

Inborn errors of canonical autophagy in neurodegenerative diseases

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Abstract

Neurodegenerative disorders (NDDs), characterized by a progressive loss of neurons and cognitive function, are a severe burden to human health and mental fitness worldwide. A hallmark of NDDs such as Alzheimer's disease, Huntington's disease, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and prion diseases is disturbed cellular proteostasis, resulting in pathogenic deposition of aggregated protein species. Autophagy is a major cellular process maintaining proteostasis and integral to innate immune defenses that mediates lysosomal protein turnover. Defects in autophagy are thus frequently associated with NDDs. In this review, we discuss the interplay between NDDs associated proteins and autophagy and provide an overview over recent discoveries in inborn errors in canonical autophagy proteins that are associated with NDDs. While mutations in autophagy receptors seems to be associated mainly with the development of ALS, errors in mitophagy are mainly found to promote PD. Finally, we argue whether autophagy may impact progress and onset of the disease, as well as the potential of targeting autophagy as a therapeutic approach. Concludingly, understanding disorders due to inborn errors in autophagy—"autophagopathies"—will help to unravel underlying NDD pathomechanisms and provide unique insights into the neuroprotective role of autophagy, thus potentially paving the way for novel therapeutic interventions.

Keywords: autophagy; neurodegenerative diseases; innate immunity; monogenic diseases

Introduction

Neurodegenerative diseases as proteinopathies

Neurodegenerative diseases (NDDs) are a heterogeneous group of neurological conditions characterized by a progressive loss of neurons [1–3]. This erosion of neuronal circuits eventually leads to a collapse of neurocognitive features including impaired motor function, cognitive decline, memory loss and behavioral alterations. Currently, more than 55 million people are affected by NDDs, with ageing as the greatest risk factor [4]. Considering the overall ageing population, the impact of NDDs on human health worldwide is expected to increase. Besides age, genetic factors are crucial contributors to the development and progression of NDDs. A hallmark of several prominent NDDs is the loss of proteostasis and pathological aggregation of cellular proteins [1]. These toxic aggregates often form insoluble amyloid fibers and are thought to be a key pathological mechanism. According to the aggregating protein, NDDs can also be classified into amyloidoses, tauopathies, α -synucleinopathies, TDP-43-associated proteinopathies and prion diseases (Fig. 1A).

Aggregated small **amyloid β ($A\beta$)** peptides are the major component of amyloid plaques in Alzheimer's disease (AD) [5]. They are derived from the amyloid- β precursor protein (APP) by cleavage during the amyloidogenic pathway by β -secretases and

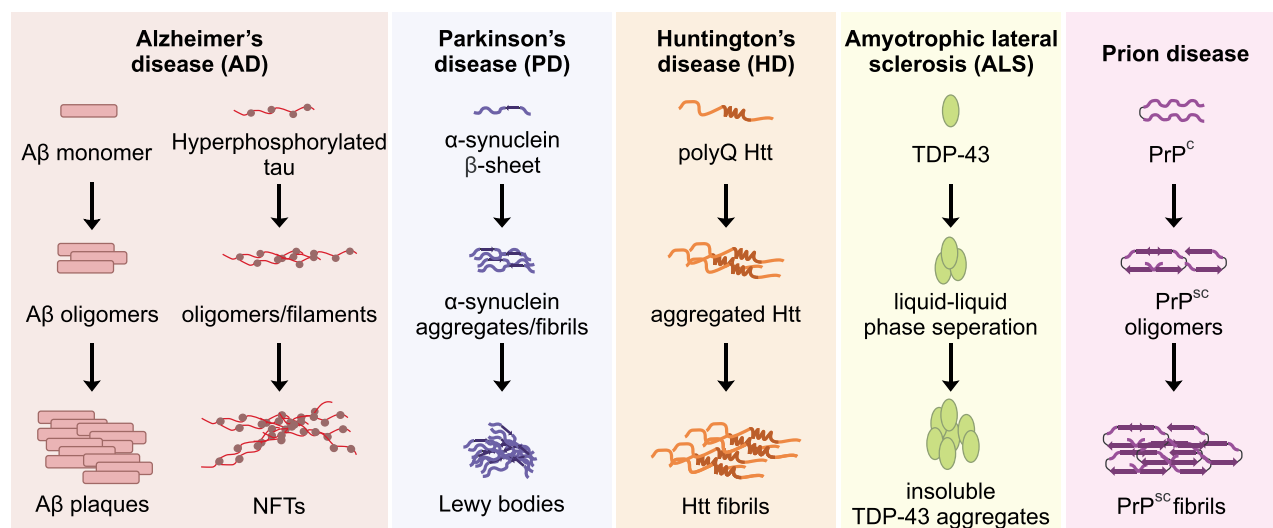
γ -secretases. Of note, while $A\beta$ oligomers and deposits have been considered as the molecular driver of Alzheimer's pathogenesis and progression, therapeutic targeting of $A\beta$ has repeatedly failed [6]. Filamentous lesions and deposits of fibrils of the microtubule-associated protein **tau** are defining features of AD and related tauopathies such as frontotemporal dementia (FTD) [7]. Aberrant hyperphosphorylation of tau in neurons leads to its aggregation into helical filaments. Notably, tauopathies are considered to be responsible for the majority of dementias [8]. **Alpha-synuclein (α -syn)** is a small 14 kDa cellular protein mainly expressed in the central and peripheral nervous system. While mainly α -helical in its non-pathogenic form, it transitions into a pathological β -sheet conformation, that eventually aggregates to form amyloid-like fibrils accumulating in neurons in Lewy bodies in Parkinson's disease (PD) or dementia with Lewy bodies (LBD) [9]. In multiple system atrophy (MSA) α -syn aggregates form mainly in glia cells [9]. α -syn aggregation/co-aggregation is observed in ~30%–50% of AD cases [10–12]. Mutated **Huntingtin (Htt)** with a polymorphic locus containing more than 36 CAG codons (coding glutamine, Q) is thought to be the main molecular cause of Huntington's disease (HD). The number of CAG repeats directly correlated with the onset and severity of the disease. Polyglutamine (polyQ) expanded Htt forms amyloid-like protein aggregates [13] which may be part of the HD pathogenesis. **TAR DNA-binding protein 43**

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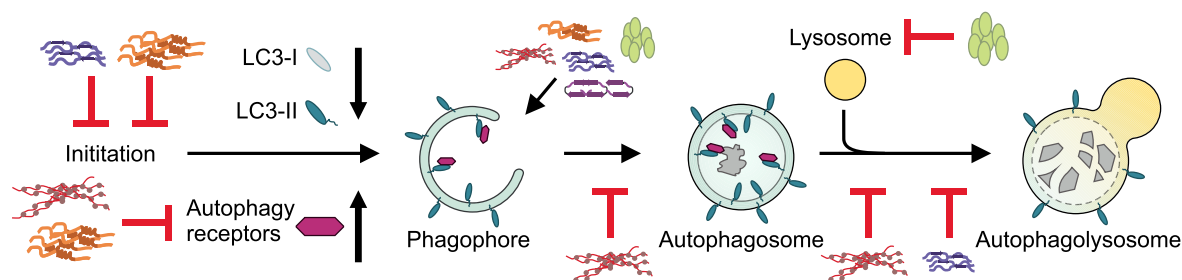


Figure 1. The interplay between autophagy and NDDs. (A) Aβ and hyperphosphorylated tau protein, β-sheet α-synuclein, Polyglutamine (polyQ) huntingtin (Htt), neurofibrillary tangles (NFTs), TDP-43 and misfolded prion protein (PrP^{Sc}) are aggregated proteins found in proteinopathies. Alzheimer's disease (AD), column 1 (red); Parkinson's disease (PD), column 2 (blue); Huntington's disease (HD), column 3 (orange); amyotrophic lateral sclerosis (ALS), column 4 (green); prion disease, column 5 (purple). (B) Proteins (aggregates) associated with NDDs interact with the autophagic pathway at multiple levels. Autophagy initiation is inhibited by Htt fibrils and α-synuclein fibrils. After LC3B-I to LC3B-II conversion, cargo like misfolded/aggregated NDD associated proteins are recruited to the budding phagophore by specific autophagy receptors. In turn autophagy receptor function is disturbed by Htt fibrils and NFTs. To degrade the cargo, the closed autophagosome fuses with a lysosome to form an autophagolysosome. Insoluble TDP-43 interferes with lysosome function, whereas NFTs and α-synuclein fibrils perturb autophagosome to autophagolysosome transition.

(TDP-43) is a highly conserved nucleic acid binding protein and its aggregation is a common feature of several NDDs including amyotrophic lateral sclerosis (ALS), FTD and AD [14]. TDP-43 pathology is most prominent in ALS, with more than 90% of the cases exhibiting cytoplasmic aggregates that spread in characteristic stages [15]. Misfolding of cellular **Prion protein (PrP^C)** into β-sheet rich, aggregation-prone infectious **PrP^{Sc}** is the underlying cause of fatal Prion diseases or “transmissible spongiform encephalopathies” among them Creutzfeld-Jacob disease [16]. As opposed to other proteinopathies, prion protein aggregates have been shown to transmit the disease [17].

Taken together, it is conceivable that disturbance of proteostasis governs the onset and/or promotes progression of proteinopathic NDDs. The core of proteostasis maintenance in cells are chaperones that reduce misfolding, catabolic systems that degrade misfolded proteins such as the ubiquitin-proteasome system (UPS), and autophagy [18].

Autophagy maintains proteostasis

Macroautophagy (hereafter referred to as autophagy) is an evolutionary ancient catabolic pathway that is active in virtually every cell [19]. It is essential to maintain cellular proteostasis and an important part of innate immunity, providing defenses against pathogens. In a nutshell, autophagy facilitates (targeted)

lysosome-associated degradation of obsolete/damaged organelles (e.g. degradation of mitochondria in mitophagy) and cellular or pathogen-associated proteins [20, 21]. Initiation is guided by the activity of kinases. AMP-activated protein kinase (AMPK1) promotes, whereas mammalian target of rapamycin (mTOR) and Casein kinase 2 (CSNK2) oppose autophagy activation [21–23]. Initiation converges in the activation of the Unc-51-like autophagy-activating kinases 1 (ULK1) complex that promotes the formation of a double-membranous structure called the phagophore [24]. The membranes are recruited by autophagy-related protein 9 (ATG9) in a Vacuolar protein sorting ortholog 35 (Vps35)-dependent manner [25, 26]. The budding autophagic vesicle (=autophagosome) is decorated with lipidated-ATG8 like proteins such as LC3B-II, promoting elongation of the autophagosome and the engulfment of cytoplasmic cargo. In selective autophagy, highly specific autophagy receptors recruit cargo earmarked by ubiquitin, among them Sequestosome-1 (SQSTM1/p62), Calcium-binding and coiled-coil domain-containing protein 2 (CALCOCO2/NDP52) or optineurin (OPTN) [27]. A master regulator of autophagy receptor phosphorylation and activation is Tank-binding kinase 1 (TBK1) [28, 29]. During mitophagy, obsolete mitochondria are recognized by PTEN-induced kinase 1 (PINK1), which causes activation of the E3 ligase parkin, that decorates the outer mitochondrial membrane with ubiquitin, which is in turn

recognized by OPTN [30]. Finally, the autophagosome matures and fuses with a lysosome to form the autophagolysosome followed by degradation of the inner membrane and the cargo by acidic hydrolases [31].

Main text

The interplay between autophagy and NDD-associated proteins

The majority of NDD-associated proteins were shown to be targeted by autophagy [32] (Fig. 1B). $A\beta$, insoluble tau and mouse Htt were shown to be cleared by activation of autophagy dependent on p62 [33–36]. Conversely, autophagy-deficient mice show increased extracellular $A\beta$ deposition and intracellular $A\beta$ accumulation [37–39], while lack of autophagy in microglia aggravates tau pathology [40]. Pharmacological activation of autophagy mitigates cognitive defects of AD model mice [38]. Curiously, it was also reported that autophagy may promote extracellular $A\beta$ secretion and deposition [41] and the autophagy adaptor TRIAD3A was suggested to promote tau fibrillation [42], suggesting a more complex role of autophagy in AD. Inhibition of autophagy attenuated the clearance of Htt aggregates both in cell culture and rats [35, 43–45]. α -syn is targeted for degradation by both autophagy and the proteasome [46, 47]. TDP-43 pathology was reduced in an ALS mouse model by autophagy induction [48, 49] and TDP-43 aggregates were found in autophagosomes [50]. Of note, TDP-43 secretion may be promoted by autophagy-associated processes [51]. The impact of autophagy on prion diseases is less well explored, however, older studies suggest that protease resistant prion may be targeted by autophagy [52] and induction of autophagy counteracts prion mediated neurotoxicity [53, 54].

In turn, NDD-associated protein aggregates impact autophagy. $A\beta$ fibrils reduce autophagic flux in AD models and AD patients [55–57] which may additionally fuel deposition of fibrils [58]. Similarly, tau accumulation inhibits autophagy via impairing ESCRT-III complex formation [59] and disturbance of the autophagosomal-lysosomal axis is associated with tauopathies [60]. Prolonged polyQ tracts in Htt outcompete binding of ataxin-3 to beclin-1, leading to beclin-1 degradation and inhibition of autophagy initiation [61]. Of note, tau as well as Htt fibrils escape clearance by autophagy by stealthing in p62 coats [62, 63]. α -syn impacts autophagy by impairing SNAP29-mediated autophagosome-lysosome fusion [64]. Pathogenic, mutated α -syn (A53T) is able to bind to tuberous sclerosis protein (TSC) 2, which destabilizes the TSC1-TSC2 complex, eventually leading to aberrant mTOR activity [65]. Of note, soluble α -syn (SNCAx3) impairs autophagy/mitophagy [66] and may disturb autophagy by accumulation at the ER, eventually leading to the aggregation of immature β -glucocerebrosidase (GCase) [67]. TDP-43 was shown to impair autophagosome turnover by increasing TFEB activity [68] and interfering with lysosomal function thus presumably preventing its own degradation [69]. The prion protein was also suggested to play a role in autophagy [70], and human prion-induced autophagy flux was reported to contribute to primary neuron cell damage [71].

Improving our understanding of the intricate relationship between autophagy and NDDs may open up new avenues for both molecular insights and therapeutic interventions.

Patient variants in autophagy genes linked to NDDs

In the recent years, monogenic variants of core autophagy proteins have been causally linked to the development of

neurodegenerative pathology providing genetic evidence for the importance of autophagy and may help to unravel the complex pathomechanisms [72]. Here we focused on recently discovered mutations in core autophagy proteins associated with five major NDDs: AD, PD, HD, ALS and Prion diseases (Fig. 2, overview in [73]).

Alzheimer's disease (AD) is a form of dementia mainly characterized by symptoms like memory loss and cognitive dysfunction [74] linked to hyperphosphorylated tau proteins, that aggregates to neurofibrillary tangles [7], and $A\beta$ aggregates derived from APP cleavage [5]. Although more than 90% of AD cases are sporadic, AD was associated with mutations in presenilin-1 and -2 (PSEN1; PSEN2) and Sortilin Related Receptor 1 (SORL1) [75, 76]. Recently, AD-associated variant p.N299S in acetyl-Coenzyme A acyltransferase 1 (ACAA1) was described to eventually lead to impaired autophagosome-lysosome fusion and function [77]. In all cases autophagy was disturbed, however, the mutations did not affect core autophagy genes.

Parkinson's disease (PD) is associated with accumulation and aggregation of α -syn within dopaminergic neurons. Although mainly sporadic (85%–90%) patient-associated variants are frequently found in genes involved in mitophagy (Fig. 2), usually in the E3 ligases parkin and PINK1. Of note, PINK1 mutations are especially common in early onset PD [78–80]. However, it should be noted that many mutations in PARKIN, may lead to a disturbance of α -syn proteasomal degradation and may not affect mitophagy. Variants of parkin include more than 10 likely pathogenic missense mutations (Table 1 and [120]), often resulting in dysfunctional gene expression. More recently, analyses of cells from a PD patient with mutated PARKIN (B125: homozygous c.1072Tdel) revealed a destabilization of active Rab7, leading to decreased mitochondria-lysosome contact, and thus amino acid accumulation in lysosomes [118]. Systematic analyses of PINK1 mutations associated with PD showed that more than 16 missense variants could be considered pathogenic [129]. Most recently, further putatively pathogenic variants were discovered, including p.F385S [130]. Mutated VPS35 p.D620N associated with PD patients, was shown to impair mitophagy and autophagy [123]. In addition, VPS35 p.D620N disturbed Rab9 dependent non-canonical autophagy [131]. PD associated mutations in the lysosomal GBA (p.N370S, p.L444P) lead to lysosomal dysfunction, inhibiting chaperone mediated autophagy, however simultaneously maintaining normal autophagy levels via increase in ULK1 phosphorylation, highlighting a possible compensatory mechanism [124].

Huntington's disease (HD) is defined by the aberrant presence of CAG repeat expansions in the Htt gene, resulting in the expression of Htt with expanded polyQ tracts at the C-terminus. These variants are prone to aggregation, formation of amyloid-like fibrils as well as neurofibrillary tangles [13]. Of note, the risk variant p.V471A in the core autophagy gene ATG7 is associated with early onset of HD, suggesting that functional autophagy may clear pathological aggregation [126, 127]. Recently it was shown that the expression of the miRNA miR-29b-3p targeting STAT3 and ultimately reducing autophagy, increases with age potentially increasing HD symptom severity [132].

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of upper and lower motoneurons, leading to death within 3–5 years after symptoms onset [15]. Even though the associated risk genes are functionally heterogeneous, intracellular TDP-43 aggregates characterize up to 97% of the cases [133]. Of note, as a large number of mutations in core autophagy genes were identified as risks in the past years (Table 1, Fig. 2), we discuss here the most recent findings. For example,

Table 1. Variants of core autophagy genes, mechanism and associated diseases.

Disease	Genes	Mutation	Identified in	Mechanism
ALS	SQSTM1	p.L341V	[81]	Reduced binding affinity to ubiquitin [82] Reduction in binding affinity to hATG8 [83]
		p.P392L		Reduced binding affinity to ubiquitin [82]
		p.G425R		Loss of binding to ubiquitin [82]
		p.A16V p.D80E p.E81K p.V90M p.I99L p.R107W p.R212C p.G219V p.S226P p.P232T p.N239K p.D258N p.E280* p.G297S p.R312H p.A33V p.V153I p.P228L p.V234V p.H261H p.S318P		NA
		p.R110C		Reduced phosphorylation of Ser-403 and Ser-349 [84] necessary for SQSTM1/KEAP1 interaction, also reducing NRF2 activity (Oxidative Stress response)
		p.D129N		Reduce affinity towards N-degron [85]
		p.K238del	[86]	Loss of function, reduced mitochondrial respiration, increased cytosolic ROS production, reduced complex 1 activity of the electron transport chain and lowered mitochondrial membrane potential [87]
		p.R321C		Increased levels of p62 and LC3B suggesting autophagy blockage [88]
		p.G411S		Loss of mono- and polyubiquitin-binding [89]
		p.A53T p.M87V p.E155K p.K238E p.E274D p.P296P p.S318S p.V346V p.A390* p.P438L p.P439L p.V346V p.S370P	[90]	NA
		p.K102E		Impaired formation of disulphide-linked conjugates upon oxidative stress and thus induction of autophagy [91]
		p.P348L		Reduced Keap1 binding [92]
	OPTN	p.E478G	[93]	Inhibits autophagosome maturation [94] Impaired mitophagy [95]
		p.G23* p.Q165* p.S262* p.L568L		NA
		p.Q398*		Loss of ubiquitin binding [96]
		p.L430Rfs*16 p.K440Nfs*8 p.D220Mfs*12		NA
		p.148_184del-, Ex5del		NA
		p.H3Y p.P16A p.K59N p.A93P p.R96L		NA

(continued)

Table 1. Continued.

Disease	Gene	Mutations	Identified in	Mechanism
	TBK1	p.D127R		
		p.A136V		
		p.V161M		
		p.R271C		
		p.T282P		
		p.V295F		
		p.Q314L		
		p.E322K		
		p.K395R		
		p.M447R		
		p.I451T		
		p.M468R		
		p.A481V		
		p.K489E		
		p.L494W		
		p.L500P		
		p.E516Q		
		p.R545Q		
		p.K557T		
		p.Q564H		
		p.Q454E		Dysregulation of NF- κ B and mitophagy [97]
		p.E696K	[98]	Impairing lysosomal degradation, Rab7 phosphorylation, OPTN binding, and mitophagy [30, 99–102]
		p.R573G		Reduction in kinase activity [103]
		p.I43V		NA
		p.L62P		
		p.L94S*		
		p.D118N		
		p.G121D*		
		p.R143C*		
		p.R229S		
		p.G244V		
		p.I246T		
		p.R271L*		
		p.K291E		
		p.G294D		
		p.L306I*		
p.H322Y				
p.I334T				
p.R384W*				
p.I397T				
p.K401E				
p.I418V				
p.I475T*				
p.E476K*				
p.I515T				
p.A535T				
p.K570R				
p.T320Qfs*40		Loss of function [104]		
p.I450Kfs*15				
p.V479Gfs*4				
p.Y185*				
p.T77Wfs*4				
p.A417*				
690-713del				
p.R440*				
p.R47H		loss of kinase activity and loss of IRF3 activation [105]		
p.R357Q		abolish OPTN binding and phosphorylation, failure to oligomerize and reduced steady-state-level [105]		
p.M559R		loss of kinase activity and dimerization, loss of OPTN binding [99, 104, 105]		
p.G217R				
p.I305T		NA		
p.A571V				
p.M598V				

(continued)

Table 1. Continued.

Disease	Genes	Mutations	Identified in	Mechanism
PD	CCNF	p.Y105C p.R308Q	[106]	Impaired interaction with OPTN and reduced p62 phosphorylation [105]
		p.M623fs p.Q629fs p.T31A p.R358H		NA
		p.E643del		NA
		p.Q2del p.R117del p.R357del p.R440del p.R444del p.S499del	[107]	NA
		p.L10S p.S151F p.I257T p.L277V p.T331I p.T343S p.R440Q p.C471Y p.I522M p.R573H p.Q580H p.I710N		NA
		p.N22H p.S151C		Reduced p62 S403 phosphorylation [105]
		p.R25H p.R47H p.R134H p.R228H p.V132E p.N129D		Reduced kinase activity/loss of function [105]
		p.R228H		Reduced autophosphorylation/partial loss of function [108]
		p.Q565P		Reduced phosphorylation of p62 [105]
		p.Y394D		Early onset of the disease/mechanism not known yet [107]
		p.S621G	[109]	Inhibits p62 foci formation [109]
	UBQLN2	p.P497H p.P509S	[110]	Reduced mitophagy [110]
	C9ORF72	G4C2 repeats		Impaired autophagic flux, autophagosome biogenesis, and fusion with lysosomes. Perturbed lysosome metabolism (axonal transport, maturation (enlarged and reduced numbers)) Increased phosphorylated TBK1 (S172) levels [111–113]
	PFN1	p.C71G p.M114T	[114]	Impaired endolysosomal processing [114]
	PINK1	p.K219A p.G309D p.L347P p.D362A p.D384A p.G386A p.G409V p.E417G	[115]	Reduced kinase activity [115]
		p.Q456*	[116]	No kinase activity [116]
		p.V170G	[117]	Reduced activity/partial loss of function [117]
	PRKN	p.C431S	[118]	Decrease in mitochondrial lysosome contact [118]
		p.Q34Rfs*5 p.Q34Rfs*10 p.V3Efs*3 p.A138Gfs*7 p.N52Mfs*29 p.G34Rfs*5 p.P113Tfs*51 p.D18Vfs*26 p.R156Sfs*29	mdsgene.org	NA

(continued)

Table 1. Continued.

Disease	Genes	Mutations	Identified in	Mechanism
		p.W445* p.C446F p.Q311*		NA
		p.R275W		Reduced PRKN translocation/loss of function [119]
		p.C212Y p.C253Y		Reduced protein stability/loss of function [120]
		p.T240M p.T240R		Residue necessary for E2-binding/likely loss of function [121]
		p.G430D		Residue necessary for RING2 domain function/likely loss of function [121]
		p.R42P p.V56E		Reduced protein stability/likely loss of function [121]
		p.T415N		Loss of function [122]
		p.W453*		Loss of substrate ubiquitination [121]
	VPS35	p.D620N	[123]	Impaired auto- and mitophagy [123]
	GBA	p.N370S p.L444P	[124]	Lysosomal dysfunction [124]
		p.E326K	[125]	Reduced TFEB activity, increase in p-RPS6 levels and decrease in DEPTOR [125]
HD	ATG7	p.V471A	[126]	Loss of function [126–128]
AD	ACAA1	p.N299S	[77]	Impaired lysosome function and lysosome-autophagosome fusion [77]

several mutations (p.L341V, p.P392L, p.G425R) in one of the major cargo-recruitment receptors of autophagy, SQSTM1/p62 were shown to result in reduced binding affinity to ubiquitin, eventually leading to reduced lysophagy [82]. p.L341V in p62 lowers the binding affinity to certain hATG8 proteins [83]. (Table 1). A patient-associated variant of the autophagy receptor OPTN (p.E478G) has been shown to interfere with the maturation of autophagosomes and MYO6 recruitment, inhibiting autophagic flux [94]. This variant negatively influences OPTN and UBQLN2 recycling at endosomes [134]. Mutations in TBK1, a central regulatory kinase of autophagy, have been frequently linked to impaired autophagy and increased neuroinflammation in ALS [135]. So far more than 90 mutations in TBK1 were associated with ALS [99]. Most of the TBK1 related mutations are deleterious and most likely lead to haploinsufficiency [99, 104] (Table 1). Lower TBK1 levels prevent efficient maturation of autophagosomes due to reduced phosphorylation of autophagy receptors [136]. Many missense mutations in TBK1 show a loss of function phenotype, however the exact mechanism for all mutations is currently not known [104] (Table 1, and [104, 137]). More recently it was reported that TBK1 p.E696K lowers its expression levels, impairs lysosomal degradation, and elevates Rab7 phosphorylation levels [100]. Of note, hyperactive Rab7 leads in turn to mTORC1 activation, further inhibiting autophagy [101]. In addition, TBK1 p.E696K has been shown to reduce binding affinity towards OPTN [99]. Another group reported that the TBK1 p.R573G mutation leads to a reduced kinase activity, yet autophagic flux levels were not altered [103]. In addition to core autophagy proteins, genetic mutations in other autophagy regulating factors were recently identified as risks genes in ALS. For example, CCNF (p.S621G) reduces p62 foci formation [109] and mutated UBQLN2 (p.P497H, p.P509S) decreases mitophagy [110]. G4C2 repeats in C9ORF72 were suggested to reduce biogenesis of autophagosomes and autophagosome-lysosome fusion [111–113]. Longer repeats cause sequestration of TBK1 into inclusion bodies, thus reducing TBK1 activity [138]. Mutant profilin 1 (PFN1) (p.C71G, p.M114T) negatively impacts endo-lysosomal processing [114].

Transmissible spongiform encephalopathies (TSE), or prion diseases, are characterized by aggregates of misfolded Prion protein, PrP^{Sc} [16]. TSEs are unique among NDDs as epidemiological evidence supports that they are transmitted between people, similar to infectious agents [17]. While the last decades have elucidated numerous pathogenic variants of the PRNP gene, up to date, there are no mutations in autophagy related genes described which are associated with prion diseases.

Concluding remarks

Molecular analysis of human genetic variants gave unique insights into the pathophysiological mechanisms of autophagy in NDDs. Patient-associated mutations have established a clear genetic link between autophagy and selected NDDs. For example, there is a clear association between PD and perturbed mitophagy. The most studied mitophagy pathway is dependent on two proteins central for familial PD, PINK1 and parkin [80, 120]. Mutations in these two proteins, which often lead to dysfunctional mitophagy are considered to be the most common genetic link to PD. Curiously, ALS seems to be predominantly associated with variants of autophagy receptors such as p62 or OPTN, or variants of autophagy-receptor activating kinases such as TBK1 [29, 134, 137]. That said, different stages/pathways of autophagy are clearly associated to specific NDDs. While the molecular details of mitophagy in PD has been extensively analyzed [9, 30, 66, 79, 118], the notable concentration of ALS-associated autophagy receptor mutations needs to be further explored. Other NDDs such as AD, HD or prion diseases were shown to impact and be impacted by autophagy, but prominent mutations in core autophagy proteins were not (yet) associated. Finally, a large number of patients develop NDDs sporadically, especially AD or PD, and no clear genetic condition could be associated. Of note, the age of onset of sporadic NDDs is usually late in life. It is tempting to speculate that waning efficiency of autophagy due to ageing may increase the chances of sporadic formation of toxic protein aggregates.

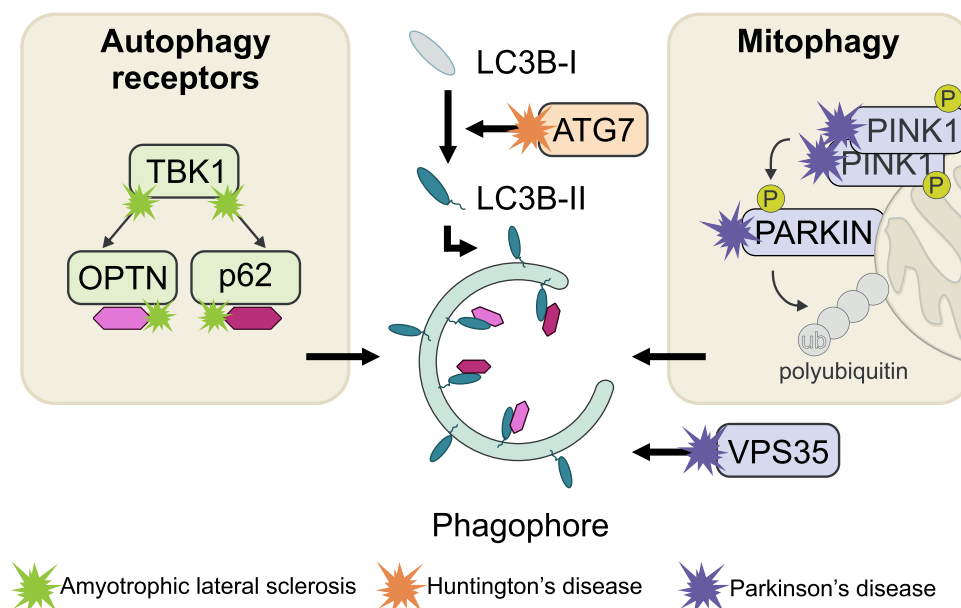


Figure 2. Mutations in autophagy initiation associated genes in NDDs. TBK1 activates autophagy receptors such as OPTN and p62 by phosphorylation, which in turn recruit cargo to the budding phagosome. Conversion of LC3B-I to membrane-associated LC3B-II is facilitated by ATG7 and required for autophagosome elongation and closure. Phagosome formation is promoted by membrane recruitment via VPS35. During mitophagy, damaged or obsolete mitochondria are recognized by PINK1, which in turn activates the E3 ubiquitin ligase parkin. Parkin catalyzes the ubiquitination of the mitochondrial outer membrane. The earmarked mitochondria are then recruited to autophagosomes by OPTN. NDD associated mutations that impair function in autophagy are highlighted in respective color.

However, the role of age-impaired autophagy in the development and progression of NDDs needs to be analyzed.

NDD-associated defects in genes related to autophagy may not automatically show involvement of canonical autophagy. For example, non-canonical autophagy such as LC3-associated phagocytosis [139] was shown to facilitate AD in mouse models [39]. In addition, many autophagy proteins play secondary roles in secretory processes [140], which can impact cytokine secretion during neuroinflammation [141] or direct secretion of fibrils [41, 51]. While canonical autophagy is considered to solely lead to the degradation of intracellular proteins/aggregates, autophagy-like processes may also target extracellular NDD-associated proteins and aggregates. It is thus crucial for future studies to delineate the exact role and impact of NDD associated gene variants in autophagy, autophagy-like processes or even non-autophagy-related processes.

Emerging evidence suggests that autophagy is a cellular defense mechanism against NDDs, preventing detrimental oligomer formation, or even clearing aggregates [142, 143]. Convincing preclinical data highlighting the therapeutic potential of autophagy modulation is currently accumulating [142, 144]. For example, activation of autophagy by the mTOR inhibitor Temsirolimus decreased Htt aggregates in mouse studies [145]. Similarly, targeting mTOR was shown to reduce progression of AD in models [146–148]. Along these lines, modulation of the activity of kinases involved in the activation of autophagy such as AMPK were also shown to decrease aggregate formation in cellular and mouse models of AD, PD, and HD. In HD, therapeutic strategies based on targeting mitophagy, such as ROCK inhibitors [149, 150], were tested in proof-of-principle experiments. Unfortunately, despite ongoing clinical trials, there are currently no approved therapeutic interventions based on autophagy modulation. The reasons are likely multifactorial, and include strong side-effects, little autophagy-specificity and difficult central nervous system delivery of currently available compounds.

Despite rapid progress in the identification of NDD-associated mutations in recent years, the underlying molecular mechanism(s) and how do they contribute to the onset and progression of the disease often remain understudied. Importantly, it also needs to be addressed, why do certain mutations lead to a specific type of NDD. In addition, insights from understanding the molecular basis of mendelian NDD-associated autophagopathies will improve our understanding of the protective role of autophagy, and help unravel the molecular biology of NDD onset and progression. Most importantly, inspired by our understanding of the molecular details the way for direly needed novel therapeutic interventions in NDDs is paved.

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