PORTLAND
PRESS

Review Article

Dysregulation of autophagy as a common mechanism in lysosomal storage diseases

Elena Seranova[1,*], Kyle J. Connolly[1,*], Malgorzata Zatyka[1], Tatiana R. Rosenstock[2], Timothy Barrett[1], Richard I. Tuxworth[1] and Sovan Sarkar[1]

[1]Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, U.K.; [2]Department of Physiological Science, Santa Casa de São Paulo School of Medical Science, São Paulo, SP 01221-020, Brazil

Correspondence: Sovan Sarkar (s.sarkar@bham.ac.uk) or Richard I. Tuxworth (r.i.tuxworth@bham.ac.uk)

OPEN ACCESS

The lysosome plays a pivotal role between catabolic and anabolic processes as the nexus for signalling pathways responsive to a variety of factors, such as growth, nutrient availability, energetic status and cellular stressors. Lysosomes are also the terminal degradative organelles for autophagy through which macromolecules and damaged cellular components and organelles are degraded. Autophagy acts as a cellular homeostatic pathway that is essential for organismal physiology. Decline in autophagy during ageing or in many diseases, including late-onset forms of neurodegeneration is considered a major contributing factor to the pathology. Multiple lines of evidence indicate that impairment in autophagy is also a central mechanism underlying several lysosomal storage disorders (LSDs). LSDs are a class of rare, inherited disorders whose histopathological hallmark is the accumulation of undegraded materials in the lysosomes due to abnormal lysosomal function. Inefficient degradative capability of the lysosomes has negative impact on the flux through the autophagic pathway, and therefore dysregulated autophagy in LSDs is emerging as a relevant disease mechanism. Pathology in the LSDs is generally early-onset, severe and life-limiting but current therapies are limited or absent; recognizing common autophagy defects in the LSDs raises new possibilities for therapy. In this review, we describe the mechanisms by which LSDs occur, focusing on perturbations in the autophagy pathway and present the latest data supporting the development of novel therapeutic approaches related to the modulation of autophagy.

Introduction

Our perspective of the lysosome has shifted remarkably from its standing as a simple, terminal organelle for the degradation of cellular components to become a critical mediator of fundamental metabolic processes. Lysosomes coordinate signals from growth factors and cellular stressors and are sensitive to various metabolites, such as amino acids, glucose, lipids and cholesterol, to pivot cells between anabolic and catabolic processes, including autophagy [1-3]. The importance of the lysosome for cellular function is apparent from the large number of disorders associated with lysosomal failure: collectively known as the lysosomal storage disorders (LSDs), more than 50 inherited conditions affect lysosomal function and many are early-onset and fatal [4-7].

A key cellular homeostatic pathway implicated in several LSDs and myriad human diseases is autophagy. Autophagy is an intracellular degradation pathway essential for cellular survival and organismal health [8-10]. This process is vital for the maintenance of energy and tissue homeostasis by degrading damaged or excess intracellular components such as aggregation-prone proteins, lipids and organelles, and recycling the breakdown products [11]. There are
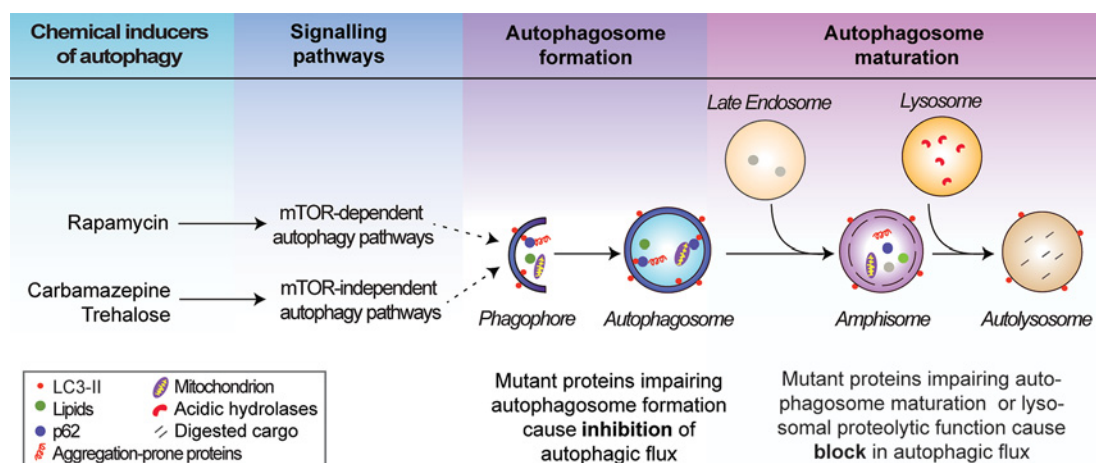
Figure 1. Schematic representation of the autophagy pathway
Autophagy initiates by the *de novo* synthesis and elongation of phagophores, which engulf cytosolic materials (autophagic cargo) to form autophagosomes. Autophagosomes predominantly fuse with the late endosomes to form amphisomes and subsequently with the lysosomes to form autolysosomes where the autophagic cargo is degraded by the lysosomal hydrolases. Autophagy can be stimulated by chemical inducers acting via the mTOR-dependent and mTOR-independent pathways regulating autophagy. Defects in autophagic flux at the autophagosome formation and maturation stages are indicated.

three types of autophagy: macroautophagy (herein referred to as autophagy), microautophagy and chaperone-mediated autophagy (CMA); each type requires functional lysosomes in the final stage for degradation of the cargo [12]. Consequently, disruption of the hydrolytic functions of lysosomes impairs autophagic flux and, conversely, lysosomal function probably requires normal flux through autophagy [13]. Deregulation of autophagy is a common disease mechanism in many LSDs [14,15]. This review will describe the connections between autophagy and the LSDs, highlight common stages of autophagy disrupted in different disorders and discuss autophagy as a potential therapeutic intervention for treating certain LSDs.

Autophagy machinery and signalling

Autophagy encompasses several vesicle fusion events leading to the eventual degradation of its cargo; a dynamic process termed autophagic flux (Figure 1). Multiple autophagy (*Atg*) genes encoding components of the autophagic machinery are required for the initiation of autophagy, which is marked by the *de novo* formation of double-membrane structures called phagophores. Two ubiquitin-like conjugation systems involving the Atg5–Atg12–Atg16 complex and phosphatidylethanolamine-conjugated microtubule-associated protein 1 light chain 3 (LC3-II) are required for the initiation step [16,17]. Cytosolic components such as macromolecules (including specific substrates like p62) and organelles (including mitochondria) are sequestered in the expanding phagophores to form double-membrane vesicles called autophagosomes. LC3-II associates with autophagosome membranes throughout their lifespan and is hence used as a marker for autophagy [18]. Autophagosomes then undergo maturation into autolysosomes by one of two routes: the predominant route is a multi-step process, in which autophagosomes fuse first with late endosomes to form amphisomes, then subsequently with lysosomes; alternatively, autophagosomes can fuse directly with lysosomes [19,20]. This enables delivery of the autophagic cargo to the autolysosomes where these materials are degraded by acidic lysosomal hydrolases (Figure 1). The breakdown products are then exported via lysosomal transporters for recycling [21].

There are several molecular mediators of autophagosome maturation, including Rab7, Beclin1–Vps34–Vps15 complex and SNAREs (*N*-ethylmaleimide-sensitive factor-attachment protein receptors). Beclin1-interacting partners like Atg14L, Ambra1 and UVRAG promote autophagosome maturation whereas Rubicon inhibits this step [20,22,23]. Recently, gene knockout studies in mammalian cells have shown that the Atg8 family proteins (LC3 and GABARAP subfamilies) are crucial for autophagosome–lysosome fusion [24]. GABARAPs were found to preferentially recruit PLEKHM1 (Pleckstrin homology domain containing protein family member 1) [24], which associates with the homotypic fusion and protein sorting (HOPS) complex to mediate autophagosome maturation [25]. The SNARE proteins are critical for membrane tethering and fusion; for example, autophagosomal Syntaxin-17, Atg14 and SNAP-29 interact with late endosomal/lysosomal VAMP8 to mediate autophagosome maturation [26-28]. Upon autolysosome

formation, the ATG conjugation system has been suggested to play a role in the degradation of the inner autophagosomal membrane [29]. The late stage of autophagy involving autophagosome maturation is primarily affected in many LSDs; the perturbations in the autophagy pathway in these conditions are described in subsequent sections (Table 1).

Transcriptional regulation of autophagy occurs via the transcription factor EB (TFEB), which drives the expression of genes related to autophagy and lysosomal biogenesis [30,31]. In turn, Ca^{2+} stored in the lysosomal lumen has been shown to modulate autophagy by promoting the nuclear localization of TFEB through the activation of calcineurin [32]. Various signalling pathways influence autophagy by acting upstream of the autophagic machinery. These include the mechanistic target of rapamycin complex 1 (mTORC1) signalling pathway (through which growth factors, nutrients and energy status influence autophagy) and mTOR-independent pathways (including inositol and IP_3, cAMP, Ca^{2+}, calpain) that negatively regulate autophagy [12,33-38]. Both mTOR-dependent and mTOR-independent signalling are amenable to chemical perturbations for modulating autophagy [34,37,39-41] (Figure 1). For instance, the mTORC1 inhibitors like rapamycin and torin1, and mTOR-independent compounds including trehalose and carbamazepine can stimulate autophagy [35,42-44]. Chemical inducers of autophagy have been tested in some LSDs as a possible therapeutic intervention (Table 2) and are described below.

Lysosomal storage diseases associated with defective autophagy

LSDs are caused by abnormal lysosomal function leading to accumulation of undegraded metabolites [4-7]. The composition of accumulated materials in the lysosomes varies substantially between the LSDs and, while all LSDs are inherited conditions and in many cases mutations are found in genes encoding lysosomal proteins, the types of proteins affected are also varied. As a consequence, the underlying cell biology changes occurring in the LSDs may vary, but each results (directly or indirectly) in reduced clearance of aggregates and diminished cellular homeostasis and survival. Autophagic dysregulation is commonly found in LSDs but again, a spectrum of defects is seen at various stages of the autophagic pathway in different LSDs (Table 1) [14,15]. Here, we will concentrate on some of the better-studied LSDs and detail the different autophagic defects identified.

Neuronal ceroid lipofuscinosis

While defective autophagy has been implicated in the pathology of many different disorders, including cancer and various cardiovascular, metabolic, pulmonary and infectious diseases [10,45], the nervous system appears to be particularly susceptible [46,47]. This is likely due to a combination of the long-lived nature of post-mitotic neurons placing particular stress on protein-clearing processes, the extreme polarization of many neurons and the high metabolic requirements of neurons leading to higher levels of oxidative damage in lysosomes via the Fenton reaction. Consistent with this, dysregulated autophagy has been identified in almost every late-onset neurodegenerative disorder [34,47,48], and similar defects are now being recognized in the neuronal ceroid lipofuscinoses (NCLs), a sub-group of the LSDs that are collectively the most common causes of childhood-onset neurodegeneration [5,49,50]. To date, 13 disease-causing genes have been identified to cause NCL pathology. Several of these genes encode lysosomal proteins, including soluble enzymes/proteins (CLN1/PPT1, CLN2/TPP1, CLN5, CLN10/CTSD and CLN13/CTSF) and membrane proteins (CLN3, CLN7/MFSD8 and CLN12/ATP13A2), but others include endoplasmic reticulum (ER) membrane proteins (CLN6 and CLN8), cytosolic proteins (CLN4/DNAJC5 and CLN14/KCTD7) and one expressed in the secretory pathway (CLN11/GRN) [51-53]. The diverse nature of these proteins, their substrates or cargos and locations within the cell are reflective of the LSDs as a whole: multiple different cell biological processes are probably affected but with each terminating in common pathology. However, as autophagic perturbations start to be identified in NCL disease models (Figure 2), the possibility that each disease hinges on defective autophagy becomes more plausible.

Several studies in NCL mouse models have indicated deregulation of autophagy *in vivo*. A recent study in *Cln7*-deficient mice reported accumulation of autophagosomes and autophagic substrates coupled with lysosomal dysfunction in the brain, suggesting that a block in autophagic flux may be occurring [54]. A similar build-up of autophagic compartments and substrates was also seen in mouse models of CLN5 and CLN6 diseases, as well as in CLN6 patient-derived fibroblasts [55-57]. The most common form of NCL is Batten disease, caused by mutations in the membrane protein, CLN3 [51]. Defective autophagosome maturation associated with increased autophagic and lysosomal compartments was observed in the *Cln3*-deficient mouse that models Batten disease, and also in patient fibroblasts as well as patient-specific induced pluripotent stem cell (iPSC)-derived neuronal cells [58-60]. The mechanism underpinning the changes to autophagy in CLN3-deficient cells is not fully understood, and the functions of the CLN3 protein remain unknown, but one potential mechanism may be due to altered Ca^{2+} homeostasis and

PORTLAND PRESS

Table 1 Overview of defective autophagy in lysosomal storage disorders

Disease	Gene	Protein	Function	Storage material	Autophagy phenotype	Autophagic flux	Mechanism
NEURONAL CEROID LIPOFUSCINOSES							
CLN2	CLN2/TPP1	Tripeptidyl peptidase 1	Serine protease	ATPase subunit c, lipofuscin	Inhibition of autophagosome formation; Reduction in autophagosomes and autophagic degradation [60]	Inhibition	Up-regulation of mTOR signalling [60]
CLN3	CLN3	CLN3	Unknown function; Lysosomal membrane protein	ATPase subunit c, lipofuscin	Defect in autophagosome maturation; Accumulation of autophagosomes and autophagic cargo [58-60]	Block	Not known; Possibly due to alteration in Ca^{2+} homeostasis [61] and deregulation of ARF1–Cdc42 pathway [62]
CLN5	CLN5	CLN5	Unknown function; Lysosomal protein	ATPase subunit c, lipofuscin	Accumulation of autophagosomes and autophagic cargo [56]	Block	Not known
CLN6	CLN6	CLN6	Unknown function; ER membrane protein	ATPase subunit c, lipofuscin	Accumulation of autophagosomes and autophagic cargo [55,57]	Block	Not known
CLN7	CLN7	CLN7	Putative lysosomal transporter	ATPase subunit c, lipofuscin	Accumulation of autophagosomes and autophagic cargo [56]	Block	Not known; Possibly due to impairment in lysosomal function [54]
CLN10	CLN10/CTSD	Cathepsin D	Aspartyl protease	ATPase subunit c, saposins A/D, lipofuscin	Accumulation of autophagosomes and autophagic cargo [64,66]	Block	Not known; Possibly due to loss of cathepsin D function [64]
SPHINGOLIPIDOSES							
Niemann–Pick type C1	NPC1	NPC1	Cholesterol transporter	Unesterified cholesterol, sphingolipids	Defect in autophagosome maturation; Accumulation of autophagosomes and autophagic cargo [73-80]	Block	Disruption in SNARE machinery [73]; Reduction in sphingosine kinase activity and VEGF [80]
Niemann–Pick type C2	NPC2	NPC2	Putative role in cholesterol metabolism and transport	Unesterified cholesterol, sphingolipids	Accumulation of autophagosomes and autophagic cargo [84]	Block	Not known; Possibly due to impairment in lysosomal function [84]
Gaucher disease	GBA1	Glucocerebrosidase	Sphingolipid degradation	Glucosylceramide	Defect in autophagosome maturation; Accumulation of autophagosomes and autophagic cargo [91,93-97]	Block	Not known; Possibly due to down-regulation of TFEB and reduction in lysosomes [91]
	PSAP	Prosaposin, saposin C	Sphingolipid hydrolase cofactor	Glucosylceramide	Defect in autophagosome maturation; Accumulation of autophagosomes and autophagic cargo [92,93]	Block	Not known; Possibly due to reduction in cathepsin B/D activity [92]
Mucolipidosis type IV	MCOLN1	TRPML1	Late endo-lysosomal Ca^{2+} transporter	Gangliosides, phospholipids, mucopolysaccharides	Accumulation of autophagosomes and autophagic cargo [104,105,109]	Block	Not known; Possibly due to impairment in lysosomal function [107]
GLYCOGENOSES							
Pompe disease	GAA	Acid α-glucosidase	Glycogen degradation	Glycogen	Accumulation of autophagosomes and autophagic cargo [116-118]	Block	Not known; Possibly due to defects in lysosomal acidification [116]

736

Table 1 Overview of defective autophagy in lysosomal storage disorders (Continued)

Disease	Gene	Protein	Function	Storage material	Autophagy phenotype	Autophagic flux	Mechanism
Danon disease	*LAMP2*	Isoform LAMP2b	Putative role in autophagosome–lysosome fusion	Glycogen	Accumulation of autophagosomes and autophagic cargo [123-126]	Block	Not known; Possibly due to defects in lysosomal function [124]
X-linked myopathy with excessive autophagy	*VMA21*	VMA21	Regulates v-ATPase	Glycogen	Accumulation of autophagosomes [128,129]	Block	Not known; Possibly due to defects in lysosomal acidification and function [128]

The list in Table 1 highlights selected LSDs where defective autophagy has been demonstrated.

Table 2 Beneficial effects of the chemical inducers of autophagy in models of lysosomal storage disorders

Autophagy inducer	Mechanism of autophagy induction	LSD	Beneficial effects in LSD models
mTOR-DEPENDENT AUTOPHAGY INDUCER			
Rapamycin [42]	Inhibition of mTORC1 [42]	NPC1	Rescue of autophagic flux and improvement in cell viability in mutant *Npc1* MEFs [73], mouse neurons with *Npc1* knockdown [73] and NPC1 patient iPSC-derived neuronal and hepatic cells [74]
		NPB	Reduction in mitochondrial ROS and lipid droplets, and induction of lysosomal exocytosis in NPB patient B lymphocytes [148]
		PD	Reduction in muscle glycogen in *Gaa*-deficient mice when treated together with recombinant human GAA [167]; Improved autophagic flux and GAA maturation in Pompe disease patient myotubes [117]
		GD	Improvement in lifespan and locomotor activity in GD *Drosophila* model [97]
mTOR-INDEPENDENT AUTOPHAGY INDUCER			
Trehalose [44]	Inhibition of SLC2A glucose transporters [145]; Activation of TFEB by Akt inhibition [144]	NPC1	Rescue of autophagic flux and improvement in cell viability in NPC1 patient iPSC-derived neurons [74]
		CLN3	Clearance of ceroid lipopigment deposits in CLN3 patient fibroblasts, and attenuation of neuropathology and extension of lifespan in *Cln3*-deficient mice [144]
Carbamazepine [35]	Reduction in inositol and IP$_3$ levels [35]	NPC1	Rescue of autophagic flux and improvement in cell viability in NPC1 patient iPSC-derived neurons and hepatic cells [74]
Lithium [35]	Inhibition of IMPase and reduction in inositol and IP$_3$ levels [35]	NPC1	Rescue of autophagic flux in mutant *Npc1* MEFs [73]
		CLN3	Rescue of autophagic flux and improvement in cell viability in mutant *Cln3* cerebellar cells [146]
L-690,330 [35]	Inhibition of IMPase and reduction in inositol and IP$_3$ levels [35]	CLN3	Rescue of autophagic flux and improvement in cell viability in mutant *Cln3* cerebellar cells [146]
Verapamil [36]	Inhibition of L-type Ca^{2+} channel and reduction in cytosolic Ca^{2+} [36]	NPC1	Rescue of autophagic flux and improvement in cell viability in NPC1 patient iPSC-derived neurons [74]
BRD2716, BRD5631, BRD34009 [142]	Unknown	NPC1	Rescue of autophagic flux and improvement in cell viability in NPC1 patient iPSC-derived neurons [142]

Abbreviations: CLN, ceroid lipofuscinosis neuronal disease; GD, Gaucher disease; IMPase, inositol monophosphatase; IP3, inositol 1,4,5-trisphosphate; iPSC, induced pluripotent stem cells; LSD, lysosomal storage disorder; mTORC1, mechanistic target of rapamycin complex I; NPB, Niemann–Pick type B disease; NPC1, Niemann–Pick type C1 disease; PD, Pompe disease; SLC2A, Solute carrier 2A; TFEB, Transcription factor EB.
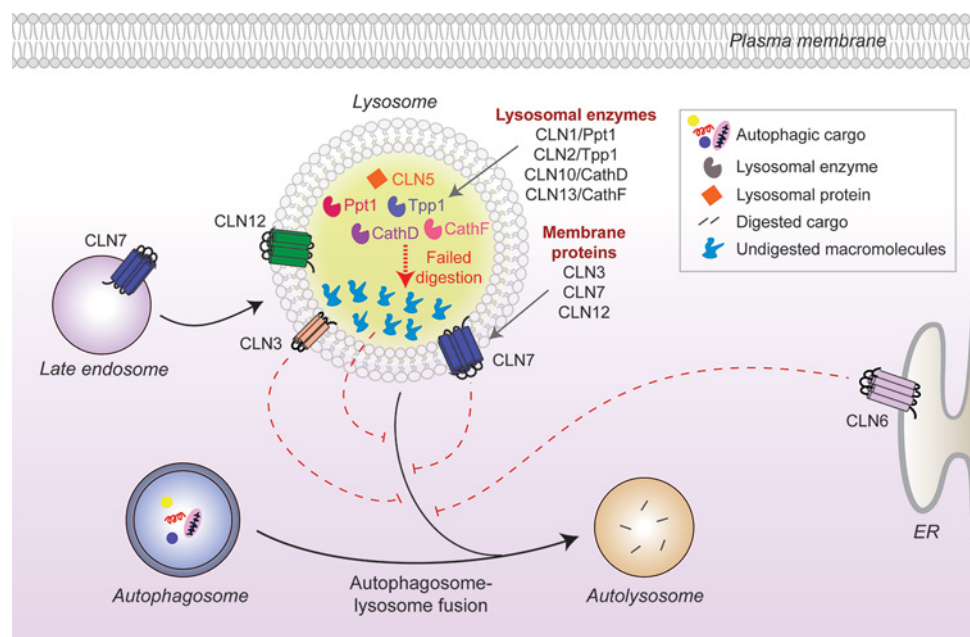
Figure 2. CLN protein distribution and their link to autophagy defects in neuronal ceroid lipofuscinoses
Many CLN proteins reside in the lysosomal matrix (CLN1, 2, 5, 10, 13) or at the lysosomal membrane (CLN3, 7), while others localize to different cellular compartments such as the ER membrane (CLN6). Disease-causing mutations in some of the CLN proteins inhibit autophagosome maturation (dashed red lines) and block autophagic flux, but the underlying mechanisms are unknown. Mutated lysosomal hydrolases (CLN1, 2, 10, 13) are unable to degrade autophagic cargo, which subsequently accumulate and impair lysosomal function.

deregulation of the ARF1–Cdc42 pathway identified in *CLN3* mutant cells that can impact on vesicular trafficking [61,62].

Several forms of NCL are caused by mutations in lysosomal proteases, including Ppt1 (CLN1), Ppt2 (CLN2) and Cathepsin D (CLN10) [53,63]. Loss of cathepsin function commonly leads to dysregulated autophagy, presumably by reducing autophagic flux due to defective cargo clearance. Autophagosomes and mitochondrial proteins accumulate in cultured neurons of mice lacking cathepsin D or B and L [64-66], while *in vivo*, cathepsin D-deficient mice exhibit widespread accumulation of storage material in the lysosomes (indicating lysosomal dysfunction) and accumulation of autophagosomes. These changes likely lead to the neurodegeneration present in these mice that mimics the human disease [66]. However, a reduction in autophagic flux has been reported in CLN2 patient fibroblasts due to inhibition of autophagosome formation, which is linked to increased ROS and Akt–mTOR signalling pathway [60].

Sphingolipidoses

Sphingolipids are a major class of lipids enriched in the nervous system and are critical for neural development and function [67,68]. Sphingolipid turnover is therefore tightly regulated with their degradation mediated by a multi-step process requiring numerous lysosomal hydrolases [69]. The sphingolipidoses are a class of LSDs caused by deficiencies in functional hydrolases, culminating in the accumulation of wholly or partially undegraded sphingolipids [68]. Members of the sphingolipidoses include Niemann–Pick, Gaucher and Fabry diseases, mucolipidosis, and GM1/2 gangliosidoses such as Tay-Sachs and Sandhoff diseases [68]. Alterations in autophagy have been reported in some of the sphingolipidoses; the most extensively studied being Niemann–Pick type C1 (NPC1) disease.

Niemann–Pick disease

Niemann–Pick disease is a recessively inherited neurodegenerative condition characterized by an accumulation of unesterified cholesterol deposits in various tissues, particularly the brain. The disease is subdivided into types A, B, C1 and C2. Types A and B are caused by mutations in *SMPD1*, leading to loss of acid sphingomyelinase activity, whereas types C1 and C2 are due to defective cholesterol transport [70]. Ninety-five per cent of cases are attributed to
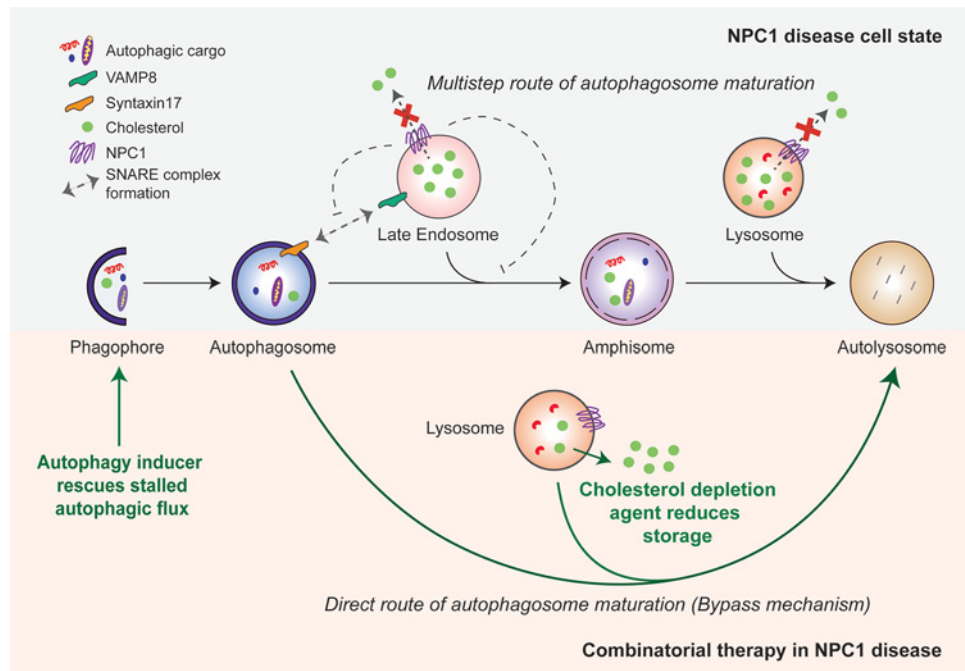
Figure 3. Autophagy defects in NPC1 disease and the bypass mechanism of autophagosome maturation for restoring autophagic flux

Mutant NPC1 protein prevents cholesterol efflux from the endo-lysosomal compartments and impairs autophagosome maturation in the multi-step route due to failure in the SNARE machinery. Induction of autophagy by chemical inducers bypasses this block and restores autophagic flux via direct autophagosome–lysosome fusion. A combinatorial treatment strategy is shown with cholesterol depletion agents. The green arrows indicate therapeutic effects of autophagy induction and cholesterol depletion.

mutations in the lysosomal cholesterol transporter NPC1, with the lysosomal glycoprotein NPC2 that possibly aids in cholesterol trafficking affected in the remainder [71,72].

A number of studies have demonstrated a major role for autophagy in the pathology of NPC1 disease and potentially in treatment options. Various experimental platforms, including *Npc1* mutant mice and disease-relevant cells (such as neurons) differentiated from human embryonic stem cells (hESCs) with *NPC1* knockdown or patient-derived iPSCs, have revealed an accumulation of autophagosomes and lysosomes both *in vivo* and *in vitro* [73-80]. We previously reported that this autophagy phenotype is related to a block in autophagic flux (Figure 3). Consistent with this, we characterized a failure in the Syntaxin17/VAMP8 SNARE machinery that retards amphisome formation and significantly stalls the multi-step route of autophagosome maturation [73]. While it is possible that loss-of-function of the NPC1 protein, which normally resides on the late endosomal/lysosomal compartments, directly perturbs amphisome formation, accumulation of lysosomal cholesterol has also been reported to cause aberrant sequestration of SNAREs and prevent autophagosome maturation [81]. Another study, although consistent with dysfunctional autophagosome maturation, reported a distinct mechanism in which diminished sphingosine kinase activity and reduced levels of vascular endothelial growth factor (VEGF) lead to the accumulation of sphingosine that, in turn, impairs autophagic flux [80]. In addition to these mechanisms, depletion of lysosomal Ca^{2+} stores due to sphingosine storage was seen in NPC1 disease models, and this could possibly influence autophagy via calcineurin or calpain [32,36,82,83]. Similar to the autophagy phenotype in NPC1 disease, a block in autophagic flux has been reported in NPC2 disease as evident by the accumulation of autophagosomes and autophagic substrates as well as impaired lysosomal activity in *Npc2*-knockdown mouse adipocytes [84].

The reduced cell viability seen in NPC1 mouse and patient-specific iPSC models has been attributed to dysregulated autophagy [73,74,78,80]. Importantly, since autophagy regulates the clearance of lipids including cholesterol (via lipophagy), autophagy-deficient $Atg5^{-/-}$ cells also exhibited accumulation of these materials similar to the cellular phenotypes observed in NPC1 disease [73,85]. Therefore, the underlying autophagy defects in NPC1 disease can further aggravate the accumulation of lipids and cholesterol, and thus, defective autophagy should be considered a major disease mechanism in Niemann–Pick disease.
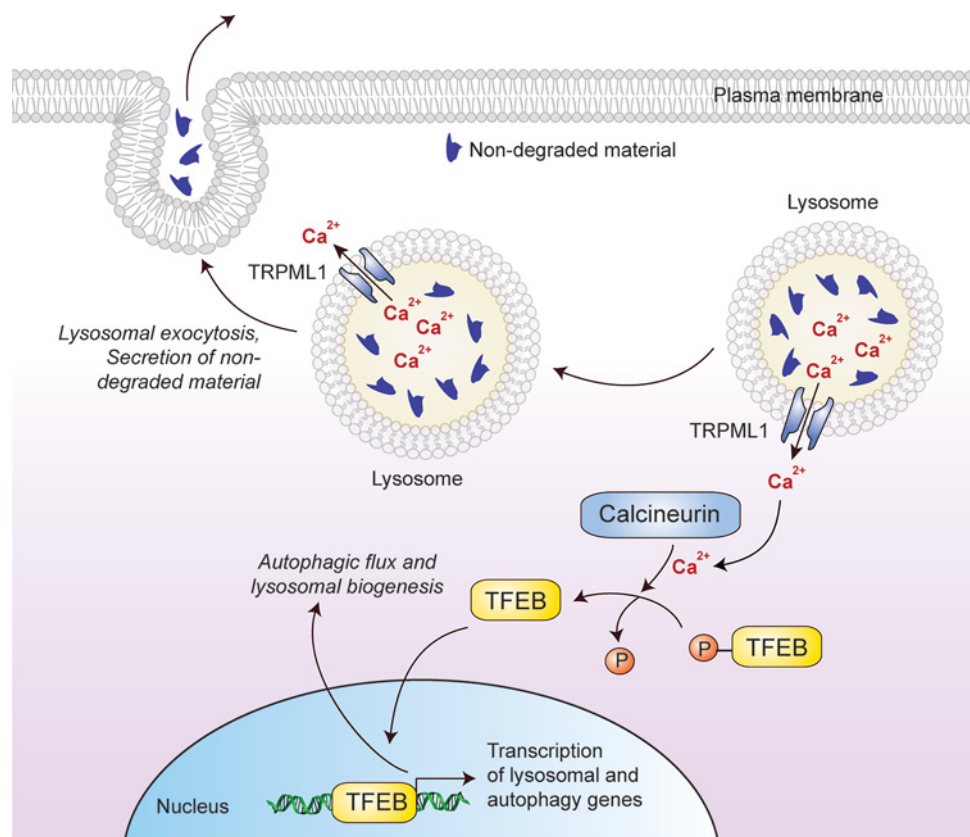
Figure 4. Cellular effects of TFEB that might be of therapeutic benefit in lysosomal storage disorders

Lysosomal Ca^{2+} efflux through TRPML1 activates the Ca^{2+}-dependent phosphatase calcineurin, which mediates dephosphorylation-dependent nuclear translocation of TFEB. Nuclear TFEB up-regulates the transcription of genes involved in lysosome biogenesis and autophagy, thereby enhancing autophagic flux. In addition, Ca^{2+} efflux from peripheral lysosomes promotes lysosomal exocytosis and the secretion of non-degraded materials.

Gaucher disease

Gaucher disease is the most common form of sphingolipidosis [86], and is caused by a deficiency in glucocerebrosidase (GCase) activity which catalyses the final step in glycosphingolipid degradation [87]. The loss of enzyme function in Gaucher disease directly impairs this process leading to accumulation of glucocerebroside and widespread pathology that particularly affects the liver and, in severe cases, the CNS [88]. Gaucher disease is most frequently caused by mutations in the *GBA1* gene which encodes GCase [87]; however, there are rare cases of Gaucher disease caused instead by mutations in the *PSAP* gene [89,90]. *PSAP* encodes the lysosomal glycoprotein prosaposin, the precursor saposins A-D, which are essential cofactors for various sphingolipid hydrolases. Saposin C is required for GCase activity, and thus mutations in *PSAP* that lead to saposin C deficiency also cause Gaucher disease [89,90].

Various lines of evidence suggest defective autophagy in Gaucher disease. Impaired autophagosome maturation and down-regulation of TFEB, including a reduction in lysosomal gene expression, were found in neurons differentiated from patient-specific iPSCs [91]. A similar impairment in autophagosome degradation was also seen in primary fibroblasts deficient in saposin C; in this case associated with reduced cathepsin B/D activity [92]. Mouse models of Gaucher disease have also revealed similar autophagy phenotypes: accumulation of various autophagic cargo such as dysfunctional mitochondria, ubiquitinated protein aggregates, insoluble α-synuclein and p62, together with autophagosomes and lysosomes were found in the brain or neurons and astrocytes cultured from mice deficient for *Gba*, *Psap* or glucosylceramidase [93-96]. Recently, a *Drosophila* model of neuropathic Gaucher disease generated by knocking out the *Gba* gene revealed severe lysosomal defects, GCase accumulation and a block of autophagic flux in the brain, resulting in reduced lifespan, neurodegeneration and age-dependent locomotor deficits [97]. Together, multiple model systems highlight deregulation of autophagy in Gaucher disease.

Mucolipidosis type IV

Mucolipidosis type IV (MLIV) is a neurodegenerative condition, caused by mutations in the *MCOLN1* gene that result in the loss of TRPML1 function [98,99]. TRPML1 is an inward rectifying, non-selective cation channel of the transient receptor potential family that transports divalent cations, such as Ca^{2+}, Fe^{2+} and Zn^{2+}, from the lysosomal intraluminal space into the cytosol [100-102]. TRPML1 and TFEB positively regulate each other and together have the potential to impact on autophagy: Ca^{2+} release by TRPML1 activates the Ca^{2+}-dependent phosphatase, Calcineurin, which mediates dephosphorylation-dependent nuclear translocation of TFEB leading, in turn, to increased expression of several lysosomal and autophagy-related proteins including TRPML1 [32] (Figure 4). Indeed, enhancing TRPML1 activity either by overexpression or by pharmacological stimulation increases autophagic flux [103].

Several models of MLIV have highlighted a defect in autophagy. Accumulation of lysosomes, autophagosomes and autophagy substrates including polyubiquitinated protein aggregates was reported in patient-derived primary fibroblasts, in *Mcoln1*-deficient mice, and in *Caenorhabditis elegans* and *Drosophila* models [104-108]. These studies also implicated a role for wild-type TRPML1 in regulating lysosomal acidification and autophagosome maturation (alkalinization of lysosomes, for instance by inhibiting the vacuolar H^+ATPase, inhibits hydrolase function). Furthermore, defects in CMA have been reported in MLIV, possibly through the interaction between TRPML1 with the molecular chaperones Hsc70 and Hsc40 that are required for protein translocation to the lysosome during CMA [109].

Glycogenoses

The glycogenoses are LSDs with profound autophagy defects that particularly affect skeletal and cardiac muscle [110,111]. As such, these diseases are often referred to as autophagic vacuolar myopathies (AVMs), although neuropathology is also present in some cases [112]. Diseases that lead to AVM are linked to genes involved in lysosome acidification, glycogen hydrolysis, and autophagosome maturation and fusion with lysosomes [113]. They include Pompe disease, Danon disease and X-linked myopathy with excessive autophagy (XMEA).

Pompe disease

Pompe disease is caused by mutations in acid α-glucosidase (GAA), a lysosomal glycogen hydrolase [114]. Enzyme activity is completely absent in Pompe disease and consequently the accumulation of intra-lysosomal glycogen, as well as of autophagosomes, is a hallmark of the disease [115]. Primary myoblasts from *Gaa*-deficient mice have enlarged endosomes, lysosomes and autophagic vacuoles, in addition to delayed endosomal acidification and mobility [116]. These features have been attributed to a block in autophagic flux since elevated levels of the autophagosomal marker LC3-II and autophagic substrate p62 were observed in *Gaa*-deficient mice and in the myotubes of infantile-onset patients [117,118]. Interestingly, autophagy has been shown to play a role in the maturation of GAA [117] and the clearance of glycogen [119,120], and thus, defective autophagy is implicated as a disease mechanism in Pompe disease.

Danon disease

Danon disease is an X-linked disorder caused by mutations in the *LAMP2* gene, which encodes a lysosomal membrane protein [121]. While LAMP2a is required for protein translocation in CMA [122], mutations in the LAMP2b isoform are associated with Danon disease [121]. Various tissues including liver, skeletal and heart muscle from *Lamp2*-deficient mice displayed accumulation of autophagic compartments, along with a decline in the lysosomal degradation of long-lived proteins in the hepatocytes that are unresponsive to amino acid starvation [123,124]. Muscle biopsies from patients also exhibit accumulation of LC3-II$^+$ autophagic vesicles and large p62 aggregates [125]. These studies point to a likely block in flux affecting the late stage of autophagy. Consistent with this scenario, impaired autophagy associated with defective mitochondrial clearance (mitophagy) was found in cardiomyocytes differentiated from patient-specific iPSC cells with *LAMP2* mutations as well as in *Lamp2*-deficient mice [126].

X-linked myopathy with excessive autophagy

X-linked myopathy with excessive autophagy (XMEA) is characterized by progressive vacuolation and atrophy of skeletal muscle, and is caused by mutations in the *VMA21* gene [127]. The VMA21 protein regulates the assembly of the vacuolar ATPase (v-ATPase) required for lysosomal acidification, and thus XMEA is associated with a reduction in the activity of lysosomal hydrolases [128]. Skeletal muscle biopsies show diminished lysosomal degradation and accumulation of autophagic compartments, suggesting a likely block in autophagic flux [128,129].

Why is defective autophagy relevant for LSD pathology?

Deregulation of autophagy has been observed in myriad human diseases including many forms of cancer and neurodegeneration [10,45,47]. Despite appearing at first glance to have diverse changes in both the cell biology and the genetics underpinning them, deregulated autophagy has been reported in most (or possibly all) LSDs where investigated (Table 1) [14,15]. Potentially, dysfunctional autophagy is the common link between the LSDs but why is impaired autophagy such an important component of disease and how does it contribute to cell death? Clues have emerged from *in* vivo experiments aimed at depleting basal autophagy in a tissue-specific manner in mice. These result in degeneration and dysfunction of the targeted organ, indicating an essential role for autophagy in maintaining tissue homeostasis [130]. For example, brain-specific deletion of *Atg5* or *Atg7* in mice results in a neurodegenerative phenotype in the absence of any disease-causing, aggregation-prone proteins [131,132]. These genetic studies suggest that the decline in autophagic capability in the LSDs is likely a major contributory factor to the neuropathology that characterizes many of these disorders. Secondly, some of the non-degraded materials in LSDs such as lipids, cholesterol and glycogen are cleared by autophagy [85,119,120]. Hence, deficient autophagy will contribute to the build-up of these components with ever increasing impact on lysosomal function and autophagic flux. For example, as discussed in NPC1 disease where lipids and cholesterol are the primary non-degraded materials, impairment in autophagy will lead to a further accumulation of these macromolecules by reducing lipophagy [73]. This is because lipophagy is retarded due to loss of autophagy, as evident in autophagy-deficient mouse models [85,133]. Finally, impairment in functional autophagic flux can retard the clearance of autophagic cargo including damaged mitochondria and other undesirable materials, which leads to enhanced oxidative damage of lipids and proteins within the lysosomal membrane and a further reduction in efficiency of clearance [134-137]. Therefore, targeting this common pathway underlying several LSDs could be a promising therapeutic intervention.

Autophagy as a potential therapeutic intervention for LSDs

Autophagy has been exploited for therapeutic benefits in diverse transgenic models of human diseases, including several neurodegenerative disorders, certain liver diseases, myopathies and infectious diseases [34,40,41,138,139]. In most of the conditions studied, induction of autophagy ameliorated the disease phenotypes and, in many cases, improved organismal longevity [140,141]. Multiple lines of evidence suggest that autophagy could also be targeted in LSDs as a treatment strategy [14,15] (Table 2). The most suitable disease contexts for this approach are likely to be LSDs where the lysosomal hydrolytic function is not overtly compromised (e.g., by direct mutation of an enzyme) so that the accumulated autophagic cargo can still be digested efficiently if autophagic flux is stimulated. Some of the mechanisms of how autophagy might be beneficial in LSDs are discussed below.

Bypassing stalled autophagosome maturation by small-molecule autophagy inducers

The dysregulated autophagy underpinning many of the LSDs occurs primarily due to failure of autophagosome maturation, which stalls autophagic flux (Table 1). This phenotype probably arises due to a block in the multi-step route of autophagosome maturation. However, this block may not be absolute because we have recently characterized a bypass mechanism in NPC1 disease models which restores functional autophagic flux [73]. We have shown that induction of autophagy facilitates direct autophagosome–lysosome fusion without requiring the formation of amphisomes in the multi-step route (Figure 3). Restoration of autophagic flux enables cargo clearance, which correlates with improved cell viability in primary mouse neurons with *Npc1* knockdown and in NPC1 patient iPSC-derived neuronal and hepatic cells [73,74]. Interestingly, we observed cell-type specificity of small molecule autophagy enhancers in these patient-derived cells: rapamycin and carbamazepine rescued cell death and defective autophagy in both iPSC-derived neuronal and hepatic cells but other autophagy inducers including trehalose, verapamil, BRD2716, BRD5631 and BRD34009 were effective only in neurons [74,142]. Strikingly, enhanced autophagic flux did not reduce the cholesterol load in NPC1 mutant cells, meaning a combination strategy coupling autophagy induction with low doses of a cholesterol-depletion agent such as 2-hydroxypropyl-β-cyclodexterin (HPβCD) may be especially effective for NPC1 disease [73,74,143].

Treatment with autophagy inducers has also been found to be beneficial in other LSDs, including CLN3 disease (Batten disease). Administration of trehalose, an mTOR-independent autophagy inducer [44], in a mouse model of CLN3 disease promoted cellular clearance of undegraded materials, attenuated the neuropathology and extended lifespan [144]. Interestingly, although the autophagy-inducing property of trehalose has been recently attributed to the inhibition of the SLC2A family of glucose transporters [145], trehalose was found to activate TFEB by inhibiting Akt independently of mTORC1 [144]. Furthermore, inositol monophosphatase (IMPase) inhibitors such as lithium

and L-690,330, which induce mTOR-independent autophagy by reducing inositol and IP$_3$ levels [35], rescued autophagic flux and improved cell viability in mutant *Cln3* cerebellar cells [146]. However, further studies are required to determine whether the bypass mechanism of restoring autophagic flux is mediating the beneficial effects of autophagy induction in these contexts.

Induction of autophagy via the classical mTORC1 pathway also had some beneficial effects in LSD models. Although TORC1 activity is dysregulated in a *Drosophila* model of Gaucher disease [97], the mTORC1 inhibitor rapamycin [42] partially corrects the shortened lifespan and locomotor defects [97], whereas reactivation of dysregulated mTORC1 by knockdown of the Rheb inhibitor, TSC2, in a mouse model of Pompe disease rescued the muscular atrophy and autophagy defects [147]. Rapamycin, while commonly used in research contexts, particular in cell-based assays *in vitro*, is unlikely to be useful clinically in all LSDs, in part because its efficacy appears to be context-dependent *in vivo* and subject to feedback loops. Even *in vitro* rapamycin has differential effects. For example, it reduces mitochondrial ROS and lipid droplets and induces lysosomal exocytosis in Niemann–Pick type B lymphocytes [148], and improves autophagic flux and GAA maturation in Pompe disease patient myotubes [117], but is toxic in neuronal cells differentiated from Gaucher disease patient-derived iPSCs [91]. Since mTOR governs critical cellular function like cell growth and translation [38], the use of mTOR inhibitors for induction of autophagy can produce potential side-effects [149]. Consequently, the mTOR-independent autophagy inducers are considered more desirable for clinical applications in patients [34,40,41,138].

TFEB promotes lysosomal biogenesis and exocytosis

Genetic up-regulation of autophagy via overexpression of TFEB has shown potential as a therapeutic strategy. Overexpression of TFEB enhances the clearance of proteinaceous aggregates in transgenic models of late-onset neurodegeneration disorders [150-152], and improves autophagy and lysosomal defects while rescuing disease severity in some LSDs [153,154]. Apart from mediating the transcription of genes required for autophagy and lysosomal biogenesis [30,31], TFEB also facilitates lysosomal exocytosis via the recruitment and fusion of the lysosomes to the plasma membrane by increasing local Ca^{2+} concentrations through its target, the TRPML1/MCOLN1 cation channel (Figure 4) [32,155]. This mechanism may underpin some of the beneficial effects of overexpression because enhanced secretion may enable the clearance of pathologically enlarged lysosomes and undegraded materials associated with LSDs that have an impact on autophagic flux.

Experimentally, overexpression of TFEB reduces glycogen load and the size of lysosomes, and promotes autophagosome maturation and lysosomal exocytosis in immortalized myogenic cells from Pompe disease patients [156] and rescues tissue pathology *in vivo* in autophagy-dependent manner [155,156]. In addition, overexpression of TFEB together with GAA cooperatively facilitated glycogen clearance and improved pathology in skeletal muscles differentiated from Pompe disease patient-derived iPSCs [157]. Similarly, lysosomal exocytosis and reduction in cellular vacuolization occur after TFEB overexpression in various cell and mouse models of LSDs, including multiple sulfatase deficiency (MSD) and mucopolysaccharidosis type IIIA (MPS-IIIA) where a block in autophagy was previously reported [155,158]. Overexpression of TFEB also causes reduction in substrate accumulation in various cell models of Pompe disease, MSD, MPS-II, MPS-IIIA and Batten disease [150,155,156], although it did not rescue the lysosomal depletion phenotype in neuronal cells differentiated from Gaucher disease patient-derived iPSCs [91]. However, TFEB did enhance lysosomal biogenesis in the presence of recombinant GCase in these cells [91]. Taken together, these studies highlight TFEB as a promising therapeutic target with the potential to be manipulated in multiple LSDs.

Combining ERT with autophagy induction

Enzyme replacement therapy (ERT) to replace the absent or non-functional hydrolytic enzyme is approved for patients with Gaucher, Pompe and Fabry diseases and some mucopolysaccharidoses, including MPS type I [159,160]. For Gaucher disease, ERT for the GCase enzyme is a relatively mature therapy with several drugs on the market, including imiglucerase, velaglucerase alfa and taliglucerase alfa [161-163], along with two substrate reduction therapies, miglustat and eliglustat [164,165]. Recent data demonstrate that ERT for the GAA enzyme deficient in Pompe disease improves muscle mass and autophagic flux in muscle biopsies of some patients after treatment [117,166]. As with the Gaucher disease experiments described above [91], combination strategies that include induction of autophagy may prove more effective in Pompe disease and similar LSDs than ERT alone, in this case possibly by stimulating clearance of the accumulating glycogen and facilitating GAA maturation [117,157]. Indeed, experiments with a Pompe mouse model *in vivo* demonstrated increasing autophagic flux with rapamycin or its analogue CCI-779 alongside ERT for the GAA enzyme reduced muscle glycogen levels compared with either treatment alone [167]. These emerging data highlight the potential benefits of combinatorial treatment strategies in the LSDs.

Conclusions

The LSDs are characterized by accumulation of undegraded macromolecules due to defective or absent lysosomal hydrolases, or because of dysregulated endosomal–lysosomal processes, including altered vesicular trafficking, autophagosome maturation, lysosomal acidification or transport of molecules across the lysosomal membrane. The separate classes of LSDs represent several different defects in lysosomal biology and to some extent require tailored therapies to overcome them. However, one feature apparent in many of the LSDs is dysregulated autophagy (Table 1), and this may be the common therapeutic target that can be exploited similarly in different disorders. Based on the common blockage of autophagic flux, therapies designed to stimulate autophagy and alleviate the block are a logical option to trial. While autophagy inducers have already shown benefit in a few LSDs (Table 2), a broader evaluation of autophagy modulation in the LSDs is urgently required. Autophagy induction shows particular promise in combination with existing therapeutic options such as ERT. However, ERT is applicable to only a subset of the LSDs and the treatment costs are high, and therefore, identifying novel therapeutic interventions for the LSDs that are cheaper and more widely useful is essential. Ultimately, different combinations of therapies are likely to be required for each of the LSDs but autophagy regulators may play a significant role in many.

Summary

- Autophagy is a vital cellular process requiring the degradative function of lysosomes.

- Abnormal lysosomal function leading to accumulation of undegraded metabolites occurs in the LSDs.

- Defects in autophagy are emerging to be a common disease mechanism underlying LSDs.

- Stimulation of autophagy is a potential therapeutic intervention in LSDs.

Competing Interests

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

Abbreviations

ERT, enzyme replacement therapy; GAA, acid α-glucosidase; GCase, glucocerebrosidase; iPSC, induced pluripotent stem cells; LAMP2, lysosome-associated membrane protein 2; LSD, Lysosomal storage disorder; MLIV, mucolipidosis type IV; MPS, mucopolysaccharidosis; MSD, multiple sulfatase deficiency; mTORC1, mechanistic target of rapamycin complex 1; NCL, neuronal ceroid lipofuscinosis; NPC1, Niemann–Pick type C1 disease; ROS, reactive oxygen species; SNARE, *N*-ethylmaleimide-sensitive factor-attachment protein receptors; TFEB, transcription factor EB; XMEA, X-linked myopathy with excessive autophagy.

References

1 Luzio, J.P., Pryor, P.R. and Bright, N.A. (2007) Lysosomes: fusion and function. *Nat. Rev. Mol. Cell Biol.* **8**, 622–632

2 Perera, R.M. and Zoncu, R. (2016) The lysosome as a regulatory hub. *Annu. Rev. Cell Dev. Biol.* **32**, 223–253

3 Xu, H. and Ren, D. (2015) Lysosomal physiology. *Annu. Rev. Physiol.* **77**, 57–80

4 Futerman, A.H. and van Meer, G. (2004) The cell biology of lysosomal storage disorders. *Nat. Rev. Mol. Cell Biol.* **5**, 554–565

5 Platt, F.M., Boland, B. and van der Spoel, A.C. (2012) The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction. *J. Cell Biol.* **199**, 723–734

6   Parenti, G., Andria, G. and Ballabio, A. (2015) Lysosomal storage diseases: from pathophysiology to therapy. *Annu. Rev. Med.* **66**, 471–486

7   Boustany, R.M. (2013) Lysosomal storage diseases–the horizon expands. *Nat. Rev. Neurol.* **9**, 583–598

8   Mizushima, N., Levine, B., Cuervo, A.M. and Klionsky, D.J. (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069–1075

9   Ravikumar, B., Sarkar, S., Davies, J.E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z.W. et al. (2010) Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol. Rev.* **90**, 1383–1435

10   Levine, B. and Kroemer, G. (2008) Autophagy in the pathogenesis of disease. *Cell* **132**, 27–42

11   Stolz, A., Ernst, A. and Dikic, I. (2014) Cargo recognition and trafficking in selective autophagy. *Nat. Cell Biol.* **16**, 495–501

12   Boya, P., Reggiori, F. and Codogno, P. (2013) Emerging regulation and functions of autophagy. *Nat. Cell Biol.* **15**, 713–720

13   Zhou, J., Tan, S.H., Nicolas, V., Bauvy, C., Yang, N.D., Zhang, J. et al. (2013) Activation of lysosomal function in the course of autophagy via mTORC1 suppression and autophagosome-lysosome fusion. *Cell Res.* **23**, 508–523

14   Lieberman, A.P., Puertollano, R., Raben, N., Slaugenhaupt, S., Walkley, S.U. and Ballabio, A. (2012) Autophagy in lysosomal storage disorders. *Autophagy* **8**, 719–730

15   Ward, C., Martinez-Lopez, N., Otten, E.G., Carroll, B., Maetzel, D., Singh, R. et al. (2016) Autophagy, lipophagy and lysosomal lipid storage disorders. *Biochim. Biophys. Acta* **1861**, 269–284

16   Klionsky, D.J. and Schulman, B.A. (2014) Dynamic regulation of macroautophagy by distinctive ubiquitin-like proteins. *Nat. Struct. Mol. Biol.* **21**, 336–345

17   Mizushima, N., Noda, T., Yoshimori, T., Tanaka, Y., Ishii, T., George, M.D. et al. (1998) A protein conjugation system essential for autophagy. *Nature* **395**, 395–398

18   Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T. et al. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO* **19**, 5720–5728

19   Mizushima, N. (2007) Autophagy: process and function. *Genes Dev.* **21**, 2861–2873

20   Ganley, I.G. (2013) Autophagosome maturation and lysosomal fusion. *Essays Biochem.* **55**, 65–78

21   Saftig, P. and Klumperman, J. (2009) Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat. Rev. Mol. Cell Biol.* **10**, 623–635

22   Reggiori, F. and Ungermann, C. (2017) Autophagosome maturation and fusion. *J. Mol. Biol.* **429**, 486–496

23   Levine, B., Liu, R., Dong, X. and Zhong, Q. (2015) Beclin orthologs: integrative hubs of cell signaling, membrane trafficking, and physiology. *Trends Cell Biol.* **25**, 533–544

24   Nguyen, T.N., Padman, B.S., Usher, J., Oorschot, V., Ramm, G. and Lazarou, M. (2016) 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

25   McEwan, D.G., Popovic, D., Gubas, A., Terawaki, S., Suzuki, H., Stadel, D. et al. (2015) PLEKHM1 regulates autophagosome-lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell* **57**, 39–54

26   Wang, Y., Li, L., Hou, C., Lai, Y., Long, J., Liu, J. et al. (2016) SNARE-mediated membrane fusion in autophagy. *Semin. Cell Dev. Biol.* **60**, 97–104

27   Itakura, E., Kishi-Itakura, C. and Mizushima, N. (2012) The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. *Cell* **151**, 1256–1269

28   Diao, J., Liu, R., Rong, Y., Zhao, M., Zhang, J., Lai, Y. et al. (2015) ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes. *Nature* **520**, 563–566

29   Tsuboyama, K., Koyama-Honda, I., Sakamaki, Y., Koike, M., Morishita, H. and Mizushima, N. (2016) The ATG conjugation systems are important for degradation of the inner autophagosomal membrane. *Science* **354**, 1036–1041

30   Settembre, C., Di Malta, C., Polito, V.A., Arencibia, M.G., Vetrini, F., Erdin, S. et al. (2011) TFEB links autophagy to lysosomal biogenesis. *Science* **332**, 1429–1433

31   Settembre, C., Fraldi, A., Medina, D.L. and Ballabio, A. (2013) Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat. Rev. Mol. Cell Biol.* **14**, 283–296

32   Medina, D.L., Di Paola, S., Peluso, I., Armani, A., De Stefani, D., Venditti, R. et al. (2015) Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* **17**, 288–299

33   He, C. and Klionsky, D.J. (2009) Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* **43**, 67–93

34   Sarkar, S. (2013) Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers. *Biochem. Soc. Trans.* **41**, 1103–1130

35   Sarkar, S., Floto, R.A., Berger, Z., Imarisio, S., Cordenier, A., Pasco, M. et al. (2005) Lithium induces autophagy by inhibiting inositol monophosphatase. *J. Cell Biol.* **170**, 1101–1111

36   Williams, A., Sarkar, S., Cuddon, P., Ttofi, E.K., Saiki, S., Siddiqi, F.H. et al. (2008) Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat. Chem. Biol.* **4**, 295–305

37   Kim, Y.C. and Guan, K.L. (2015) mTOR: a pharmacologic target for autophagy regulation. *J. Clin. Invest.* **125**, 25–32

38   Saxton, R.A. and Sabatini, D.M. (2017) mTOR signaling in growth, metabolism, and disease. *Cell* **168**, 960–976

39   Sarkar, S. (2013) Chemical screening platforms for autophagy drug discovery to identify therapeutic candidates for Huntington's disease and other neurodegenerative disorders. *Drug Discover. Today* **10**, e137–e144

40   Levine, B., Packer, M. and Codogno, P. (2015) Development of autophagy inducers in clinical medicine. *J. Clin. Invest.* **125**, 14–24

41   Rubinsztein, D.C., Codogno, P. and Levine, B. (2012) Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discover.* **11**, 709–730

42   Blommaart, E.F.C., Luiken, J.J.F.P., Blommaart, P.J.E., van Woerkom, G.M. and Meijer, A.J. (1995) Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. *J. Biol. Chem.* **270**, 2320–2326

43   Thoreen, C.C., Kang, S.A., Chang, J.W., Liu, Q., Zhang, J., Gao, Y. et al. (2009) An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. *J. Biol. Chem.* **284**, 8023–8032

44   Sarkar, S., Davies, J.E., Huang, Z., Tunnacliffe, A. and Rubinsztein, D.C. (2007) Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α-synuclein. *J. Biol. Chem.* **282**, 5641–5652

45   Jiang, P. and Mizushima, N. (2014) Autophagy and human diseases. *Cell Res.* **24**, 69–79

46   Rubinsztein, D.C. (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**, 780–786

47   Nixon, R.A. (2013) The role of autophagy in neurodegenerative disease. *Nat. Med.* **19**, 983–997

48   Menzies, F.M., Moreau, K. and Rubinsztein, D.C. (2011) Protein misfolding disorders and macroautophagy. *Curr. Opin. Cell Biol.* **23**, 190–197

49   Nita, D.A., Mole, S.E. and Minassian, B.A. (2016) Neuronal ceroid lipofuscinoses. *Epileptic Disord.* **18**, 73–88

50   Palmer, D.N., Barry, L.A., Tyynela, J. and Cooper, J.D. (2013) NCL disease mechanisms. *Biochim. Biophys. Acta* **1832**, 1882–1893

51   Mole, S.E. and Cotman, S.L. (2015) Genetics of the neuronal ceroid lipofuscinoses (Batten disease). *Biochim. Biophys. Acta* **1852**, 2237–2241

52   Simonati, A., Pezzini, F., Moro, F. and Santorelli, F.M. (2014) Neuronal ceroid lipofuscinosis: the increasing spectrum of an old disease. *Curr. Mol. Med.* **14**, 1043–1051

53   Cárcel-Trullols, J., Kovács, A.D. and Pearce, D.A. (2015) Cell biology of the NCL proteins: What they do and don't do. *Biochim. Biophys. Acta* **1852**, 2242–2255

54   Brandenstein, L., Schweizer, M., Sedlacik, J., Fiehler, J. and Storch, S. (2016) Lysosomal dysfunction and impaired autophagy in a novel mouse model deficient for the lysosomal membrane protein Cln7. *Hum. Mol. Genet.* **25**, 777–791

55   Thelen, M., Damme, M., Schweizer, M., Hagel, C., Wong, A.M.S., Cooper, J.D. et al. (2012) Disruption of the autophagy-lysosome pathway is involved in neuropathology of the nclf mouse model of neuronal ceroid lipofuscinosis. *PLoS One* **7**, e35493, https://doi.org/10.1371/journal.pone.0035493

56   Leinonen, H., Keksa-Goldsteine, V., Ragauskas, S., Kohlmann, P., Singh, Y., Savchenko, E. et al. (2017) Retinal degeneration in a mouse model Of CLN5 disease is associated with compromised autophagy. *Sci. Rep.* **7**, 1597

57   Cannelli, N., Garavaglia, B., Simonati, A., Aiello, C., Barzaghi, C., Pezzini, F. et al. (2009) Variant late infantile ceroid lipofuscinoses associated with novel mutations in CLN6. *Biochem. Biophys. Res. Commun.* **379**, 892–897

58   Cao, Y., Espinola, J.A., Fossale, E., Massey, A.C., Cuervo, A.M., MacDonald, M.E. et al. (2006) Autophagy is disrupted in a knock-in mouse model of juvenile neuronal ceroid lipofuscinosis. *J. Biol. Chem.* **281**, 20483–20493

59   Lojewski, X., Staropoli, J.F., Biswas-Legrand, S., Simas, A.M., Haliw, L., Selig, M.K. et al. (2014) Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. *Hum. Mol. Genet.* **23**, 2005–2022

60   Vidal-Donet, J.M., Cárcel-Trullols, J., Casanova, B., Aquado, C. and Knecht, E. (2013) Alterations in ROS activity and lysosomal pH account for distinct patterns of macroautophagy in LINCL and JNCL fibroblasts. *PLoS One* **8**, e55526, https://doi.org/10.1371/journal.pone.0055526

61   Chandrachud, U., Walker, M.W., Simas, A.M., Heetveld, S., Petcherski, A., Klein, M. et al. (2015) Unbiased cell-based screening in a neuronal cell model of batten disease highlights an interaction between Ca^{2+} homeostasis, autophagy, and CLN3 Protein function. *J. Biol. Chem.* **290**, 14361–14380

62   Schultz, M.L., Tecedor, L., Stein, C.S., Stamnes, M.A. and Davidson, B.L. (2014) CLN3 Deficient cells display defects in the ARF1-Cdc42 pathway and actin-dependent events. *PLoS One* **9**, e96647, https://doi.org/10.1371/journal.pone.0096647

63   Shacka, J.J. and Roth, K.A. (2005) Cathepsin deficiency as a model for neuronal ceroid lipofuscinoses. *Am. J. Pathol.* **167**, 1473–1476

64   Koike, M., Nakanishi, H., Saftig, P., Ezaki, J., Isahara, K., Ohsawa, Y. et al. (2000) Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin in mouse CNS neurons. *J. Neurosci.* **20**, 6898–6906

65   Koike, M., Shibata, M., Waguri, S., Yoshimura, K., Tanida, I., Kominami, E. et al. (2005) Participation of autophagy in storage of lysosomes in neurons from mouse models of neuronal ceroid-lipofuscinoses (Batten disease). *Am. J. Pathol.* **167**, 1713–1728

66   Shacka, J.J., Klocke, B.J., Young, C., Shibata, M., Olney, J.W., Uchiyama, Y. et al. (2007) Cathepsin D deficiency induces persistent neurodegeneration in the absence of Bax-dependent apoptosis. *J. Neurosci.* **27**, 2081–2090

67   Olsen, A.S.B. and Færgeman, N.J. (2017) Sphingolipids: membrane microdomains in brain development, function and neurological diseases. *Open Biol.* **7**, https://doi.org/10.1098/rsob.170069

68   Platt, F.M. (2014) Sphingolipid lysosomal storage disorders. *Nature* **510**, 68–75

69   Sandhoff, K. and Kolter, T. (2003) Biosynthesis and degradation of mammalian glycosphingolipids. *Philos. Trans. R. Soc. B Biol. Sci.* **358**, 847–861

70   Schuchman, E.H. and Desnick, R.J. (2017) Types A and B Niemann-Pick disease. *Mol. Genet. Metab.* **120**, 27–33

71   Vanier, M.T. (2010) Niemann-Pick disease type C. *Orphanet J. Rare Dis.* **5**, 16

72   Storch, J. and Xu, Z. (2009) Niemann–Pick C2 (NPC2) and intracellular cholesterol trafficking. *Biochim. Biophys. Acta* **1791**, 671–678

73   Sarkar, S., Carroll, B., Buganim, Y., Maetzel, D., Ng, A.H.M., Cassady, J.P. et al. (2013) Impaired autophagy in the lipid-storage disorder Niemann-Pick Type C1 disease. *Cell Rep.* **5**, 1302–1315

74   Maetzel, D., Sarkar, S., Wang, H., Abi-Mosleh, L., Xu, P., Cheng, A.W. et al. (2014) Genetic and chemical correction of cholesterol accumulation and impaired autophagy in hepatic and neural cells derived from Niemann-Pick Type C patient-specific iPS cells. *Stem Cell Rep.* **2**, 866–880

75   Boland, B., Smith, D.A., Mooney, D., Jung, S.S., Walsh, D.M. and Platt, F.M. (2010) Macroautophagy is not directly involved in the metabolism of amyloid precursor protein. *J. Biol. Chem.* **285**, 37415–37426

76   Liao, G., Yao, Y., Liu, J., Yu, Z., Cheung, S., Xie, A. et al. (2007) Cholesterol accumulation is associated with lysosomal dysfunction and autophagic stress in Npc1$^{-/-}$ mouse brain. *Am. J. Pathol.* **171**, 962–975

77   Pacheco, C.D., Kunkel, R. and Lieberman, A.P. (2007) Autophagy in Niemann-Pick C disease is dependent upon Beclin-1 and responsive to lipid trafficking defects. *Hum. Mol. Genet.* **16**, 1495–1503

78   Meske, V., Erz, J., Priesnitz, T. and Ohm, T.G. (2014) The autophagic defect in Niemann-Pick disease type C neurons differs from somatic cells and reduces neuronal viability. *Neurobiol. Dis.* **64**, 88–97

PORTLAND
PRESS

79 Ordonez, M.P., Roberts, E.A., Kidwell, C.U., Yuan, S.H., Plaisted, W.C. and Goldstein, L.S. (2012) Disruption and therapeutic rescue of autophagy in a human neuronal model of Niemann Pick type C1. *Hum. Mol. Genet.* **21**, 2651–2662

80 Lee, H., Lee, J.K., Park, M.H., Hong, Y.R., Marti, H.H., Kim, H. et al. (2014) Pathological roles of the VEGF/SphK pathway in Niemann-Pick type C neurons. *Nat. Commun.* **5**, 5514

81 Fraldi, A., Annunziata, F., Lombardi, A., Kaiser, H.J., Medina, D.L., Spampanato, C. et al. (2010) Lysosomal fusion and SNARE function are impaired by cholesterol accumulation in lysosomal storage disorders. *EMBO* **29**, 3607–3620

82 Lloyd-Evans, E., Morgan, A.J., He, X., Smith, D.A., Elliot-Smith, E., Sillence, D.J. et al. (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat. Med.* **14**, 1247–1255

83 Kiselyov, K., Yamaguchi, S., Lyons, C.W. and Muallem, S. (2010) Aberrant Ca^{2+} handling in lysosomal storage disorders. *Cell Calcium* **47**, 103–111

84 Guo, H., Zhao, M., Qiu, X., Deis, J.A., Huang, H., Tang, Q.Q. et al. (2016) Niemann-Pick type C2 deficiency impairs autophagy-lysosomal activity, mitochondrial function, and TLR signaling in adipocytes. *J. Lipid Res.* **57**, 1644–1658

85 Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M. et al. (2009) Autophagy regulates lipid metabolism. *Nature* **458**, 1131–1135

86 Mistry, P.K., Lopez, G., Schiffmann, R., Barton, N.W., Weinreb, N.J. and Sidransky, E. (2017) Gaucher disease: progress and ongoing challenges. *Mol. Genet. Metab.* **120**, 8–21

87 Hruska, K.S., LaMarca, M.E., Scott, C.R. and Sidransky, E. (2008) Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (*GBA*). *Hum. Mutat.* **29**, 567–583

88 Wong, K., Sidransky, E., Verma, A., Mixon, T., Sandberg, G.D., Wakefield, L.K. et al. (2004) Neuropathology provides clues to the pathophysiology of Gaucher disease. *Mol. Genet. Metab.* **82**, 192–207

89 Vaccaro, A.M., Motta, M., Tatti, M., Scarpa, S., Masuelli, L., Bhat, M. et al. (2010) Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting. *Hum. Mol. Genet.* **19**, 2987–2997

90 Tamargo, R.J., Velayati, A., Goldin, E. and Sidransky, E. (2012) The role of saposin C in Gaucher disease. *Mol. Genet. Metab.* **106**, 257–263

91 Awad, O., Sarkar, C., Panicker, L.M., Miller, D., Zeng, X., Sgambato, J.A. et al. (2015) Altered TFEB-mediated lysosomal biogenesis in Gaucher disease iPSC-derived neuronal cells. *Hum. Mol. Genet.* **24**, 5775–5788

92 Tatti, M., Motta, M., Di Bartolomeo, S., Scarpa, S., Cianfanelli, V., Cecconi, F. et al. (2012) Reduced cathepsins B and D cause impaired autophagic degradation that can be almost completely restored by overexpression of these two proteases in Sap C-deficient fibroblasts. *Hum. Mol. Genet.* **21**, 5159–5173

93 Sun, Y., Liou, B., Ran, H., Skelton, M.R., Williams, M.T., Vorhees, C.V. et al. (2010) Neuronopathic Gaucher disease in the mouse: viable combined selective saposin C deficiency and mutant glucocerebrosidase (V394L) mice with glucosylsphingosine and glucosylceramide accumulation and progressive neurological deficits. *Hum. Mol. Genet.* **19**, 1088–1097

94 Osellame, L.D., Rahim, A.A., Hargreaves, I.P., Gegg, M.E., Richard-Londt, A., Brandner, S. et al. (2013) Mitochondria and quality control defects in a mouse model of gaucher disease—links to Parkinson's disease. *Cell Metab.* **17**, 941–953

95 Y-h, Xu, K, Xu, Sun, Y., Liou, B., Quinn, B., R-h, Li et al. (2014) Multiple pathogenic proteins implicated in neuronopathic Gaucher disease mice. *Hum. Mol. Genet.* **23**, 3943–3957

96 Farfel-Becker, T., Vitner, E.B., Kelly, S.L., Bame, J.R., Duan, J., Shinder, V. et al. (2014) Neuronal accumulation of glucosylceramide in a mouse model of neuronopathic Gaucher disease leads to neurodegeneration. *Hum. Mol. Genet.* **23**, 843–854

97 Kinghorn, K.J., Grönke, S., Castillo-Quan, J.I., Woodling, N.S., Li, L., Sirka, E. et al. (2016) A *Drosophila* model of neuronopathic gaucher disease demonstrates lysosomal-autophagic defects and altered mTOR Signalling and is functionally rescued by rapamycin. *J. Neurosci.* **36**, 11654–11670

98 Bassi, M.T., Manzoni, M., Monti, E., Pizzo, M.T., Ballabio, A. and Borsani, G. (2000) Cloning of the gene encoding a novel integral membrane protein, mucolipidin—and identification of the two major founder mutations causing mucolipidosis Type IV. *Am. J. Hum. Genet.* **67**, 1110–1120

99 Bargal, R., Avidan, N., Ben-Asher, E., Olender, Z., Zeigler, M., Frumkin, A. et al. (2000) Identification of the gene causing mucolipidosis type IV. *Nat. Genet.* **26**, 118–123

100 LaPlante, J.M., Ye, C.P., Quinn, S.J., Goldin, E., Brown, E.M., Slaugenhaupt, S.A. et al. (2004) Functional links between mucolipin-1 and Ca^{2+}-dependent membrane trafficking in mucolipidosis IV. *Biochem. Biophys. Res. Commun.* **322**, 1384–1391

101 Eichelsdoerfer, J.L., Evans, J.A., Slaugenhaupt, S.A. and Cuajungco, M.P. (2010) Zinc dyshomeostasis is linked with the loss of mucolipidosis IV-associated TRPML1 ion channel. *J. Biol. Chem.* **285**, 34304–34308

102 Dong, X.-P., Cheng, X., Mills, E., Delling, M., Wang, F., Kurz, T. et al. (2008) The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature* **455**, 992–996

103 De Leo, M.G., Staiano, L., Vicinanza, M., Luciani, A., Carissimo, A., Mutarelli, M. et al. (2016) Autophagosome-lysosome fusion triggers a lysosomal response mediated by TLR9 and controlled by OCRL. *Nat. Cell Biol.* **18**, 839–850

104 Micsenyi, M.C., Dobrenis, K., Stephney, G., Pickel, J., Vanier, M.T., Slaugenhaupt, S.A. et al. (2009) Neuropathology of the *Mcoln1*$^{-/-}$ knockout mouse model of mucolipidosis Type IV. *J. Neuropathol. Exp. Neurol.* **68**, 125–135

105 Venkatachalam, K., Long, A.A., Elsaesser, R., Nikolaeva, D., Broadie, K. and Montell, C. (2008) Motor deficit in a *Drosophila* model of mucolipidosis Type IV due to defective clearance of apoptotic cells. *Cell* **135**, 838–851

106 Miedel, M.T., Rbaibi, Y., Guerriero, C.J., Colletti, G., Weixel, K.M., Weisz, O.A. et al. (2008) Membrane traffic and turnover in TRP-ML1–deficient cells: a revised model for mucolipidosis type IV pathogenesis. *J. Exp. Med.* **205**, 1477–1490

107 Thompson, E.G., Schaheen, L., Dang, H. and Fares, H. (2007) Lysosomal trafficking functions of mucolipin-1 in murine macrophages. *BMC Cell Biol.* **8**, 54, https://doi.org/10.1186/1471-2121-8-54

108 Curcio-Morelli, C., Charles, F.A., Micsenyi, M.C., Cao, Y., Venugopal, B., Browning, M.F. et al. (2010) Macroautophagy is defective in mucolipin-1-deficient mouse neurons. *Neurobiol. Dis.* **40**, 370–377

109 Venugopal, B., Mesires, N.T., Kennedy, J.C., Curcio-Morelli, C., LaPlante, J.M., Dice, J.F. et al. (2009) Chaperone-mediated autophagy is defective in mucolipidosis type IV. *J. Cell. Physiol.* **219**, 344–353

110 Oldfors, A. and DiMauro, S. (2013) New insights in the field of muscle glycogenoses. *Curr. Opin. Neurol.* **26**, 544–553

111 Godfrey, R. and Quinlivan, R. (2016) Skeletal muscle disorders of glycogenolysis and glycolysis. *Nat. Rev. Neurol.* **12**, 393–402

112 Nishino, I. (2003) Autophagic vacuolar myopathies. *Curr. Neurol. Neurosci. Rep.* **3**, 64–69

113 Malicdan, M.C. and Nishino, I. (2012) Autophagy in lysosomal myopathies. *Brain Pathol.* **22**, 82–88

114 Raben, N., Plotz, P. and Byrne, B.J. (2002) Acid α-glucosidase deficiency (glycogenosis Type II, Pompe disease). *Curr. Mol. Med.* **2**, 145–166

115 Dasouki, M., Jawdat, O., Almadhoun, O., Pasnoor, M., McVey, A.L., Abuzinadah, A. et al. (2014) Pompe disease: literature review and case series. *Neurol. Clin.* **32**, 751–776, ix

116 Fukuda, T., Ewan, L., Bauer, M., Mattaliano, R.J., Zaal, K., Ralston, E. et al. (2006) Dysfunction of endocytic and autophagic pathways in a lysosomal storage disease. *Ann. Neurol.* **59**, 700–708

117 Nascimbeni, A.C., Fanin, M., Masiero, E., Angelini, C. and Sandri, M. (2012) The role of autophagy in the pathogenesis of glycogen storage disease type II (GSDII). *Cell Death Differ.* **19**, 1698–1708

118 Raben, N., Hill, V., Shea, L., Takikita, S., Baum, R., Mizushima, N. et al. (2008) Suppression of autophagy in skeletal muscle uncovers the accumulation of ubiquitinated proteins and their potential role in muscle damage in Pompe disease. *Hum. Mol. Genet.* **17**, 3897–3908

119 Zirin, J., Nieuwenhuis, J. and Perrimon, N. (2013) Role of autophagy in glycogen breakdown and its relevance to chloroquine myopathy. *PLoS Biol.* **11**, e1001708, https://doi.org/10.1371/journal.pbio.1001708

120 Kotoulas, O.B., Kalamidas, S.A. and Kondomerkos, D.J. (2004) Glycogen autophagy. *Microsc. Res. Tech.* **64**, 10–20

121 Nishino, I., Fu, J., Tanji, K., Yamada, T., Shimojo, S., Koori, T. et al. (2000) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* **406**, 906–910

122 Bandyopadhyay, U., Kaushik, S., Varticovski, L. and Cuervo, A.M. (2008) The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. *Mol. Cell. Biol.* **28**, 5747–5763

123 Tanaka, Y., Guhde, G., Suter, A., Eskelinen, E.-L., Hartmann, D., Lullmann-Rauch, R. et al. (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* **406**, 902–906

124 Eskelinen, E.-L., Illert, A.L., Tanaka, Y., Schwarzmann, G., Blanz, J., von Figura, K. et al. (2002) Role of LAMP-2 in lysosome biogenesis and autophagy. *Mol. Biol. Cell* **13**, 3355–3368

125 Nascimbeni, A.C., Fanin, M., Angelini, C. and Sandri, M. (2017) Autophagy dysregulation in Danon disease. *Cell Death Dis.* **8**, e2565

126 Hashem, S.I., Murphy, A.N., Divakaruni, A.S., Klos, M.L., Nelson, B.C., Gault, E.C. et al. (2017) Impaired mitophagy facilitates mitochondrial damage in Danon disease. *J. Mol. Cell Cardiol.* **108**, 86–94

127 Dowling, J.J., Moore, S.A., Kalimo, H. and Minassian, B.A. (2015) X-linked myopathy with excessive autophagy: a failure of self-eating. *Acta Neuropathol. (Berl.)* **129**, 383–390

128 Ramachandran, N., Munteanu, I., Wang, P., Ruggieri, A., Rilstone, J.J., Israelian, N. et al. (2013) VMA21 deficiency prevents vacuolar ATPase assembly and causes autophagic vacuolar myopathy. *Acta Neuropathol. (Berl.)* **125**, 439–457

129 Munteanu, I., Kalimo, H., Saraste, A., Nishino, I. and Minassian, B.A. (2017) Cardiac autophagic vacuolation in severe X-linked myopathy with excessive autophagy. *Neuromuscul. Disord.* **27**, 185–187

130 Mariño, G., Madeo, F. and Kroemer, G. (2011) Autophagy for tissue homeostasis and neuroprotection. *Curr. Opin. Cell Biol.* **23**, 198–206

131 Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I. et al. (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441**, 880–884

132 Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R. et al. (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441**, 885–889

133 Singh, R. and Cuervo, A.M. (2012) Lipophagy: connecting autophagy and lipid metabolism. *Int. J. Cell Biol.* **2012**, 282041

134 Youle, R.J. and Narendra, D.P. (2011) Mechanisms of mitophagy. *Nat. Rev. Mol. Cell Biol.* **12**, 9–14

135 Green, D.R. and Levine, B. (2014) To be or not to be? how selective autophagy and cell death govern cell fate. *Cell* **157**, 65–75

136 Schneider, J.L. and Cuervo, A.M. (2014) Autophagy and human disease: emerging themes. *Curr. Opin. Genet. Dev.* **26**, 16–23

137 Lee, J., Giordano, S. and Zhang, J. (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochem. J.* **441**, 523–540

138 Sarkar, S., Ravikumar, B., Floto, R.A. and Rubinsztein, D.C. (2009) Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. *Cell Death Differ.* **16**, 46–56

139 Sarkar, S., Perlstein, E.O., Imarisio, S., Pineau, S., Cordenier, A., Maglathlin, R.L. et al. (2007) Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. *Nat. Chem. Biol.* **3**, 331–338

140 Madeo, F., Zimmermann, A., Maiuri, M.C. and Kroemer, G. (2015) Essential role for autophagy in life span extension. *J. Clin. Invest.* **125**, 85–93

141 Morselli, E., Galluzzi, L., Kepp, O., Criollo, A., Maiuri, M.C., Tavernarakis, N. et al. (2009) Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. *Aging* **1**, 961–970

142 Kuo, S.Y., Castoreno, A.B., Aldrich, L.N., Lassen, K.G., Goel, G., Dančík, V. et al. (2015) Small-molecule enhancers of autophagy modulate cellular disease phenotypes suggested by human genetics. *Proc. Natl Acad. Sci. U.S.A.* **112**, E4281–E4287

143 Sarkar, S., Maetzel, D., Korolchuk, V.I. and Jaenisch, R. (2014) Restarting stalled autophagy a potential therapeutic approach for the lipid storage disorder, Niemann-Pick type C1 disease. *Autophagy* **10**, 1137–1140

144 Palmieri, M., Pal, R., Nelvagal, H.R., Lotfi, P., Stinnett, G.R., Seymour, M.L. et al. (2017) mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. *Nat. Commun.* **8**, 14338

145 DeBosch, B.J., Heitmeier, M.R., Mayer, A.L., Higgins, C.B., Crowley, J.R., Kraft, T.E. et al. (2016) Trehalose inhibits solute carrier 2A (SLC2A) proteins to induce autophagy and prevent hepatic steatosis. *Sci. Signal.* **9**, ra21

PORTLAND PRESS

146 Chang, J.-W., Choi, H., Cotman, S.L. and Jung, Y.-K. (2011) Lithium rescues the impaired autophagy process in Cb*Cln3*$^{\Delta ex7/8/\Delta ex7/8}$ cerebellar cells and reduces neuronal vulnerability to cell death via IMPase inhibition. *J. Neurochem.* **116**, 659–668

147 Lim, J.-A., Li, L., Shirihai, O.S., Trudeau, K.M., Puertollano, R. and Raben, N. (2017) Modulation of mTOR signaling as a strategy for the treatment of Pompe disease. *EMBO Mol. Med.* **9**, 353–370

148 Canonico, B., Cesarini, E., Salucci, S., Luchetti, F., Falcieri, E., Di Sario, G. et al. (2016) Defective autophagy, mitochondrial clearance and lipophagy in Niemann-Pick Type B lymphocytes. *PLoS One* **11**, e0165780, https://doi.org/10.1371/journal.pone.0165780

149 Pallet, N. and Legendre, C. (2013) Adverse events associated with mTOR inhibitors. *Expert Opin. Drug Saf.* **12**, 177–186

150 Sardiello, M., Palmieri, M., di Ronza, A., Medina, D.L., Valenza, M., Gennarino, V.A. et al. (2009) A gene network regulating lysosomal biogenesis and function. *Science* **325**, 473–477

151 Decressac, M., Mattsson, B., Weikop, P., Lundblad, M., Jakobsson, J. and Björklund, A. (2013) TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *Proc. Natl Acad. Sci. U.S.A.* **110**, E1817–E1826

152 Martini-Stoica, H., Xu, Y., Ballabio, A. and Zheng, H. (2016) The autophagy–lysosomal pathway in neurodegeneration: a TFEB perspective. *Trends Neurosci.* **39**, 221–234

153 Sardiello, M. (2016) Transcription factor EB: from master coordinator of lysosomal pathways to candidate therapeutic target in degenerative storage diseases. *Ann. N.Y. Acad. Sci.* **1371**, 3–14

154 Napolitano, G. and Ballabio, A. (2016) TFEB at a glance. *J. Cell Sci.* **129**, 2475–2481

155 Medina Diego, L., Fraldi, A., Bouche, V., Annunziata, F., Mansueto, G., Spampanato, C. et al. (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev. Cell* **21**, 421–430

156 Spampanato, C., Feeney, E., Li, L., Cardone, M., Lim, J.-A., Annunziata, F. et al. (2013) Transcription factor EB (TFEB) is a new therapeutic target for Pompe disease. *EMBO Mol. Med.* **5**, 691–706

157 Sato, Y., Kobayashi, H., Higuchi, T., Shimada, Y., Ida, H. and Ohashi, T. (2016) TFEB overexpression promotes glycogen clearance of Pompe disease iPSC-derived skeletal muscle. *Mol. Ther.–Methods Clin. Dev.* **3**, 16054

158 Settembre, C., Fraldi, A., Jahreiss, L., Spampanato, C., Venturi, C., Medina, D. et al. (2008) A block of autophagy in lysosomal storage disorders. *Hum. Mol. Genet.* **17**, 119–129

159 Desnick, R.J. and Schuchman, E.H. (2012) Enzyme replacement therapy for lysosomal diseases: lessons from 20 years of experience and remaining challenges. *Annu. Rev. Genomics Hum. Genet.* **13**, 307–335

160 Ratko, T.A., Marbella, A., Godfrey, S. and Aronson, N. (2013) *Enzyme-Replacement Therapies for Lysosomal Storage Diseases. Agency for Healthcare Research and Quality*, Comparative Effectiveness Technical Briefs, Rockville (MD), Report No.: 12(13)-EHC154-EF

161 Cox, T.M. (2010) Recommendations for treating patients with Gaucher disease with emerging enzyme products. *Blood Cells Mol. Dis.* **44**, 84–85

162 Aerts, J.M.F.G., Yasothan, U. and Kirkpatrick, P. (2010) Velaglucerase alfa. *Nat. Rev. Drug Discover.* **9**, 837–838

163 Hollak, C.E. (2012) An evidence-based review of the potential benefits of taliglucerase alfa in the treatment of patients with Gaucher disease. *Core Evidence* **7**, 15–20

164 McEachern, K.A., Fung, J., Komarnitsky, S., Siegel, C.S., Chuang, W.-L., Hutto, E. et al. (2007) A specific and potent inhibitor of glucosylceramide synthase for substrate inhibition therapy of Gaucher disease. *Mol. Genet. Metab.* **91**, 259–267

165 Bennett, L.L. and Mohan, D. (2013) Gaucher disease and its treatment options. *Ann. Pharmacother.* **47**, 1182–1193

166 Ripolone, M., Violano, R., Ronchi, D., Mondello, S., Nascimbeni, A., Colombo, I. et al. (2017) Effects of short-to-long term enzyme replacement therapy (ERT) on skeletal muscle tissue in late onset Pompe disease (LOPD). *Neuropathol. Appl. Neurobiol.*

167 Ashe, K.M., Taylor, K.M., Chu, Q., Meyers, E., Ellis, A., Jingozyan, V. et al. (2010) Inhibition of glycogen biosynthesis via mTORC1 suppression as an adjunct therapy for Pompe disease. *Mol. Genet. Metab.* **100**, 309–315