Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. J. Cell Biol 186, 773–782 (2009). [PubMed: 19797076]
253. Funderburk SF, Wang QJ & Yue Z The Beclin 1–VPS34 complex–at the crossroads of autophagy and beyond. Trends Cell Biol. 20, 355–362 (2010). [PubMed: 20356743]
254. Tracey TJ, Steyn FJ, Wolvetang EJ & Ngo ST Neuronal lipid metabolism: multiple pathways driving functional outcomes in health and disease. Front. Mol. Neurosci 11, 10 (2018). [PubMed: 29410613]
255. Levine B & Kroemer G Biological functions of autophagy genes: a disease perspective. Cell 176, 11–42 (2019). [PubMed: 30633901]

256. New J & Thomas SM Autophagy-dependent secretion: mechanism, factors secreted, and disease implications. Autophagy 15, 1682–1693 (2019). [PubMed: 30894055]

257. Martinez J et al. Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. Proc. Natl Acad. Sci. USA 108, 17396–17401 (2011). [PubMed: 21969579]

258. Florey O, Kim SE, Sandoval CP, Haynes CM & Overholtzer M Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. Nat. Cell Biol 13, 1335–1343 (2011). [PubMed: 22002674]

259. Martinez J et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat. Cell Biol 17, 893–906 (2015). [PubMed: 26098576]

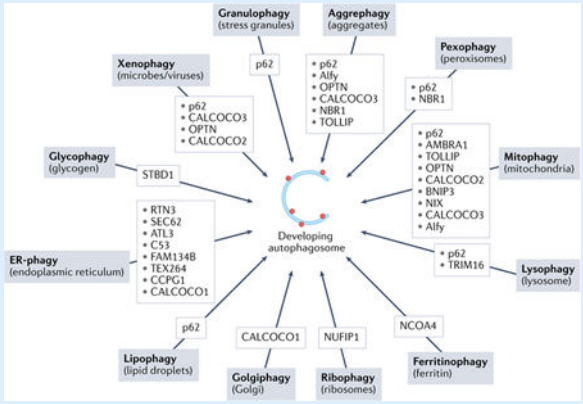
260. Kim JY et al. Noncanonical autophagy promotes the visual cycle. Cell 154, 365–376 (2013). [PubMed: 23870125]

261. Negrete-Hurtado A et al. Autophagy lipidation machinery regulates axonal microtubule dynamics but is dispensable for survival of mammalian neurons. Nat. Commun 11, 1535 (2020). [PubMed: 32210230]

Box 1 |

Types and mechanisms of selective autophagy

Autophagy can non-selectively degrade bulk cytosol, classically described in peripheral tissues under conditions of starvation. We now appreciate that certain molecular events can promote autophagic degradation of select structures. To undergo selective autophagy, cargoes are scaffolded to the autophagosome membrane by adaptor proteins, which recognize signalling moieties on cargo, one of the best characterized being ubiquitin chains. Across phyla, various cargoes and adaptor proteins for selective autophagy have been described[46,47] (see the figure). A given adaptor may scaffold multiple cargo types, and a given cargo type may involve multiple adaptor proteins. Importantly, some adaptors have shown functional redundancy (for example, p62 (also known as SQSTm1) and optineurin (oPTN)), complicating our understanding of the role of these pathways in physiological and pathological settings. In the mammalian CNS, the turnover of protein aggregates (aggrephagy), mitochondria (mitophagy), endoplasmic reticulum (eR-phagy) and stress granules (granulophagy) are the most studied, especially in neurons. Aggrephagy has been described from yeast to mammals and can engage multiple adaptor proteins[247] (see the figure). Although a hallmark of many neurodegenerative diseases, the direct connection between protein aggregates and pathology remains an active area of investigation[58]. mitophagy can occur by various mechanisms depending on signalling context. The most prominent mitophagy pathway involves the Parkinson disease-associated proteins PINK1 and parkin. After mitochondrial depolarization, PINK1 stabilizes on the outer mitochondrial membrane and recruits the e3 ubiquitin ligase parkin to ubiquitylate mitochondrial proteins, which attract adaptors oPTN, CAlCoCo2 (also known as NDP52) and p62. PINK1–parkin-independent and ubiquitin-independent mitophagy have also been described, involving other autophagy adaptors[24] (see the figure). Additionally, the inner mitochondrial membrane phospholipid cardiolipin has been implicated in mitophagy by coordinating autophagy machinery upon its externalization to the outer mitochondrial membrane in neuronal cells[248]. The relative contribution of these in the CNS under basal and pathological conditions remains to be defined. Several ER-phagy adaptors have been identified (see the figure) and studied in the context of the CNS[46,213], as they have also been associated with neurological disorders (TABLE 2). of note, although it has not been demonstrated that p62 is required for ER-phagy, it is associated with ER proteins[249] and p62 knockout in the mouse CNS produces large neurites filled with smooth ER[56]. Whether potential relationships between the turnover of various cargoes exist remains an open question. Further cargoes and their adaptor proteins not shown on the figure have also been identified[44,45].



Box 2 |

Membrane trafficking fates and lipid kinase regulation

In cells of the CNS, membrane trafficking and phospholipid dynamics are inextricably linked to proper function[250]. Central to its regulation are three classes (I, II, III) of phosphoinositide 3-kinases (PI3Ks), each with distinct roles in cellular health. The three classes generate different phosphoinositides, which recruit effector proteins to carry out membrane remodelling events[251]. The autophagy pathway is mainly regulated by the class III PI3K vPS34 in complex with vPS15 and beclin 1 (FIG. 1), with PI3P availability and PI3K function impacting autophagy at multiple points along the pathway, including isolation membrane initiation and maturation–expansion[32,252]. The vPS34 complex itself and autophagy overall exhibit vast crosstalk with endosomal, phagocytic and lysosomal pathways[253], illustrating complex relationships between membrane sources and downstream use. understanding how these different compartments interact is particularly salient for cells of the CNS due to their composition, architecture and functional needs. Given that neural membranes are especially rich in cholesterol and sphingolipids, how cargoes interact with these membranes and specify their degradative fate is essential[254]. For example, for synaptic vesicles or neurotransmitter receptors in neurons, or for transmembrane proteins in oligodendrocytes, we must understand how these species are internalized and reach the lysosome under physiological and, possibly, pathological conditions.

Box 3 |

Non-autophagic roles of autophagy machinery

The term ‘canonical autophagy’ refers to the molecules and functions involved in autophagosome biogenesis, maturation and fusion to the lysosome. Some pathway components, particularly the lipid conjugation machinery, carry out similar functions but do so in distinct membrane trafficking events, including internalization and ATG-dependent secretion (reviewed in REFS[250,255,256]). In particular, studies by Green and others have shown that changes in the regulation of the vPS34 kinase complex can impact which membranes are ultimately conjugated to lC3, including those that enter the cell via phagocytosis or endocytosis: lC3-associated phagocytosis (lAP) in peripheral macrophages[257–259] and retinal pigment epithelial cells[260] involves lC3 conjugation and lysosomal degradation of distinct internalized substrates. lAP requires some but not all autophagy machinery components, including ATG7, ATG5 and certain vPS34 kinase complex members. lC3-associated endocytosis (lANDo) has been identified in microglia, in which internalized RAB-positive and clathrin-positive structures conjugate to lC3 and are degraded, and similarly requires a subset of autophagy machinery[117]. Focusing on the CNS, this suggests that at least phagocytic cells can shuttle membranes down degradative paths with distinct molecular requirements and regulation. This has raised questions surrounding the interpretation of studies that have primarily focused on *Atg5* and *Atg7* deletions (TABLE 1), which cannot distinguish between canonical autophagy, lAP and lANDo. Considering the high endocytic activity of neurons, glia and cells of the neurovasculature, the connection between endosomal systems and either autophagy[36] or lAP and lANDo requires further study, and will be essential to dissect the relative importance of these membrane fates in specific cells of the CNS. members of the core autophagy machinery can also have non-autophagic roles outside of membrane trafficking, including in apoptotic or necroptotic pathways (for example, FIP200 and ATG7), innate immune signalling (for example, the ATG12–ATG5 complex), microtubule dynamics (for example, ATG5 and ATG16l1) and cell cycle regulation[255,261]. With implications for disease mechanisms, the autophagic and non-autophagic roles of these molecular players should be considered in future investigations.

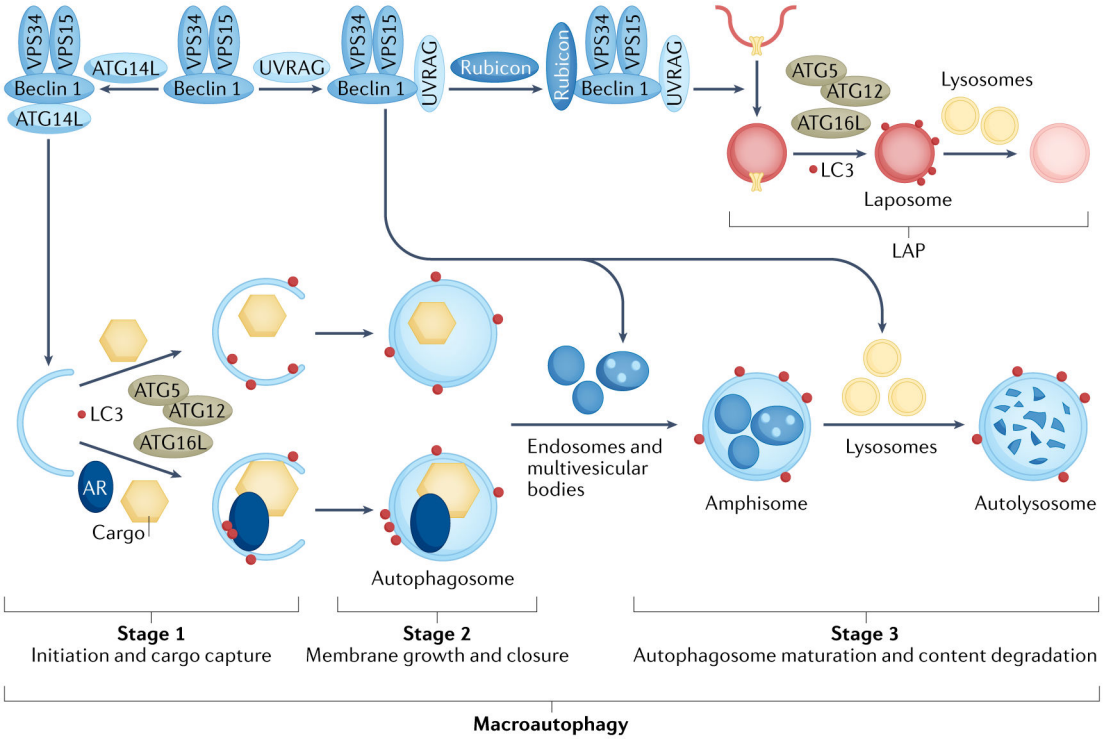


Fig. 1 |. Molecular overview of autophagy and related membrane trafficking events.
Autophagy initiates with ULK complex-mediated autophagosome biogenesis, promoting formation of the isolation membrane, or phagophore, by a series of enzymatic reactions involving autophagy-related (ATG) proteins and incorporation of ATG8 homologues (for example, LC3) into the growing membrane. Autophagosomes form either non-selectively around bulk cytosol or coordinate with adaptor proteins or autophagy receptors (ARs) to selectively capture specific cargo. Nascent autophagosomes can fuse to endosomes and multivesicular bodies to form the intermediate structure, called the amphisome, or directly with lysosomes to form the autolysosome. Ultimately, lysosomal hydrolases degrade the cargo captured by the autophagosome. Autophagy exhibits crosstalk with other membrane trafficking events. Critical for this crosstalk is the regulation of phosphatidylinositol 3-monophosphate (PI3P) by the lipid kinase complex VPS34–VPS15–beclin 1. Depending on binding partners, PI3P can be shunted to autophagy, endosome–lysosome formation or non-canonical membrane fates, including ATG-dependent secretion and LC3-associated phagocytosis (LAP) or LC3-associated endocytosis (not shown).

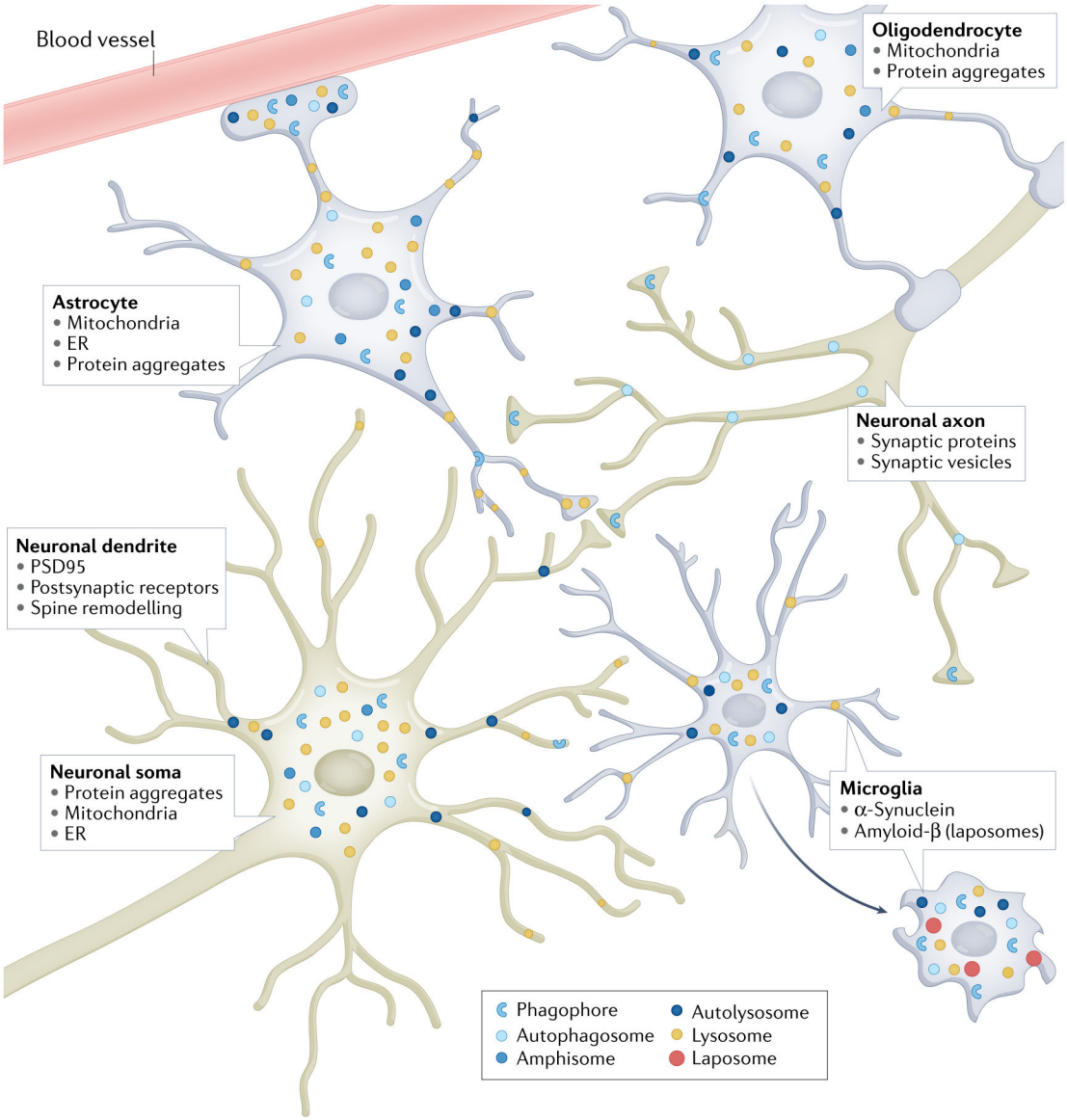


Fig. 2 |. Autophagy in cells of the CNS.

Schematic depicting spatial dynamics and example cargo captured by autophagosomes in neurons, astrocytes, oligodendrocytes and microglia. The boxes indicate the cell type and the most prevalently described cargoes. Blue c-shaped structures represent newly forming autophagosomes (phagophores) to indicate potential sites of autophagosome biogenesis; differently shaded blue circles are autophagosomes, amphisomes and autolysosomes; yellow circles indicate the localization of lysosomes. In microglia, red circles represent vesicles formed by LC3-associated phagocytosis and endocytosis, a feature noted in such cells. Only in neuronal axons, the newly formed autophagosomes must traffic in a retrograde manner to the soma to permit fusion to lysosomes, which are concentrated at the soma. Autophagosome biogenesis in the dendritic and somatic compartments likely represents capture of postsynaptic cargo, mitochondria and endoplasmic reticulum (ER).

Compartmentalization does not seem to be as rigid for astrocytes and oligodendrocytes as autophagosome biogenesis and lysosomal fusion occurs throughout these cells.

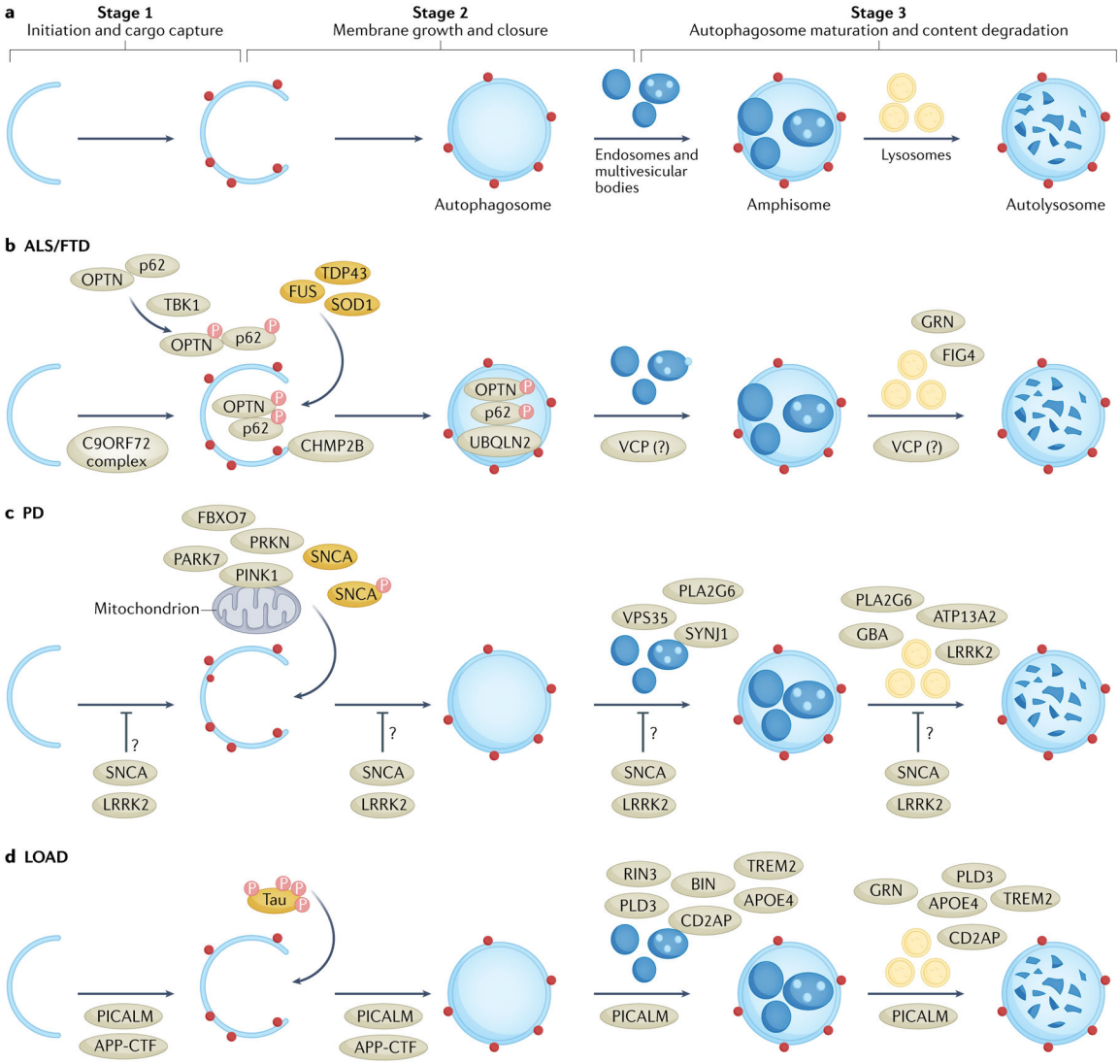


Fig. 3 |. Models of autophagy dysfunction in neurodegenerative diseases.

The neurodegenerative diseases amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Parkinson disease (PD) and late-onset Alzheimer disease (LOAD) occur predominantly without known genetic causes. Even within the small proportion of cases for which monogenic aetiologies or risk loci have been identified, convergent pathophysiology remains elusive. To begin to understand the role of autophagy in these mechanisms of disease, disease-causative, disease-associated and disease-susceptibility genes for familial or known genetic forms of ALS/FTD, PD and LOAD are mapped onto their putative sites of function in the autophagy pathway. **a** | Schematic of autophagosome biogenesis and cargo capture, autophagosome maturation, and fusion of structures to lysosomes for degradation. **b** | In ALS/FTD, mutations in *FUS* (encoding fused in sarcoma (FUS)), *TARDBP* (encoding TAR DNA-binding protein 43 (TDP43)) and *SOD1* (encoding superoxide dismutase 1 (SOD1)) can cause disease, and their protein products as well as UBQLN2 have been identified as autophagic cargo and can accumulate in tissue from idiopathic cases and cases with other genetic aetiologies. Other genetic aetiologies include hexanucleotide repeat

expansion at the *C9ORF72* locus, whose protein product forms a complex that is implicated in autophagosome biogenesis. Optineurin (OPTN) and p62 are known autophagy adaptors, which are phosphorylated by TANK-binding kinase 1 (TBK1), an event essential for targeting these adaptors and their associated cargoes for autophagic degradation. VCP, progranulin (GRN) and FIG4 are implicated in membrane trafficking and lysosome function. **c** | In PD, mutations in *PINK1* and *PRKN* (encoding parkin) can cause autosomal recessive, early-onset PD, with cell biological studies connecting them to mitophagy. *SNCA* encodes α-synuclein (SNCA), a cargo for autophagy and a pathological component of Lewy bodies in brain tissue from patients with idiopathic PD and other synucleinopathies. SNCA and leucine-rich repeat kinase 2 (LRRK2) have been broadly associated with different steps of autophagy. Other known causes of familial PD are primarily associated with autophagosome maturation. **d** | The genetic determinants of AD are not well understood, but a number of risk loci for the development of LOAD have been identified. The risk-associated genes have roles in the regulation of endocytic transport or are localized to the lysosome. These include *TREM2*, *APOE4*, *BIN1*, *CD2AP*, *PICALM* and *PLD3*. Orange ovals depict protein cargoes that are degraded by autophagy and can be mutated in disease, and beige ovals represent all other types of molecular players. BIN1, bridging integrator 1; CD2AP, CD2-associated protein; CHMP2A, charged multivesicular body protein 2A; PINK1, PTEN-induced kinase 1; PLD3, phospholipase D3; SYNJ1, synaptojanin 1.

Table 1 |

Autophagy loss-of-function studies in the mammalian CNS

Gene	Nature of disruption (Cre, expression onset age)	Autophagy pathway	Loss-of-function consequences on the CNS	Refs
Ambra1	Global KO	Autophagosome biogenesis	Embryonic lethality, neural tube defects, cell proliferation in fetal brain, increased apoptosis	Fimia et al. (2007)[206], Cullup et al. (2013)[140]
Atg5	CNS/PNS KO (*Nes*–Cre, E15.5)	Autophagosome biogenesis	Protein inclusions, neurodegeneration, motor defects	Hara et al. (2006)[11]
Atg5	Global KO	Autophagosome biogenesis	Perinatal lethality	Kuma et al. (2004)[207], Qu et al. (2007)[208]
Atg5	Purkinje cells (*Pcp2*–Cre, P6)	Autophagosome biogenesis	Dystrophic neurons, axonal degeneration, ataxia	Nishiyama et al., 2007[98]
Becn1	Global KO	Autophagosome biogenesis	Early embryonic lethality, ectodermal defects	Yue et al. (2003)[209], Qu et al. (2007)[208]
Atg7	CNS/PNS KO (*Nes*–Cre, E15.5)	Autophagosome biogenesis	Protein inclusions, neurodegeneration, axonal degeneration, motor defects	Komatsu et al. (2006)[10]
Atg7	Purkinje cells (*Pcp2*–Cre, P6)	Autophagosome biogenesis	Dystrophic neurons, axonal degeneration, ataxia	Komatsu et al. (2007)[13]
Atg7	Dopaminergic neurons (*Slc6a3*(DAT)–Cre, E15; *Th*–Cre, E16.5; *En1*–Cre, E9)	Autophagosome biogenesis	Striatal dopamine depletion, dysfunctional presynaptic terminals, protein inclusions, neurodegeneration	Hernandez et al. (2012)[67], Friedman et al. (2012)[15], Ahmed et al. (2012)[14], Inoue et al. (2013)[16], Sato et al. (2018)[17]
Atg7	Hypothalamic AGRP neurons (*Agrp*–Cre, E4)	Autophagosome biogenesis	Impaired hypothalamic response to starvation	Kaushik et al. (2011)[63]
Atg7	Hypothalamic POMC neurons *(Pomc*–Cre, P0)	Autophagosome biogenesis	Protein inclusions, axonal defects, impaired lipid catabolism	Kaushik et al. (2012)[210], Coupe et al. (2012)[134]
Atg7	Global KO	Autophagosome biogenesis	Perinatal lethality	Komatsu et al. (2005)[61]
Atg7	Direct, indirect MSNs (*Drd1*–Cre, E17; *Adora2a*–Cre, E15.5)	Autophagosome biogenesis	Defects in dendritic structure, intrinsic excitability, motor learning	Lieberman et al. (2020)[94]
Atg7	Spinal motor neurons (*Chat*–Cre, E15.5)	Autophagosome biogenesis	Protein inclusions, presynaptic dysfunction at the neuromuscular junction	Rudnick et al. (2017)[97]
Atg7	Forebrain pyramidal cells (*Camk2a*–Cre, P0)	Autophagosome biogenesis	Protein inclusions, impaired long-term potentiation and fear memory	Inoue et al. (2012)[204]
Atg7	Microglia (*Cx3cr1*–CreER, induced P60)	Autophagosome biogenesis	Protein inclusions	Choi et al. (2020)[105]
Rb1cc1	CNS/PNS KO (*Nes*–Cre, E15.5)	Autophagosome biogenesis	Protein inclusions, axonal degeneration, neurodegeneration, motor defects	Liang et al. (2010)[211]
Epg[5]	Global KO	Autophagosome maturation	Protein inclusions, neurodegeneration, motor defects	Cullup et al. (2013)[140], Zhao et al. (2013)[142], Byrne et al. (2016)[141]
Snx14	Global KO	Autophagosome maturation	Autophagosome accumulation, lysosomal dysfunction	Thomas et al. (2014)[147], Akizu et al. (2015)[148]
Vps15	Global KO	Autophagosome maturation	Protein inclusions, neurodegeneration	Gstrein et al. (2018)[151]

Gene	Nature of disruption (Cre, expression onset age)	Autophagy pathway	Loss-of-function consequences on the CNS	Refs
Wdfy3	Global KO	Cargo capture	Midline crossing and cell migration defects	Dragich et al. (2016)[132], Orosco et al. (2014)[133]
Fam134b	Global KO	Cargo capture	Endoplasmic reticulum expansion, peripheral neuropathy	Kurth et al. (2009)[212], Khaminets et al. (2015)[213]
Optn	Global KO	Cargo capture	Protein inclusions	Maruyama et al. (2010)[157], Kurashige et al. (2021)[57]
Sqstm1	Global KO	Cargo capture	None	Komatsu et al. (2007)[56]
Prkn	Global KO	Cargo capture	Mild physiology and behaviour changes	Goldberg et al. (2003)[193], Perez & Palmiter (2005)[194]
Pink1	Global KO	Cargo capture	Mild physiology and behaviour changes	Oliveras-Salva et al. (2011)[214]

AGRP, agouti-related protein; DAT, dopamine transporter; E, embryonic day; KO, knockout; MSN, medium spiny neuron; P, postnatal day; PNS, peripheral nervous system; POMC, pro-opiomelanocortin.

Table 2 |

Autophagy adaptor proteins associated with neurological disease

Adaptor or receptor	Autophagic cargo	Non-autophagic functions	Neurological disorders associated with genetic variants	Neurological disorders associated with pathology	Refs
p62 (also known as SQSTM1)	Protein aggregates, mitochondria, peroxisomes, microbes	NF-κB signalling, apoptosis	ALS/FTD, AD	ALS/FTD, AD, PD, HD	Matsumoto et al. (2011)[215], Teyssou et al. (2013)[156], Rea et al. (2014)[216], Cuyvers et al. (2015)[217], Komatsu et al. (2012)[218], Moscat et al. (2009)[219], Moscat et al. (2016)[220]
OPTN	Mitochondria, microbes, non-ubiquitylated inclusions	Interferon signalling	ALS/FTD	HD, PD	Padman et al. (2019)[221], Heo et al. (2015)[222], Schwab et al. (2012)[223], Maruyama et al. (2010)[157], Mankouri et al. (2010)[224], Outlioua et al. (2018)[225]
NBR1	Protein aggregates	None known	None known	PD, HD	Odagiri et al. (2012)[226], Rue et al. (2013)[227]
Alfy (also known as WDFY3)	Protein aggregates	None known	ASD, schizophrenia, microcephaly	ALS/FTD, AD, PD, HD	Fox et al. (2020)[58], Stessman et al. (2017)[228], Iossifov et al. (2014)[229], Clausen et al. (2010)[230], Filimonenko et al. (2010)[59]
CALCOCO2 (also known as NDP52)	Mitochondria, protein aggregates, microbes	NF-κB, interferon signalling	None known	AD	Padman et al. (2019)[221], Heo et al. (2015)[222], Kim et al. (2014)[231], Fan et al. (2020)[232], Till et al. (2013)[233], Jin et al. (2018)[234]
NUFIP1	Ribosomes	Nucleocytoplasmic mRNA transport	PEHO syndrome	None known	Sabaie et al. (2020)[235], Wyant et al. (2019)[236], Bardoni et al. (2003)[237]
ATL3	ER	Vesicle trafficking, viral replication	HSAN-I	None known	Chen et al. (2019)[238], Kornak et al. (2014)[239], Neufeldt et al. (2019)[240]
RTN3	ER	Apoptosis, proteolysis	AD	None known	Zou et al. (2018)[241], Murayama et al. (2006)[242], Kuang et al. (2005)[243], Tang et al. (2007)[244]
FAM134B	ER	Cell cycle, apoptosis, viral replication	HSAN-II	None known	Kurth et al. (2009)[212], Khaminets et al. (2015)[213], Mo et al. (2020)[245], Chiramel et al. (2016)[246]

AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; ASD, autism spectrum disorder; ER, endoplasmic reticulum; FTD, frontotemporal dementia; HD, Huntington disease; HSAN-I/II, hereditary sensory and autonomic neuropathy type I/II; OPTN, optineurin; PD, Parkinson disease; PEHO, progressive encephalopathy with oedema, hypsarrhythmia and optic atrophy.