Mechanisms of Autophagy–Lysosome Dysfunction in Neurodegenerative Diseases

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Abstract

Autophagy is a lysosome-based degradative process used to recycle obsolete cellular constituents and eliminate damaged organelles and aggregate-prone proteins. Their post-mitotic nature and extremely polarized morphologies make neurons particularly vulnerable to disruptions caused by autophagy–lysosomal defects, especially as the brain ages. Consequently, mutations in genes regulating autophagy and lysosomal functions cause a wide range of neurodegenerative diseases. Here, we review the role of autophagy and lysosomes in neurodegenerative diseases such as Alzheimer disease, Parkinson disease and frontotemporal dementia. We also consider the strong impact of cellular aging on lysosomes and autophagy as a tipping point for late-age emergence of and related neurodegenerative disorders. Many of these diseases have primary defects in autophagy, e.g. affecting autophagosome formation, and in lysosomal functions, especially pH regulation and calcium homeostasis. We have aimed to provide an integrative framework for understanding the central importance of autophagic–lysosomal function in neuronal health and disease.

Introduction

Macroautophagy, which is conserved from yeast to humans, is a process whereby cells capture typically cytoplasmic material in double-membraned autophagosomes **(BOX1)** and then traffic these organelles to perinuclear sites, where lysosomes are concentrated. This is followed by autophagosome–lysosome fusion, after which the autophagic contents are degraded by lysosomal hydrolases and the building blocks such as amino acids or sugar moieties are recycled. (**Figure 1**).

Autophagy is an important intracellular clearance route for intracytoplasmic aggregate-prone proteins, certain pathogens, and dysfunctional organelles like mitochondria. The accumulation of aggregate-prone proteins is a hallmark of most neurodegenerative diseases. Usually these aggregates are intracellular but sometimes, as in the case of Alzheimer disease **[G]** (AD), β-amyloid also appears extracellularly and the intraneuronal tau **[G]** aggregates persist in the extracellular space after the neuron dies[1]. Most of these aggregate-prone proteins cause disease through toxic gain-of-function mechanisms and their pathogenicity is associated with their propensity to aggregate. Cells buffer themselves against such aggregate-prone proteins through the proteostasis network, which comprises components that regulate the synthesis, folding, aggregation and disposal of proteins, including through autophagy[2].

In addition to macroautophagy, cytoplasmic proteins can be delivered to lysosomes via other “autophagy” pathways. These include non-canonical autophagy, a poorly understood phenomenon where macroautophagy-like processes appear to occur in specific contexts in the absence of some of the core autophagy machinery[3]. In chaperone-mediated autophagy (CMA), substrates containing a KFERQ or similar pentapeptide motif are recognized by the heat shock cognate 70-kDa protein (HSC70) (ref. [4]). This allows targeting of such HSC70-bound proteins to the lysosome-associated membrane glycoprotein 2 (LAMP2A) that acts as a translocation complex to enable import into the lysosome, where the substrates are recognized by a lysosomal HSC70 (ref. [4]). Whereas CMA is not the focus of this Review, it is important to stress that the efficiency of this pathway declines with neuronal aging and contributes to the accumulation of aggregate-prone proteins in the brain[5]. Similarly, its activity is compromised by various disease-causing proteins, like alpha-synuclein **[G]** , tau and huntingtin **[G]** (reviewed in[4]). In microautophagy and endosomal microautophagy, substrates are captured by lysosomes and endosomes through the formation of invaginations of their membranes[4]. The roles of these pathways in mammalian neurodegenerative diseases are still poorly understood and will not be discussed here. In the following, we refer to macroautophagy as autophagy for simplicity.

Although it is convenient to consider the different components of the autophagy–lysosome network as separate, they frequently engage in significant crosstalk. For example, mechanistic target of rapamycin

complex 1 (mTORC1) positively regulates protein synthesis while restraining protein degradation via the autophagy–lysosome pathway[6]. Conversely, mTORC1 inhibition stimulates both autophagosome and lysosome biogenesis[6]. mTORC1 activity is enabled by the binding of its subunit Raptor to Rag proteins on the lysosome, as part of its nutrient-sensing functions[7, 8]. Indeed, lysosomal mTORC1 tethering and mTORC1-containing lysosomal localization in the cell (perinuclear versus peripheral) are critical factors regulating mTORC1 activity and consequently autophagy[9, 10]. Similarly, consideration of the key degradation pathways employed by cells shows that proteasome inhibition activates macroautophagy[11], whereas autophagy inhibition impairs flux in the ubiquitin–proteasome pathway[12] (**Figure 1**).

The autophagy–lysosome pathway is not simple or unidirectional. For example, the lysosome has emerged as a key signalling hub enabling activation of crucial autophagy regulators like mTORC1 (ref.[13]) and alterations of lysosomal distribution in cells can impact mTORC1 activity and autophagosome biogenesis, further underscoring the interconnections of this network[10]. Many cross-dependencies also exist between the autophagy–lysosome pathway and the endosomal–lysosomal pathway. For example, proper lysosome function depends on endocytosis, which is crucial for autophagosome biogenesis[14]. In addition, autophagy can impact aspects of endocytic trafficking[15]. Although this Review focuses squarely on the pathway from autophagosomes to lysosomes, it is reasonable to consider both pathways to lysosomes as an integrated endosomal–lysosomal–autophagy (ELA) network. Further details on the autophagy interface with the endocytic pathway to lysosomes are described in **BOX 2**.

In this Review, we will highlight how the autophagy–lysosome system is involved in various neurodegenerative diseases. We will provide examples that illustrate key concepts but will not aim to be encyclopaedic, as this approach risks obscuring the key broad concepts we aim to convey. We have focussed particularly on the roles of the autophagy–lysosome system in neurons. Although the roles of glia in neurodegeneration are becoming more apparent, the understanding of the autophagy–lysosome system in these cells are less developed in the context of neurodegenerative diseases. We will also discuss and speculate on the interplay between the autophagy–lysosome pathway and ageing in neurodegeneration, its impact on specific neuronal and cell type vulnerabilities in different diseases and consider some challenges when aiming to exploit the therapeutic potential of this pathway in different diseases.

[H1] The autophagy–lysosome system in neurons

Much of what we know about autophagy has come from characterizations in non-neuronal cells. Newer studies have revealed a more complex autophagy–endolysosomal topography and more dynamic

behavior in neurons compared to other cells, reflecting adaptations to accommodate the extreme asymmetrical shape of neurons and their much larger cytoplasmic volumes.

The extreme lengths of projecting axonal and some dendritic processes, combined with total volumes as much as 10-50 fold larger than that of the neuronal cell body or the average size of glial cells[16], make neurons especially dependent on efficient protein quality control mechanisms, including autophagy. This challenge is unique to neurons, as they must traffic autophagosomes and endosomes across long distances to reach the degradative lysosomes that are found in the cell body[17]. This challenge is partly the consequence of endosomes moving retrogradely along axons in vivo and only achieving mature lysosome identity and acidification as they reach their somal destination[18-20]. Once lysosomes are fully acidified and activated, they do not return to axons, explaining why lysosomes are rare in axons[18, 19] and relatively sparse in dendrites of the adult brain[21]. Instead, lysosome-associated membrane protein 1 (LAMP1)- and cathepsin-positive Golgi carriers predominate in axons and, as in other cell types, these are the vesicles that deliver lysosomal components to axonal endosomes during lysosomal maturation[18].

These adaptations make neurons especially vulnerable to autophagy–endolysosomal disruption and explain why gene mutations targeting dynein-based retrograde axonal transport mechanisms cause varied neurodegenerative disorders[22] (**Figure 2**). In addition, being predominantly post-mitotic also prevents most populations of neurons from reducing cytoplasmic waste by cell division or by being replaced with a new neuron as is possible with the mitotic cells of the brain[23]. The need of neurons to survive for the length of an individual's lifetime, yet with exceedingly limited capacity for neurogenesis, makes them exceptionally susceptible to the cumulative effects of cellular aging, especially oxidative stress[24] and to the emergence of late-life neurodegenerative diseases. The deterioration of autophagy and lysosomal functions, which is pivotal to the mechanism underlying cellular aging, is central as a precipitant of overt disease in these late-life disorders. A better understanding of the molecular basis responsible for significant deterioration of lysosomal function in aging brain, requires the development of more cell-specific probes for this incipient pathobiology applied to in vivo model systems, rather than cultured cells, to appreciate the extent of its synergies with disease factors and what interventions may be effective to preserve lysosome health in normal aging.

[H1] Brain aging in adult-onset neurodegenerative diseases

The direct neurotoxic actions of a "pathogenic protein" are often blamed as the sole or primary causes of adult-onset neurodegenerative diseases. Less commonly considered is that the toxic form of the protein is usually produced in comparable amounts throughout life and is handled effectively without evident

toxicity during early life. Disease emergence during adulthood coincides temporally with the progressive failure of proteostatic systems and especially autophagy at all levels of the pathway, which is facilitated by poorly understood aging factors. For example, autophagosome biogenesis appears to decrease in mammalian brains and neurons with age[25, 26] and the delivery of waste-carrying vesicles to lysosomes slows[26]. A particularly critical driver of cell aging in various lower species like *C. elegans*[27, 28] and likely mammals[29] is declining lysosomal function linked to failing intralumenal acidification[26], which is believed to be responsible for the initial buildup of multiple pathogenic aggregation-prone proteins (amyloid-beta precursor protein (APP) peptides (e.g. Aβ and APP-βCTF, and TAR DNA-binding protein 43 (TDP43), synuclein) in brain upon normal aging. During normal brain aging, the coalescence of ineffective autolysosomes containing hydrolysis-resistant substrates enables the compaction through extensive cross-linking modifications of the undigested cargoes within lipofuscin granules[91]. This process renders the substrates less bioactive and capable of release, which reduces but does not eliminate the damaging impact on cell function. The gradual accumulation of lipofuscin granules is a well-established correlate of neuronal age in many cell populations.

Many neurodegenerative diseases are likely driven by the accumulation of an autophagy substrate that itself compromises autophagy modestly (e.g. mutant huntingtin accelerating the degradation of the autophagy regulator Beclin-1 (ref. [30])), providing the potential for positive feedback. This is seen with alpha-synuclein in PD[31], tau in various dementias[32] and mutant huntingtin in Huntington's disease **[G]** (HD)[30]. It is likely that neurons maintain functional autophagy and/or buffer the accumulation of these proteins for many years by using other proteostasis mechanisms, including the ubiquitin–proteasome pathway and CMA. However, once these mechanisms can no longer manage the accumulating aggregate-prone proteins, more autophagy substrates will accumulate, leading to further autophagy impairment including failing mitophagy[33]. This creates a positive feedback loop as the toxic substrates accumulates more and boost oxidative stress[34]. This may explain why neurodegenerative diseases strike in late adulthood and that even individuals with autosomal-dominant mutations in huntingtin, tau or alpha-synuclein are functionally normal until disease onset.

In the absence of a highly-penetrant disease-causing gene mutation, declining function of autophagy and particularly lysosomes, known to be driven by aging-related factors such as oxidative damage to substrates and endosomal–lysosomal and autophagy (ELA) network components[24, 34-37], represents a basis for the emergence of manifest disease. In so-called sporadic forms of neurodegenerative diseases, a summation of aging-related factors such as oxidative damage to substrates and components of the endosome–lysosome–autophagy (ELA) network synergize with environmental and genetic risk factors to initiate disease. In this context, it is noteworthy that so many of the risk genes for the two most studied

major late-life neurodegenerative disorders, AD and PD, have a direct gain- or loss-of-function impact on the ELA network, the effects of which can summate or synergize. A pivotal role of aging in triggering neurodegenerative disease critically involving lysosomal mechanisms is consistent with genes that cause a congenital devastating lysosomal storage disorder **[G]** (LSD) when homozygously mutated, but are also major risk factors for a late-age onset neurodegenerative disorder, such as PD *(e.g., GB1)* or FTD *(e.g., GRN)*, when only one mutant copy is inherited[38, 39].

[H1] Defects in upstream autophagy in neurodegenerative disease

As strong genetic evidence implicates proteins of the autophagy–lysosome pathway and the broader ELA network in neurodegenerative disease pathogenesis, it is crucial to understand how this network maintains cell homeostasis and how its disruption may impact a wide range of neuronal and glial functions in disease and cause resulting phenotypes.

Many Mendelian neurological or neurodegenerative diseases, including HD, certain forms of Parkinsonism and forms of amyotrophic lateral sclerosis (ALS) reviewed previously[4], are caused by mutations that compromise autophagy either at stages of autophagosome biogenesis or at subsequent steps that orchestrate effective autophagic substrate degradation in autolysosomes. Mouse models with defects in these early steps suggest that they are sufficient to cause neurodegeneration[40, 41] and can partially explain the accumulation of aggregate-prone proteins in these conditions. However, the mutated proteins often have roles in other membrane trafficking processes, including alternative lysosomal degradation systems, and the combined deficits in multiple pathways results in multifactorial pathology.

In the following, we will provide some examples of Mendelian diseases causing defects at different stages of the macroautophagy pathway (**Figure 1**), using neurological or neurodegenerative diseases where possible. It is interesting to note that, in some cases, human mutations have helped to illuminate new steps in autophagosome biogenesis (**Box 1**). Within this network of systems available for capturing substrates, lysosomes deserve special emphasis as the only degradative compartment shared by all autophagy and endocytic delivery routes. Investigators often incorrectly equate “autophagy” with just the substrate sequestration steps of the process even though the process derives its name from its digestive ‘phagy’ (“eating”) lysosomal step. Despite its obvious redundancy, a useful convention has often been to refer to an “autophagy-lysosomal pathway” to underscore the crucial importance of the degradative step in autophagy. For the discussion below on neurodegenerative diseases, we find it useful to consider first the autophagy pathway defects affecting upstream steps of the pathway followed by the more extensive

dysfunctions affecting the digestive lysosomal stage of autophagy, bearing in mind that both are integral stages of the same pathway.

[H2] Autophagosome initiation

Homozygous mutations in *ATG5* (encoding autophagy protein 5)[42], *WIPI2* (encoding WD repeat domain phosphoinositide-interacting protein 2)[43] and *ATG7* (ref. [44]), which are involved in the biogenesis of the phagophore, i.e. the open autophagosome precursor, cause human diseases. ATG5 and ATG7 contribute to the formation of the ATG5-12–ATG16L1 complex, which serves as the E3-like enzyme for the ubiquitin-like conjugation of LC3 family members **[G]** to the lipid phosphatidylethanolamine in recycling endosome membrane, whereas the recruitment of the ATG5-12–ATG16 complex to these membranes is mediated by WIPI2 (ref. [45]). Mutation of *ATG5* causes congenital ataxia, mental retardation and developmental delay, and loss of *ATG7* is associated with complex neuro-developmental disorders with brain, muscle and endocrine involvement[44]. The loss of *WIPI2* function also causes a neurodevelopmental disorder that includes developmental delay, speech and language impairment, cardiac, neurological, thyroid and skeletal abnormalities[43]. It is surprising that the loss-of-function of these core autophagy genes results in neurodevelopmental abnormalities in humans that are not obvious in autophagy-null mice[46], where loss of autophagy causes neurodegeneration[40, 41]. However, it is possible that neurodevelopmental signs were overlooked in whole body autophagy-null mice that die soon after birth, and that humans with *ATG5, ATG7* and/or *WIPI2* mutations may develop neurodegenerative features as they age since they survive unexpectedly into quite advanced age (8th decade of life in individuals where ATG7 is reduced and 3rd decade where ATG7 is undetectable)[44]. As there are minimal longitudinal phenotypic data on such cases and no postmortem analyses available, it is not possible to know if and when any progressive neurodegeneration may start to manifest. In some of these ultrarare diseases, there may be insufficient neuropathological assessment so neurodegeneration may well have been missed.

[H2] Autophagosome closure and scission

The closure of the multiple openings between phagophore “fingers” that enables sequestration of their substrates from the cytoplasm is mediated by the endosomal sorting complexes required for transport (ESCRT) complex[47]. When this step is blocked, autophagic flux is impaired and autophagic cargo accumulates[47]. Mutations in the ESCRT complex cause diverse diseases, including forms of neurodegeneration, like FTD (e.g. *CHMP2B*), and it is possible that the autophagy defects have an important role in pathogenesis[48]. However, the ESCRT complex also has other roles, including in the

formation of multivesicular bodies in endosomes, a route for degradation of ubiquitinated cargo receptors enabled by invagination and inward budding of vesicles into endosomes[48]. After fusion of endosomes with lysosomes, these vesicles and their cargoes are degraded. The ESCRT complex has also been shown to participate in the repair of lysosomal damage[49, 50], a process in which PD-related genes such as leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2) have been implicated[51-53]. Further, disruption of the ESCRT pathway has been reported to promote endo-lysosomal escape **[G]** in tau propagation, with tau aggregation being one of the hallmarks of AD[54]. Thus, non-autophagic functions of the ESCRT complex also likely contribute to these diseases — a principle that applies to some of the examples below.

Following autophagosomes closure, they need to be released from the Ras-related protein Rab-11A (RAB11A)-based recycling endosome platform from which autophagosomes evolve[55] by dynamin 2-dependent scission[56]. A *dynamin 2* mutation that targets this recently described step in the autophagy pathway causes centronuclear myopathy[56] and has helped to define this new rate-limiting step in autophagosome formation — the scission of closed autophagosomes from the recycling endosome compartment. So far, neurodegenerative diseases have not been associated with this step specifically. However, since autophagosome closure (which is affected in various neurodegenerative diseases described above) is a prerequisite for release from the recycling endosome, such closure-defective mutations result in the accumulation of open autophagosome precursors still attached to the recycling endosome[55].

After closed autophagosomes are released from the recycling endosomes, they need to be trafficked by dyneins along microtubules in a retrograde direction to the perinuclear region of the cell where the lysosomes are clustered, to facilitate autophagosome–lysosome fusion[57] (**Figure 2**). This transport machinery is defective due to mutations in the dynein complex in Perry syndrome, which manifests with Parkinsonism[58, 59], and results in impaired autophagosome–lysosome fusion.

[H2] Defects associated with autophagy receptors

Originally, most of the focus of autophagy research was on its role as a non-selective bulk degradation process. However, it is becoming increasingly evident that autophagosomes can selectively engulf substrates including aggregate-prone proteins (aggrephagy)[60], dysfunctional mitochondria (mitophagy)[61], and endoplasmic reticulum (ER-phagy)[62]. The selectivity is mediated by so-called autophagy receptors that link the substrates (normally ubiquitinated proteins) to the autophagy machinery (like LC3 family members). An ALS- and FTD-associated mutation in p62 (also known as SQSTM1), the first of these

receptors to be described, compromises selective autophagy of ubiquitinated proteins[63]. Optineurin is a key mitophagy receptor, whose phosphorylation by the serine/threonine-protein kinase TBK1 is crucial for its receptor role in mitophagy[64]. Mutations in the genes encoding optineurin and TBK1 can cause ALS or FTD[64]. Mutations of two proteins that act together to regulate ER-phagy, reticulophagy regulator 1 (also known as FAM134B) and ADP-ribosylation factor-like protein 6-interacting protein 1 (ARL6IP1), cause sensory neuropathy[65, 66]. Such mutations have the potential to reveal important physiological roles of these forms of selective autophagy in neurological function.

Although the effect of some ER-phagy pathways on sensory neuropathies suggests the importance of this process in the peripheral nervous system, it is difficult to know how important loss of this selective autophagy subtype is for the genesis of neurodegenerative diseases of the central nervous system. Previously, much of the support for the idea that mitophagy was critical in neurons came from studies of two genes involved in early-onset Parkinsonism, *PINK1* (encoding the mitochondrial serine/threonine-protein kinase PINK1) and *Parkin*, which act in the same mitophagy pathway[67]. However, this particular mitophagy pathway may not be dominant in the brain, compared to other forms of mitophagy[68], and *PINK1* and *Parkin* may have mitophagy-independent roles as regulators of mitochondrial biogenesis[69]. Indeed, recent data suggested that nucleoid **[G]** -enriched mitochondrial fragments are predominant cargoes in mouse brain autophagosomes in the absence of autophagy receptors. The authors speculate that this is due to mitochondrial fission occurring close to sites of non-selective autophagosome formation[70]. Moreover, distinct from previously described extracellular vesicle subtypes, (e.g., microvesicles **[G]** , exosomes **[G]**), these "mitovesicles"[71] are upregulated in brains derived from a mouse model of Down syndrome, where mitochondrial damage is a prominent anomaly[71]. These studies suggest that bulk autophagic clearance of mitochondria is important for neuronal health.

[H2] Defects affecting multiple steps of the autophagy–lysosomal pathway

Some mutations associated with neurodegenerative disease impact multiple processes in autophagy and this may be more prevalent than one would imagine at first. One reason is that key regulators of autophagy, like mTORC1, act at multiple stages of the pathway[72, 73]. Mutant huntingtin may regulate autophagy at multiple stages from autophagosome biogenesis by enhancing Beclin-1 degradation[30], cargo selection possibly because of abnormal binding of mutant huntingtin to the cargo receptor, p62 (ref.[74]), to autophagosome trafficking to lysosomes[75]. In addition, the AD risk gene *PICALM*, encoding phosphatidylinositol-binding clathrin assembly protein, regulates both autophagosome biogenesis and autophagosome clearance by lysosomes by controlling the endocytosis of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) proteins[76]. Such SNARE proteins are required for

the homotypic fusion between plasma membrane-derived ATG16L-containing vesicles and heterotypic fusions between ATG16L1- and ATG9A-containing vesicles that are required prior to the initiation of autophagosome formation[77, 78]. In addition, SNAREs, whose trafficking is regulated by PICALM, impact autophagosome–lysosome fusion[76]. These upstream impairments compound the range of deficits corrupting the degradative steps in AD discussed below.

Most of the focus of the field has understandably been on cell-autonomous effects of disease-modifying or -causing factors on autophagy. However, neurons exist within an environment containing many different glial cells and recent data have suggested that surrounding cells may impact neuronal vulnerability in disease[79]. In this context, nitric oxide and chemokines like C-C motif ligand 3 (CCL3), CCL4 and CCL5, which are secreted by reactive microglia, impair neuronal autophagy and countering these effects was found to be protective in disease models[32, 80]. CCL3, CCL4 and CCL5 inhibit neuronal autophagy by binding and activating the GPCR C-C chemokine receptor type 5 (CCR5) on neurons[32], which may present the deleterious effect of chronic activation of a pathway that has been suggested to assist repair after acute neuronal injury[81]. These represent mechanisms whereby microglia can accelerate neurodegeneration in models of HD and tauopathy by compromising neuronal proteostasis, driving the accumulation of the disease-causing protein. In the context of a mouse model of AD, autophagy buffers against microglial senescence and ameliorates extracellular amyloid-plaque pathology[82].

[H1] Lysosomal defects in neurodegenerative disease

As the indispensable “downstream” degradative (“phagy”) step in autophagy (**Figure 1**) and endosomal cargo clearance pathways, lysosomes are essential for neuronal survival. Lysosomal dysfunction in adult onset neurodegenerative “proteinopathies”, such as AD, PD, HD and frontotemporal lobar degeneration **[G]** FTLD (**Figure 2**), was long presumed to be a later secondary response to eliminate aggregates and damaged organelles in severely compromised neurons. Newer evidence, however, showed that, for major disorders like AD, PD, and FTD, lysosomal components commonly are the primary target of causative and risk gene mutations[83, 84], as discussed below. The resulting lysosomal dysfunction drives disease onset by allowing pathogenic forms of the mutant protein and other substrates to resist breakdown, to become (further) covalently modified by reactive oxygen species, and to accumulate as even more toxic fragments or aggregates compared to the original pathogenic form. Combined effects from these instigating processes are essential to drive the downstream pathobiology leading to neuronal death.

The apt description of lysosomes by their discoverer, Christian DeDuve, as potential “suicide bags”, anticipated the vulnerability of this fundamental degradative process in degenerative diseases[85, 86]. This is now well validated and >40 lysosomal genes are known to cause neuron loss in inheritable neurodegenerative disorders across the age spectrum[87]. Further validating the link between lysosome-based mechanisms to adult neurodegenerative disease are lysosomal gene mutations that in homozygous form cause congenital LSD with severe neurodegenerative or neurodevelopmental phenotypes but, in heterozygous form, cause an adult-onset neurodegenerative disease or substantially raise its risk[38, 39]. Pathogenic mechanisms underlying varying major late-age onset neurodegenerative diseases are converging on common themes of pH and ion balance dysregulation that disrupt not only lysosomal hydrolytic enzyme function broadly but also the diverse signaling roles of the lysosome and related acidic vesicular compartments, as discussed below, which together instigate and further drive disease progression.

[H2] Lysosomal acidification and neuronal homeostasis failure in adult neurodegenerative diseases

Lysosomes maintain cellular homeostasis through the complex coordination of their essential roles in substrate hydrolysis to generate energy, the controlled release of nutrient transporters and signaling factors, which together enable metabolic coordination with other organelles to regulate vesicle dynamics and signaling events, and specific gene expression programmes. This range of physiological functions, beyond the scope of this Review of their implications for neurodegeneration, underscores the centrality of lysosome in metabolic regulation[7]. Among the lysosomal properties most crucial to this orchestration of neuronal functions and, arguably the one most significantly implicated in disease pathogenesis is the lysosome’s strongly acidic intraluminal pH (pH 4.3–5.0)[88], which is essential for hydrolytic enzyme activation, complete digestion of substrates, H^+-coupled nutrient and drug transport, and the varied homeostatic signaling functions[89-91]. Acidification is achieved by the ATP-dependent proton pump, vacuolar H^+-ATPase (v-ATPase)[92, 93], a 14 subunit complex regulated mainly by the association and dissociation of two subcomplexes: One of these subcomplexes (V1) is cytoplasmic and provides the ATPase activity needed to generate torque that opens a channel within the membrane-spanning V0 subcomplex (**Figure 3**). The V0a1 subunit of the V0 subcomplex is especially critical because it tethers V1 to the membrane anchored V0 subcomplex[94]. Not surprisingly, this subunit is broadly implicated in the pathogenesis of neurodegenerative diseases[95]. In addition to the v-ATPase complex, an array of ion channels in the lysosomal membrane are now known to influence lysosomal proton content and finely modulate lysosomal pH, including lysosomal-associated membrane proteins (LAMPs), which were

353 previously viewed only as structural proteins[96]. Remarkably, the varied modulators of ion balance within
354 lysosomes have themselves been recently implicated as causative for major neurological diseases
355 (**Figure 4**).

356 The need for tight regulation of lysosomal pH can be appreciated from the diverse regulatory roles that
357 vesicle acidity has in neuronal function. The widely varied pH optima of lysosomal hydrolases (3.5 to 6.0)
358 imply that a small rise in pH, as might chronically be imposed in some diseases affecting v-ATPase or
359 other ion channels, can alter patterns of substrate cleavage and promote aggregation, proteolytic
360 resistance of substrate, enzyme denaturation and turnover[97] (**Figure 5**). Hydrolytic activity in turn
361 regulates neuronal homeostasis during nutrient and lysosomal stress[98] by generating amino acids that
362 activate signaling platforms on lysosomes, thereby controlling homeostatic balance[13, 99-102]. Declining
363 proteolysis and amino acid generation, for example, releases lysosome-docked mTORC1, resulting in
364 lower mTORC1 kinase activity[13]. This decreases phosphorylation of transcription factors, like TFEB,
365 TFE3 **[G]** and others[103], by this kinase, enabling their nuclear translocation to drive expression of genes
366 encoding many components of lysosomes and additional autophagosome machinery[13, 103-105]. Feedback
367 onto mTOR also influences the crucial balance between autophagy and protein synthesis. Apart from
368 clearance functions, high levels of acidification in synapses are also required for synaptic vesicle fusion
369 and recycling[106] during neurotransmitter exocytic release[107], which may explain why lysosomal
370 enhancement via pH correction ameliorates synaptic dysfunction and cognition, as well as clearance of
371 aggregate-prone proteins, in mouse models of AD and PD[94, 108, 109].

372

373 **[H3] Lysosomal acidification failure in Alzheimer's disease**

374 V-ATPase complex vulnerability underlying neurodegenerative disease gained initial attention from
375 studies of Presenilin 1 (PSEN1), mutations of which cause the most common inherited form of AD[110].
376 This multi-span transmembrane protein[111] is the catalytic subunit of the γ-secretase complex responsible
377 for intramembrane proteolysis of many substrates[111, 112, 110], the best known being cleavage of the APP
378 carboxy-terminal fragment (APP-βCTF) to amyloid-β (Aβ) (**Figure 3**). The γ-secretase, including the
379 PSEN1 homolog PSEN2 (ref.[113]), mutations of which are also associated with AD, is enriched in
380 lysosomes[114, 115], has broad substrate specificity and becomes highly abundant in autophagic vacuoles
381 when autophagy is upregulated[116], all of which points strongly to γ-secretase having a broad role in
382 lysosomal catabolism rather a selective one. Independently of its function in the γ-secretase complex,
383 PSEN1 itself is required for complete lysosomal acidification[108, 117, 118]. The PSEN1 holoprotein located in
384 the ER acts as a chaperone for the v-ATPase subunit V0a1, the crucial anchoring subunit (**Figure 3**),
385 and enables normal v-ATPase complex assembly[108, 117-121]. *PSEN1* deletion or loss-of-function mutations

causing AD to reduce V0a1 delivery to lysosomes, lysosomal V0a1 content, v-ATPase activity and lysosomal acidification[94, 108, 117-120, 122-125]. These effects have been documented in primary skin fibroblasts from patients with early-onset AD caused by a familial autosomal dominant mutation of Presenilin 1 (PSEN1-FAD)[94, 108, 117, 118], human PSEN1-FAD neurons[125], and mouse models of PSEN1-FAD[118], and are reversed by re-establishing normal lysosomal pH directly with lysosome-targeted acidic nanoparticles[94, 108] or by pharmacologically restoring lysosomal pH[94, 119, 122-126]. An earlier unconfirmed claim that PSEN1 deletion has no effect on pH[127] was subsequently challenged on multiple technical grounds[108, 117, 128] and by the wealth of subsequent contrary evidence cited above. As discussed below, PSEN1 loss of function also releases calcium from lysosomes[19, 108, 117, 127] via transient receptor potential channel mucolipin 1 (TRPML1, also known as mucolipin-1)[19, 108] or two pore channel protein 2 (TPC2) channels[129]. Normalizing the rise in lysosomal pH in *PSEN1* knockout cells mentioned above also corrected lysosomal Ca^{2+} release , whereas blocking lysosomal Ca^{2+} release did not correct the abnormal pH rise[19, 108]. PSEN1-mediated release of calcium from the ER[123, 126] may also promote lysosomal pH elevation and reduce lysosomal function, which is partially rescued by blocking ryanodine[123, 126] or inositol triphosphate receptors[126], which are two of the multiple calcium channels mainly in ER that maintain cellular calcium homeostasis by releasing calcium and refilling depleted calcium stores in lysosomes or mitochondria[130]. Whereas several mechanisms potentially contribute to PSEN1-mediated lysosomal deacidification in still unclear ways, there is strong consensus that pH rise inhibits waste elimination by lysosomes including the two proteotoxic metabolites of APP, namely APP-βCTF and Aβ, which drive disease progression[121, 131-133] (**Figures 5, 6**).

APP, arguably the most important AD gene, also directly inhibits v-ATPase, although through a different mechanism than *PSEN1*. The initial APP cleavage product, APP-βCTF, binds selectively to the V0a1 subunit within the v-ATPase complex and reduces association of the V1 subcomplex with the V0 subcomplex, thereby lowering vATPase activity[94]. In healthy cells, small fluctuations in APP-βCTF level can tonically modulate vATPase activity and pH within a physiological range[94, 121] (**Fig. 3**). Early in AD, APP-βCTF accumulation to abnormally high levels in neuronal lysosomes more strongly inhibits the V1 subcomplex's association with the V0 subcomplex causing pathological lysosomal pH rise[121] and impaired substrate hydrolysis. In response to pathological lysosomal pH elevation in neurons of AD brain, a uniquely extreme "autophagic stress"[131] develops in the neuronal soma[131], characterized by massive accumulation of inadequately acidified autolysosomes unable to degrade substrates, including APP-βCTF and Aβ leading to intracellular formation of β-amyloid[131] (**Figures 5, 6**). Ensuing free radical-mediated and proteotoxic damage to lysosomal membranes promotes the early autophagic–lysosomal death of affected neurons containing β-amyloid aggregates and their transformation into extracellular

senile ("β-amyloid") plaques[131, 132, 134-136] (**Figure 6**). A similar neuropathological sequence also evolves early in sporadic AD[131].

Mounting evidence for the pathogenicity of APP-βCTF in AD includes its prominent role in triggering endosome dysfunction beginning at the earliest stages of AD[132, 137-140] and regulating interactions at mitochondria-associated ER membranes (MAMs) which, in AD, disrupts calcium and lipid homeostasis and alters mitochondrial behaviors that amplify oxidative phosphorylation and reactive oxygen species (ROS)[141]. Moreover, APP-βCTF is a suspected factor mediating the increased late-onset AD risk due to polymorphisms of the neuronal lysosomal protein phospholipase D3 (PLD3): PLD3, a transmembrane protein delivered from the Golgi and proteolytically cleaved in lysosomes, yields a stable soluble polypeptide with 5'-3' exonuclease activity but unclear roles in lysosomes[142]. APP-βCTF build-up in lysosomes of PLD3-over-expressing mice is associated with an AD-like phenotype characterized by robust accumulation of autophagic vacuoles in the soma and within swellings along axons resembling "dystrophic neurites" of AD, suggesting selectively impaired retrograde transport of these autophagic vacuoles[143] (**Figure 6**). A similar autophagic phenotype is accelerated in brains from individuals carrying the risk *PLD3* gene polymorphism[142, 143].

[H3] Primary lysosomal pH dysregulation is common in neurodegenerative disease

The pathogenic targeting of lysosome acidification, and particularly the V0a1 v-ATPase subunit, extends beyond Alzheimer-related conditions to mechanisms underlying the pathogenic actions of causal genes in a host of other neurodegenerative diseases[95] (**Figures 2, 3**). Analogous to *PSEN1*'s chaperone role, palmitoylation of V0a1 in the Golgi assists its maturation[144]. Loss-of-function mutations of the palmitoyl-protein thioesterase 1 gene (*PPT*) that cause a severe neurodegenerative LSD called CLN1 neuronal ceroid lipofuscinosis **[G]** (NCL), impair maturation of V0a1 and other *Ppt*-modified proteins, leading to deficient assembly and activity of lysosomal v-ATPase[145]. Also, in PD, the causative Arg1441Cys mutation in leucine-rich repeat serine/threonine-protein kinase 2 (LRRK2) disrupts a LRRK2–v-ATPase interaction leading to the observed v-ATPase deficiency and acidification defects[144]. Given the mechanism in CLN1, a possible role for v-ATPase palmitoylation in the disruption is possible but has not been demonstrated[144]. Furthermore, PD-causing LRRK2 mutations hyperactivate LRRK2 function as a kinase for a number of different Ras-related (RAB) proteins, thus likely impacting diverse components of the endolysosomal system[146]. The relationship between PD and LSDs is supported by Kufor-Rakeb syndrome, a rare neurodegenerative dementia associated with early-onset parkinsonism[147] and a cause of NCL in a strain of dogs and at least one human family[148-150]. The PD risk gene *ATP13A2,* encoding a lysosomal P5-type ATPase involved in polyamine (e.g., spermine, spermidine) transport from the

lysosome[151], thereby maintaining lysosome health by preventing toxic polyamine accumulation[151]. *ATP13A2* is considered a pH modulator given that its mutation and/or loss leads to accumulation of these polyamines[152-154], pH elevation, autophagosome and lysosome build-up[155-157] and earlier cell death by apoptosis than treated wildtype cells[151]. As polyamines act as scavengers of heavy metals, polyamine accumulation in lysosomes in PD might explain previously reported changes in heavy metal content in PD[158].

Yet another likely modulator of lysosomal pH, and a risk factor for the synucleinopathies PD and Lewy body dementia **[G]** (LBD), is the endosomal/lysosomal proton channel TMEM175 (refs.[159-161]) (**Figure 4**). The working mechanism and function of TMEM175 in different pathophysiological contexts are not yet fully resolved. Although originally reported to be a non-canonical potassium channel[162, 163], recent reports emphasize its role as a selective channel to leak H^+ out of lysosomes when the lysosomal pH drops below normal[164, 165]. Conversely, TMEM175 is proposed to expel potassium to retain H^+ if the pH rises excessively[164, 165]. TMEM175-deficient cells have hypoactive hydrolases including lysosomal acid glucosylceramidase (GBA)[144, 166] and an accumulation of pathological α-synuclein[164, 167] potentially accounting for the loss of dopaminergic neurons seen in mice with TMEM175 mutations[163, 164, 168]. Whether this phenotype reflects hyper-acidification or elevated lysosomal pH may depend on the experimental context[164, 167]. A recently discovered facet of TMEM175 biology is its association with LAMP1 and LAMP2. LAMPS were originally believed just to be structural proteins that protect lysosomal membrane from resident hydrolytic enzymes, although evidence now reveals that they have a key physiological role in suppressing proton leak through TMEM175 channels to facilitate re-acidification when lysosomal pH rises inappropriately[96].

Another example of lysosomal dysfunction and pH disruption as central to the mechanism of adult neurodegenerative disease is FTLD, the most common, inherited form of which is caused by mutations of *GRN*, the gene encoding progranulin (PGRN) and causing the disorder known as FTLD-GRN[169] (**Figure 5**). Growing evidence indicates PGRN regulates multiple aspects of lysosomal function, including cathepsin and GCase activities and lysosomal pH[39, 170]. Recent unbiased multimodal proteomics and functional analyses of lysosomes in PGRN-deficient neurons or human PGRN-mutant induced pluripotent stem cells **[G]** (iPSC) neurons uncovered a marked impairment of lysosomal acidification resulting in impaired hydrolytic activity despite an upregulated expression of acidification machinery proteins[171]. Loss of PGRN is proposed to impede attachment of the V1 subcomplex to the membrane-anchored V0 domain[172] (**Figure 3**). Furthermore, PGRN directly interacts with the lysosomal pH regulator, transmembrane protein 106B (TMEM106B), a ubiquitous type 2 (endo)lysosomal integral membrane protein expressed at especially high levels in neurons and oligodendroglial cells[173-175]. Single-nucleotide

TMEM106B polymorphisms modify the risk and lower the age of onset of FTLD-GRN[176-178]. TMEM106 gene deletion downregulates V0a1, V0c, and V0d1 and dramatically exacerbates CNS neurodegeneration and autophagic stress when combined with loss of PGRN in mice, presumably compounding the acidification deficit common to both genetic manipulations[179-181]. *Tmem106b* deletion alone induces a cascade of effects stemming from insufficient lysosomal acidification[172, 179] and autophagic stress resembling effects seen in AD[182,183], as well as defective lysosomal transport[183]. Lysosomal processing of TMEM106B yields a carboxy-terminal fragment that binds to v-ATPase[172] and its accessory protein 1 (AP1) component[172] as well as to cathepsin D[179], one of approximately a dozen proteases in lysosomes that are activated at low pH and collectively are capable of degrading endogenous and internalized exogenous proteins completely to amino acids. Their range of functions in maintaining cellular homeostasis is very broad[184]. Cathepsin D, while ubiquitously expressed in cells, exhibits high expression in brain and decreases in its function are frequently suspected in mechanisms of neurodegeneration[179, 185]. Notably, a second accessory protein of the V0 domain, ATP6AP2, is a risk gene for PD[154]. Finally, the v-ATPase is also targeted in individuals with X-linked dominantly inherited amyotrophic lateral sclerosis with FTD by mutation in the *UBQLN2* gene (encoding ubiquilin2) that functions in both the proteasome and autophagy pathways by clearing misfolded proteins[186]. Mutation of *UBQLN2* reduces expression of ATP6v1G1, a critical subunit of the ATPase pump that also regulates vacuolar acidification and is required for autophagosome maturation[187]. Modeling the disease in flies and mammalian cells also revealed that ubiquilins are required to maintain proper levels of the V0a/V100 subunit of v-ATPase. Mutations or deletion of UBQLN2 elevated the lysosomal pH and lowered cathepsin B activity despite greater autophagy induction[188].

That so many disease-causing lysosomal genes converge directly on pH regulation within this vital organelle is remarkable, and novel evidence continues to emerge. It is important also to recognize that the impact of these genes on other ELA network components and most importantly on downstream neuronal functions is informative about brain disease pathogenesis and particular vulnerabilities exhibited by different neurodegenerative diseases. Several major interconnecting mechanisms are considered below.

[H2] Lysosomal calcium and neurodegeneration

Lysosomes represent a rich store of calcium in neurons, which is tightly regulated in close coordination with ER and mitochondrial calcium regulation and lysosomal pH[108, 189-191] (**Figure 4 and 5**). Calcium efflux from lysosomes influences waste clearance through regulatory actions on lysosomal motility, fusion with other organelles, and cell signaling controlling autophagy flux[192-194]. As an example for the latter role,

calcium released locally around lysosomes in response to cell stress activates calcineurin, which dephosphorylates the lysosome-docked transcription factors TF-EB and TF-E3 and enables their translocation to the nucleus[195]. There, these factors promote gene transcription supporting lysosome biogenesis, including expression of catabolic enzymes and v-ATPase complex subunits. The calcium release also promotes lysosomal exocytosis (i.e., secretion of lysosomal content upon lysosome fusion with plasma membrane), an alternative route for waste clearance[194], which is upregulated when conventional autophagy routes are impaired[194]. TRPML1, which transports mainly Ca^{2+} but also Fe^{2+} and Zn^{2+}, initiates this process[194] and its expression itself is regulated by TFEB[195]. Loss-of-function mutations in TRPML1 cause Mucolipidosis type IV (MLIV), a severe neurodegenerative LSD[196] with impaired lysosomal trafficking and hydrolysis[197].

TRPML1 (ref. [198]) can be activated by phosphatidylinositol 3,5-bisphosphate ($PI(3,5)P_2$)[199-205]. Importantly, however, TRPML1 channel opening also increases independently of agonists in response to metabolic stressors, including lysosomal deacidification[206] and oxidative stress — another major contributor to lysosomal pH elevation[207-210]. TRPML1 signaling induces lysosome biogenesis and mTORC1-mediated autophagy[211] to maintain cell homeostasis under conditions of mild stress. Sustained TRPML1 channel opening is triggered by extreme ROS generation from oxidants such as hydrogen persoxide[207] or following damage to mitochondria[212, 213], which can cause neurodegeneration by over-activating calcium-dependent calpain proteases that cleave structural proteins, dysregulate essential enzymes and thereby induce cell necrosis[108, 214, 215]. Its inhibition is neuroprotective in AD, PD, and HD models[216-218]. Calcium (and calpains) also over-activate the protein kinase CDK5, which injures mitochondria by hyper-phosphorylating key components and also can cause post-mitotic neurons to re-enter the cell cycle[108, 216-219]. A buffer against rises in cytoplasmic calcium or altered organellar calcium homeostasis is the bidirectional exchange of calcium through multiple ER channels (IP3Rs, RyR). Repleting levels of ER calcium attenuates disease-related ER stress[220], whereas refilling lysosomal calcium stores may facilitate acidification. Two-pore channels (TPC) are a class of nicotinic acid adenine dinucleotide phosphate (NAADP)-stimulated Ca^{2+} release channels that modulate varied physiological processes, including endolysosomal trafficking and plasma membrane excitability[203] and, like TRPML1, promote autophagy and possibly lysosomal exocytosis mainly under cellular stress conditions[221].

The intertwined nature of lysosomal calcium and pH or autophagy dysregulation seen in varied neurodegenerative diseases is exemplified by inherited (familial) mutations of *PSEN1* that cause AD (i.e. PSEN1 FAD) (**Figure 4**). A knock-in mouse model of PSEN1 FAD was found to develop a late-age onset AD-like axonal dystrophy stemming from selectively impaired retrograde transport of autophagic and/or amphisomal vesicles and their accumulation within focal axon swellings[19]. The defect involves pH-

dependent TRPML1-mediated efflux of calcium that activates c-jun N-terminal kinase (JNK)-mediated phosphorylation of dynein intermediate chains and thereby impedes retrograde motility[19]. Restoring proper lysosome acidity rescues the subsequent pathological events[19]. Recent findings that lysosome and autophagy defects in PSEN1-FAD models could be substantially rescued by blocking calcium release from the ER through overactivated ryanodine receptors **[G]** (RyRs)[123] raised the question of whether the release of calcium from the ER is a cause or an effect of lysosomal pH elevation and calcium release. Supporting the latter possibility, an initial Ca^{2+} release from activated TRPML1 channels can trigger subsequent Ca^{2+} release through IP3Rs or RyRs in the ER[222-225]. Moreover, activating experimentally TRPML1 channels increases TFEB-mediated transcription of v-ATPase components[225], a known neuroprotective response that is thought to rescue acidification and enhance autophagy. A primary disruption of v-ATPase and lysosomal pH in PSEN1 FAD is also supported by disruptions of v-ATPase via direct binding with the FAD-mutant pathogenic protein *PSEN1* and the β-cleaved CTF of *APP*. Interestingly, as in the FAD mouse model, lysosomal TRPML1 activation, by inducing autophagy, also promotes autophagy stress and the focal axonal swellings filled with autophagic vacuoles, which are associated with coding variants of *PLD3* that increase AD risk[142, 143]. Reduced lysosomal Ca^{2+} levels, likely reflecting excessive calcium efflux, are also seen in forms of PD caused by mutations in the lysosomal acid glucosylceramidase GBA1 (ref. [226, 227]). The accumulating mitochondrial damage and ROS generated by other causal Parkinson genes that disrupt mitophagy[226] are a further basis for abnormal pH elevation and TRPML1 over-activation (**Figures 4, 5**). In several other cellular states (e.g. LDL over-load in non-neural cells[228], APOE4 expression), by contrast, the TRPML1 agonist ML-SA1 prevents and TRPML1 silencing potentiates lysosomal dysfunction[228, 229]. These seemingly contradictory data obscure an answer to whether stimulating or preventing Ca^{+2} efflux from lysosomes would be a useful intervention in any particular neurological disease context, especially in view of the potential neurotoxicity of excessive calcium release from lysosomes[108]. Given the important cross-regulation among major calcium stores and the potential for lysosomal dysfunction by varied alterations in degenerative disease states, this interplay warrants more intensive research focus to clarify the long-term impact of modifying calcium fluxes as a disease intervention.

[H2] Lysosomal hydrolysis failure

In addition to the sizable impact of pH changes on general hydrolytic activity in some disorders, disease genes, pathogenic substrates, or a combination of both may directly disrupt lysosomal hydrolytic enzymes. Either of these two sources of disruption may induce varied responses from different enzymes, such as compensatory upregulated lysosomal gene transcription, altered hydrolase maturation and

proenzyme activation, or inactivation and buildup of the hydrolase and its specific substrates. The disease literature is replete with reports of altered cathepsin levels; however, the use of mainly artificial assay systems makes interpretation difficult and few reports document direct pathogenic protein–enzyme interactions within a specific intact cell type. As in the diseases described above, a chronically low-level rise in baseline pH can substantially inactivate the hydrolases with the most acidic optima, such as cathepsins D (CTSD) and L. Also, by inhibiting the turnover of the protease in the lysosome, elevated pH raise levels of the partially inactive enzyme, which is mistaken to an elevation in expression. That said, mutations in three of 15 cathepsins (CTSD, CTSA, and CTSF) are known to cause degenerative LSDs, including most notably neuronal ceroid lipofuscinosis 10 (CLN10). CTSD polymorphisms are additionally associated with increased risk of AD, PD, and FTD[185, 230]. Additional gene mutations and polymorphisms disrupting a specific hydrolase or calcium channel involved in autophagy–lysosome clearance expand the important theme linking pathogenesis of neurodegenerative LSDs with disorders in adults. Mutations affecting enzymes involved in lipid clearance also disrupt overall hydrolysis[231-237] and clearing these lipids can help ameliorate proteolysis deficits[238]. The physiological regulation and crosstalk among individual hydrolases, remains largely uncharted investigative territory and is likely to reveal important new roles in signaling and neuronal dysfunctions more subtle than degeneration[239, 240].

[H2] Later consequences of autophagy–lysosomal failure

In the foregoing disease scenarios, the accumulation of autophagic substrates in autophagic vacuoles, mainly autolysosomes, is a common pathological outcome in the most affected neurons in adult neurodegenerative diseases and a harbinger of eventual neuron death. This phenomenon, termed “autophagic stress”, reflects an imbalance favoring autophagosome production over rate of clearance, and its effects on the severity of autophagic vacuole build-up varies in different diseases[241]. Given the established function of autophagy as a neuroprotective cellular response through specific anti-apoptosis molecular mechanisms[242], autophagy upregulation is an early response against cell aging and the added compromise due to disease. In the case of AD, however, the persistence of autophagy induction in the face of growing lysosome failure becomes counter-productive, leading to a uniquely massive autolysosome accumulation in the most compromised neurons (**Figure 6**). In PD and HD, autophagy induction does not change and may actually be lowered, possibly accounting for why autophagic stress is much less prominent than in AD despite lysosomal dysfunction[243, 244]. Lysosomal and autolysosomal damage causing lysosomal membrane permeability (LMP) leads to gradual leakage of toxic hydrolytic enzymes. The likely final common pathway triggering a slowly progressive neuronal degeneration and cell death in the above-described diseases. LMP leading to a gradual lysosomal-associated cell death

can be mediated by lysosomal hydrolase release directly[245, 246]. Greater release via more severe LMP can trigger cascades (e.g. apoptosis, necroptosis, ferroptosis) that cause rapid demise of the neuron, which has morphological features or markers of multiple death cascades[131, 247]. LMP is often attributed to damage from the accumulated pathogenic protein (e.g. Aβ, a-synuclein); however, hydrolytic failure generates numerous damaging derivatives, most notably oxidized lipids and proteins that further inhibit hydrolases by oxidizing or otherwise damaging resident proteins[24, 35-37, 248, 249]. Oxidized lipid accumulations in lysosomes elevate Fe^{2+} entry into cells[250, 251] through the endolysosomal system[252, 253], which helps to further drive oxidative stress via the Fenton reaction[254, 255] and further promotes lysosomal deacidification[256] and promote a more acute death involving ferroptosis[257]. The oxidative attack on v-ATPase and lysosomal enzymes creates a vicious cycle of progressive lysosomal corruption that initiates other pathogenic events described in **Figure 5**. The sequence of autophagy–lysosomal-triggered pathogenic events evolving in AD highlights the protracted intraneuronal deterioration of this system. This failure precedes very early death of a select population of vulnerable neurons and the transformation of each of these dying neurons into an extracellular senile plaque (**Figure 6**). This pathological sequence of intraneuronal events aligns AD pathobiology with other aging-related neurodegenerative proteinopathies, many of which are being recognized as disrupting the autophagic–lysosomal system and inter-related endosomal-lysosomal system functions[131, 134, 241, 243, 258-260].

[H1] Future directions for clinical translation

As accumulated toxic misfolded proteins are a hallmark of most neurodegenerative diseases, translational efforts have focused on enhancing degradation of these proteins. Since lesions that cause neurodegenerative diseases may impact autophagy at different stages of the pathway, the sites of such defects need to be understood to maximize therapeutic opportunities. For example, in the case of disease where there is defective formation of autophagosomes at an early stage of the pathway but no compromise in the pathway after autophagosome formation or not enough of a defect to significantly impair subsequent stages of autophagic flux, then therapeutically boosting autophagosome formation may be suitable (e.g. in models of HD, or PD caused by α-synuclein mutations)[261]. By contrast, in diseases where there are defects after the stage of autophagosome formation, for instance in AD and LSDs, then autophagosomes may accumulate unproductively and cause toxicity especially if autophagosome formation is boosted as part of positive feedback loops aimed to promote clearance of accumulating pathogenic substrates. For example, autophagosome membranes may serve as a platform for p62-dependent caspase 8 recruitment to form an intracellular death-inducing signaling complex[262] and apoptosis may thus occur under conditions leading to excessive autophagic membranes. However, this theoretical possibility needs to be rigorously tested in different disease-relevant scenarios, as there

is evidence that inhibition of autophagic flux caused by some lysosomal defects may be amenable to amelioration with autophagosome formation inducers[263] if the compound can also unblock the lysosome[264]. Nevertheless, it is desirable to stratify diseases by genotype, where possible, in order to prioritize the cases that are most likely to benefit from induction of autophagosome formation. For example, in the case of Parkinson diseases **[G]** , patients with α-synuclein mutation may be suitable for this strategy[265], whereas those with *ATP13A2* defects affecting lysosomal activity[266] may be less suitable.

Much activity has focused on identifying drugs to induce autophagosome formation and improve lysosome function[4, 267, 268]. Frequently, such drugs act at signaling nodes that impact other pathways, hence the need to consider safety and specificity. However, one of the attractions of this strategy is that one can induce the autophagy pathway in a pulsatile fashion, which may mitigate some side-effects. This has been demonstrated with mTOR inhibition in a tauopathy mouse model[269]. The timing of autophagosome induction treatment may be critical in some diseases. Upregulated autophagosome biogenesis might be protective in the early stages of diseases like AD (as suggested by mouse studies[270]), but may be ineffective or compound the lysosome failure that becomes overt at later stages. One possible way that may enable simultaneous induction of autophagosome biogenesis along with improved lysosomal function would be by increasing TFEB and/or TFE3 activities[271]. It is also worth considering the strategy of increasing specific removal of substrates by using molecular glues to link substrates to autophagy components like LC3 (ref. [4]).

Pharmacological intervention programs have historically been wary of considering lysosomal remediation, due to the risks of overshooting normalization (e.g. decreasing pH too far in diseases where lysosomes are too alkaline) by therapeutically enhancing lysosomal proteolytic activity. However, scant evidence has been produced to justify the concern and in fact, effective targeting of lysosomal dysfunction has been demonstrated in animal models of neurodegenerative disease[260, 268, 272-277]. A growing number of strategies to abrogate pH elevation and calcium efflux abnormalities and the toxic substrate storage are suggested by the discussion above or detailed in recent reviews. Some preclinical interventions have attenuated lysosomal dysfunction and rescued additional deficits in synaptic function and cognition, attesting to the promise of this approach.

Despite enormous progress in understanding autophagy, the movement of more agents into the clinic is still impeded by a relative lack of tools to measure autophagy activity in vivo and to establish target engagement by drugs in specific cell types in intact brain[84]. Different or even reciprocal responses to a drug may well occur in different neural cell types. Biochemical analyses of tissues from preclinical models may obscure these cell type differences. For future clinical trials, surrogate biomarkers of ELA dysfunction in accessible fluids or revealed by neuroimaging are urgently needed to validate the effective modification

of an ELA-related disease target non-invasively. Nevertheless, advances towards targeting mechanisms underlying phenotypes in neurodegenerative diseases, the diversity of possible ways that autophagy might be targeted therapeutically provides optimism that autophagy modulation may ultimately prove to be an effective therapeutic approach for neurodegenerative diseases.

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Author contributions

The authors contributed equally to all aspects of the article.

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DCR is a consultant for Aladdin Healthcare Technologies Ltd., Mindrank AI, Nido Biosciences, Drishti Discoveries, Retro Biosciences, PAQ Therapeutics and Alexion Pharma Intl Ops Ltd. RAN declares no competing interests.

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Figure legends

Figure 1. Autophagy–lysosomal pathway in neurons

Macroautophagy is initiated by the formation of a double-membrane enveloping structure, the phagophore, which occurs both in the synapse and throughout the neuron. However, most autophagosome formation appears to occur in the distal axon. Cytoplasmic constituents and organelles targeted for degradation are captured within an autophagosome, which is formed as the phagophore elongates and encircles the substrates. The autophagosome is degraded upon fusion with a lysosome to form an autolysosome. This process introduces acid hydrolases and proton pumps (v-ATPase), leading to luminal acidification, thereby activating an array of hydrolases that can fully digest most substrates into their building blocks (e.g. amino acids), which are recycled for energy or new synthesis. An intermediate step particularly prevalent in the processes of neurons (both in dendrites and axons) is the fusion of an autophagosome with a Ras-related protein 7 (Rab7)-positive late endosome to form an amphisome, which shows high dynein-based retrograde motility[17, 278]. In chaperone-mediated autophagy (CMA), proteins carrying a KFERQ-like sequence are recognized by the chaperone Hsc70, which can then associate with the integral lysosome-associated membrane protein LAMP2A. This interaction triggers its oligomerization, enabling translocation of the bound substrate into the lysosome. “Heterophagy” via the endolysosomal pathway involves lysosomal degradation of plasma membrane components and exogenous substrates after they are internalized by receptor-mediated or bulk endocytosis. Cargo proteins are sorted selectively to different cellular destinations or recycled to the plasma membrane (not shown). Proteins targeted for degradation are trafficked to late endosomes or multivesicular bodies (MVB), which mature to lysosomes, enabling complete degradation of cargo proteins. A buildup of lipofuscin reflecting normal presence of internal waste storage in this compacted form increases as neurons age (see details in main text).

The figure illustrates unique features of the autophagy–lysosomal pathway in neurons, which are asymmetric post-mitotic cells that cannot be replaced and therefore must maintain efficient proteostasis over many decades. Sites of highly active autophagy and endocytosis are separated by long distances from the most active degradative capability located in the soma where lysosomes are concentrated, implying a strong reliance on efficient retrograde transport — a process commonly affected in disease. The absence of mature fully activated lysosomes along axons limits a neuron’s capability for eliminating waste during the lengthy transit of autophagosomes and amphisomes until they reach lysosomes in the soma. Anterogradely moving Golgi carrier vesicles deliver lysosomal components to the amphisomes and late endosomes to facilitate their maturation toward a lysosome identity, however this does not suffice for efficient substrate degradation. The most vulnerable neuron populations in neurodegenerative

disease are often ones with a very high axon to somal volume ratio requiring autophagic quality control over huge cytoplasmic volumes.

Figure 2. **Causal genes for adult-onset neurodegenerative disease commonly disrupt autophagic-lysosomal function**

Listed here are principally mutated genes considered causative for selected major adult-onset neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and frontotemporal dementia (FTD) as well as several examples of proteins believed to contribute to disease, where the evidence strongly pinpoints a critical pathogenic mechanism within the endosomal–lysosomal–autophagy axis. There are additional unlisted genes featuring polymorphisms or other modifications that increase disease risk but have smaller effect sizes or where the mechanism of pathway disruption is less well defined. The listed genes disrupt a broad range of lysosome-related pathways, including lysosome or autophagosome biogenesis, chaperone-mediated autophagy, substrate capture, mito- and macroautophagy and trafficking routes, but mechanistic convergence at the lysosome is most common, causing failure to properly clear potentially toxic substrates and maintain important lysosomal-related cellular homeostatic signaling mechanisms. Although the diagram and this Review focuse on this dysfunction in neurons, which is most closely linked to clinical progression of neurodegenerative disease, the less well characterized autophagy–lysosomal impairments of clearance mechanisms in microglia, astrocytes and vascular cells undoubtably contribute to disease progression.

Figure 3. v-ATPase and lysosome acidification in adult neurodegenerative disease

The v-ATPase complex is composed of an extra-lumenal V1 domain (consisting of the subunits A-F) and an integral membrane-associated V0 domain (composed of subunits a, c, c″, d, and e)[279]. ATP hydrolysis by the V1 subcomplex opens a channel within the V0 subcomplex through which protons pass. Proton pump activity is regulated by the reversible dissociation of the V1 and V0 domains. Reversible dissociation is rapid, does not require new protein synthesis, and occurs in response to diverse cellular signals that toggle v-ATPase activity up or down. A few examples are nutritional states and assembly factors such as phosphatidylinositol-3-kinase, mTORC, cAMP, PKA, glucose and amino acid levels. Due to its interactions with V1 domain subunits that mediate full complex assembly and proton pump activity, the V0a1 subunit is an especially vulnerable target in many diseases. Most of the proteins listed in the box interfere at different stages of V0a1 subunit maturation, complex assembly, or interaction with the V1 subcomplex and their mutation or loss elevates pH in disease. The figure

illustrates primary actions of two key proteins causing Alzheimer's Disease (AD), namely amyloid precursor protein **[G]** (APP) and Presenilin 1 (PSEN1). Disease variants of PSEN1 and APP directly disrupt v-ATPase activity and cause lysosomal pH elevation responsible for autophagy clearance failure, intracellular amyloid formation, and ultimately neuronal cell death and extracellular senile (amyloid) plaque formation[131] (more details in text and Figure 6). *PSEN1* deletion or mutations causing AD, which are loss of function defects, impair the activity of the presenilin 1 holoprotein in the ER, which acts as a chaperone for V0a1 subunit glycosylation (by the oligosaccharyltransferase (OST) complex) and folding. The instability of the subunit structure triggers premature degradation by endoplasmic reticulum (ER)-associated degradation **[G]** (ERAD) and results in insufficient delivery of V0a1 subunits for assembling adequate complexes on lysosomes and ensuring proper proton pumping activity. In addition, PSEN1 has a separate function in the cleavage of APP: The initial cleavage of APP by β-secretase (BACE) generates a carboxyl terminal fragment (APP-βCTF) that, in its constitutively phosphorylated form (pTYR^{682}), specifically binds to V0a1 and impedes V1 subcomplex association with V0, leading to similar pathological consequences as in the mutant PSEN1 loss of function (LOF) condition. Levels of APP-βCTF and pTYR^{682}-βCTF are substantially elevated in early-onset Alzheimer disease due to its mutation-mediated over-production and impaired turnover. In the common late-onset forms of Alzheimer disease, neuronal APP-βCTF is also elevated due to multiple factors that include over-production based on elevated β-cleavage of APP and its accumulation in the failing lysosomes of vulnerable neuron populations[121, 132, 133, 136, 139, 280-292], setting up a vicious cycle similar to that triggered in early-onset AD.

Figure 4. Lysosomal ion imbalances in neurodegenerative disease

Effective lysosomal acidification requires not only the v-ATPase described in Fig. 3 but also counterion flows involving chloride, potassium, sodium, and possibly other ions. This figure depicts components of the lysosome implicated in neurodegenerative disease that affect lysosomal ion flux, including v-ATPase, the non-selective cation channels transient receptor potential channel mucolipin 1 (TRPML1, which transports mainly Ca^{+2} but also other cations, for details see text) and two-pore channel (TPC2). TPC2 acts as a Ca^{2+}-permeable channels when activated by NAADP[190, 221, 293, 294] and can act as a Na^{+} channel when activated by $PI(3,5)P_2$. In addition, the ion transporters H(+)/Cl(-) exchange transporter 7 (ClC-7), ClC-5 that transports Cl^{-}, and the K^{+} channel TMEM175 that also acts as a proton leak channel are found in the lysosomal membrane. Arrows indicate the direction of ion fluxes. Further details on their roles in neurodegenerative diseases are provided in the text. Lysosomal pH is also influenced by Donnan particles, which are negatively charged proteins and molecules that affect ion homeostasis through

changes in the lysosomal membrane potential[295]. The buffering capacity of the luminal contents of the lysosome can also substantially affect the rate of pH changes after fusion with substrate-carrying organelles[296]. Various molecules on the lysosome surface that regulate ionic balance fine-tune the extent of v-ATPase-mediated pH changes and these can be primary targets of disease-causing genes (see text). V-ATPase-mediated acidification of lysosomes, which hyperpolarizes the lysosomal membrane, is countered by ClC-7 that exchanges two cytosolic Cl^- for one luminal H^+ [201] and TPCs that transport Na^+ from the lysosomes. Furthermore, when v-ATPase activity results in excessive number of protons, TMEM175 shuttles protons back out to prevent excessive acidification. Once the pH rises to optimal levels, TMEM175 stops releasing protons. The barrier to proton leak by TMEM175 is aided by proper levels of lysosomal-associated membrane proteins, LAMP1 and 2, which interact directly with TMEM175, blocking its proton conduction and facilitate acidification (ref.[96]). Counter-balancing the potentially toxic effects of excessive calcium release are independent possibly adaptive protective actions of calcium release from TRPML1 and TPC2 channels.

Figure 5. Pathological consequences of chronic lysosomal dysfunction

In aging neurons, gradual impairment of lysosomal acidification caused by oxidative damage to v-ATPase initiates a vicious cycle involving suppression of substrate hydrolysis, accelerated aggregation of modified proteins that are increasingly protease-resistant, and accumulation of poorly degraded oxidizable lipids, which are a major source of free radicals[248, 297]. Limited proteolysis required to activate proenzymes (e.g. pro-cathepsin D (Cat D)) and generate new bioactive polypeptides (e.g. conversion of progranulin (PRGN) to granulins (GRN)) begins to diminish. The subsequent superimposition of disease-promoting genetic and environmental factors exacerbates this pathogenic cycle. Persistent residence of these accumulated oxidized substrates inactivates autolysosomes, promoting their accumulation and enlargement and accelerating production of free radicals such as reactive oxygen species (ROS). In turn, ROS cause bidirectional toxicity to mitochondria that then release additional free radicals. Lipofuscin granules, within which undegraded substrates are compacted through a series of cross-linking events, renders this waste less toxic albeit still potentially damaging to membranes[297]. The collective injury to the lysosomal limiting membrane from these sources induces lysosomal membrane permeability (LMP) — a state of injured membranes that allows relatively small proteins like cathepsins to pass through into the cytoplasm. Calcium release via transient receptor potential channel mucolipin 1 (TRPML1) and two-pore channel 2 (TPC2) channels activates calpains and calcium-dependent protein kinases such as cyclin-dependent kinase 5 (CDK5), promoting hyper-phosphorylation of pathogenic proteins like tau and activation of RIPK1, which initiates necrosis-associated neurodegeneration. TRPML1- and TPC2-

mediated calcium release is linked to mitochondrial dysfunction, and mTORC1 activation. Figure adapted with permission from Guerrero-Navarro et al.[297].

Figure 6 Evolution of autophagy–lysosome dysfunction in Alzheimer Disease leading to neurodegeneration.

a) The primary disruptive action of elevated APP-βCTF on lysosomal acidification in Alzheimer Disease (see **Figure 3**), coupled with multiple genetic, environmental, and cell aging factors[121, 132, 133, 135, 136, 139, 280-292], begins to corrupt lysosomal function at the earliest stage of the disease and before signs of neuropathology appears. Increased autophagy induction, a neuroprotective cellular stress response, becomes counter-productive later as poorly-acidified autolysosomes (pAL) and lysosomes progressively fail to clear the growing waste burden. The result is a massive buildup of autophagic vacuoles (AVs), mainly pAL, causing a unique pattern of extreme perikaryal membrane blebbing and trafficking deficits producing AV-filled swellings along axons (dystrophic neurites). **b)** These processes also cause accelerated intra-vesicle Aβ accumulation, and the formation of fibrillar aggregates of β-amyloid within an expanding network of endoplasmic reticulum (ER) tubules (see inset) which reflects an apparent stalling of ER-phagy — a normally highly active constitutive process of ER turnover by autophagy. **c)** This intracellular pathobiology evolves within still intact neurons, preceding the advanced neuronal degeneration associated with lysosomal membrane permeability and lysosomal-associated neuronal cell death, which initiates the transformation of each dying neuron into a senile (“amyloid”) plaque. An ensuing inflammatory response involving the recruitment of reactive astrocytes and phagocytic microglia to the disintegrating neuron induces release of damaging cytokines and hydrolases from surrounding microglia in the effort to clear the extracellular debris. This microglial phagocytic process creates bystander toxicity and accelerates autophagy pathology in the nearby less affected neurons. The recruitment of these neighboring neurons to the lesion expands the senile plaque (not shown in the diagram) and gradually condenses protease-resistant debris, especially ß-amyloid, within the central “core” of the plaque. The neuron diagrams in this figure are reproduced with permission from Lee et al.131. Senile plaque adapted from a drawing by Oskar Fischer299, which highlights the projection of membrane blebs outward from the central region of the degenerating soma, as detailed in panel b.

BOX 1 – Recent insights into autophagosome formation

Autophagosomes evolve from open, finger-shaped, double-membrane structures called phagophores[55]. These originate from recycling endosome membranes where phosphatidylinositol 3-phosphate (PI(3)P)

synthesis by a Beclin 1-dependent kinase complex enables PI(3)P- and RAB11A-dependent recruitment of WD repeat domain phosphoinositide-interacting protein 2 (WIPI2)[299]. WIPI2 recruits the ATG5-12–ATG16L1 complex that conjugates ATG8 or LC3 family members to phagophore membranes — this ubiquitination-like conjugation event represents a defining step in autophagosome formation[45]. The phagophores have finger-like morphologies and do not appear to be single-opening cup-shaped structures, as they are conventionally portrayed. These “fingers” close analogous to how one would make a fist in an ESCRT-dependent manner to enable autophagosome closure, a prerequisite for subsequent release from the recycling endosome[55].

Most, if not all, autophagosomes are derived from Ras-related protein (RAB11)-positive recycling endosome membranes, while receiving inputs from other membrane compartments[299, 300]. For example, the ER is in close proximity to phagophores and regulates early stages of autophagosome biogenesis[301] (e.g. the autophagy protein ATG2 transfers lipids between ER and phagophores[302]), as do secretory pathway-derived machinery and regulators[303, 304]. However, these organelles have distinct roles from the RAB11A membranes that are conjugated by LC3 or ATG8 to evolve into autophagosomes.

Autophagosome release from the recycling endosome compartment is mediated by dynamin 2 (ref. [56]). The autophagosomes are then trafficked on microtubules by the dynein machinery to the part of the cells where the lysosomes are concentrated, close to the cell body[57]. Autophagosome–lysosome fusion appears to be mediated by kiss-and-run repeated interactions[305] although such repeated interactions between these organelles have not been studied extensively in different cell types. The productive fusion leading to autolysosome formation depends on multiple machineries, including certain Rab proteins (e.g. Rab7), phosphoinositides (e.g. PI(3,5)P_2), tethering complex components and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs)[306].

BOX 2. Autophagy and endosomal pathways are tightly integrated and equally vulnerable

Recent studies have amplified the number of known routes capable of sequestering and delivering damaged, toxic, or obsolete cellular constituents to lysosomes for degradation and recycling. This includes the endosomal–lysosomal pathway. Though not formally considered part of the autophagy system, in fact, it tightly integrates with autophagy by contributing membrane components during autophagosome formation and by providing an alternative cytoplasmic substrate sequestration site. Indeed, since autophagosomes are derived from Ras-related protein 11 (RAB11)-positive recycling endosomes, some transmembrane proteins that traffic via endocytosis from the plasma membrane to recycling endosomes, like C-C chemokine receptor type 5 (CCR5) and the transferrin receptor, are

degraded via autophagy as part of the inner autophagosome membrane after endocytosis from the plasma membrane[32, 299]. This contrasts with the canonical model of macroautophagic substrate captured by engulfment within autophagosomes. The interface with autophagy extends the role of endocytosis as a sorting mechanism for delivery of internalized cargoes to varied cellular destinations to include the routing of non-essential endocytosed materials to lysosomes for clearance.

Another interface between endo-lysosomal and autophagy pathways is when endosomes fuse with autophagosomes to form a hybrid organelle, the amphisome, which is especially important in neurons. Indeed, most of the autophagosomes generated in axonal terminal regions quickly acquire late endosomal markers (e.g. Rab7), which engage the dynein complex and accelerate their retrograde transport to the soma[17]. Endosomes also serve as a default waste clearance site by providing exit routes from cells via exocytosis and the release of cargo-containing exosomes from multivesicular bodies (MVBs). This process is stimulated when autophagosome or endosome fusion with lysosomes are compromised[307]. Autophagy also shares machinery with endocytic-like pathways as with LC3-associated endocytosis and LC3-associated phagocytosis, where components of the LC3-conjugation system (but not the entire broader canonical autophagy machinery) are co-opted to these vesicles. This pathway likely has relevance to neurodegeneration, since LC3-associated endocytosis helps clearance of amyloid-beta and protects against neurodegeneration in Alzheimer mouse models[308]. Conjugation of LC3 family membranes to single membranes **[G]** (CASM) also occurs with other scenarios, including during membrane repair processes in damaged lysosomes (CASM), and in LC3-associated micropinocytosis **[G]** [309].

Given the tight integration of the endolysosomal pathway with autophagy, bidirectional interplay between these two pathways in addition to independent pathogenic contributions may substantially impact clearance. A few examples are briefly mentioned here but are reviewed in detail elsewhere[310-313]. One example is exocytic release of cargoes from late endosomes via either exosomes[314, 315], autophagosomes or amphisomes, which is activated as a default mechanism for waste clearance when delivery to lysosomes or subsequent degradation stalls in disease states[316, 317]. Whether neurons can discard waste from lysosome-related compartments, as actively as occurs in non-neuronal cells from individuals with LSDs, is less clear though likely. Another default pathway is inter-neuronal transfer of proteins released by exosomes or other exocytic paths. This occurs physiologically at low levels[318], but is enhanced as another default clearance option that helps clear toxic proteins. However, this process may also propagate disease to other brain regions when the pathogenic protein (e.g. synuclein, tau) is endocytosed and delivered to lysosomes of unaffected neurons[319].

Glossary of Terms

Alpha-synuclein (SCNA): a highly soluble neuronal protein that regulates synaptic vesicle trafficking and subsequent neurotransmitter release and accumulates in Lewy bodies and Lewy neurites in Parkinson disease and other synucleinopathies. Mutations of SCNA are linked to familial Parkinson disease.

Alzheimers Disease (AD): The most common form of dementia involving neurodegeneration of brain regions controlling thought, memory, and language. AD progresses from memory loss to impaired language expression, comprehension and inability to perform activities of daily living. Autosomal dominant gene mutations (APP, PSEN1, PSEN2) induce adult early onset (50's), while most cases are late onset (age >65) involving influences of varied genetic and environmental risk factors.

Amyloid Precursor Protein (APP): A single-pass transmembrane protein highly expressed in the brain but of mainly unknown function. APP undergoes rapid cleavage into multiple bioactive products by sequential proteases, including the intramembranous γ-secretase complex that generates βCTF and Aβ, two polypeptides implicated in AD pathogenesis and over-produced by APP mutations that cause autosomal dominant Alzheimers Disease.

CASM (conjugation of ATG8 (LC3) to single membranes): A noncanonical autophagy pathway that shares the common ATG machinery, but bears key mechanistic and functional distinctions, and is characterized by conjugation of ATG8 (LC3) to single membranes like lysosomes and phagosomes.

***Dementia* with *Lewy bodies* (DLB):** a progressive *dementia* involving a decline in thinking, movement, behavior, and mood and associated with abnormal deposits of alpha-synuclein in brain, called Lewy bodies.

Endo-lysosomal escape: Escape of substrates from vesicles in the endocytic and lysosomal systems

Endoplasmic Reticulum (ER)-associated *degradation (*ERAD*):* The recognition of substrates in the lumen and membrane of the ER, their translocation into the cytosol, ubiquitination, and delivery to the proteasome for degradation

Exosomes: Membrane-bound extracellular vesicles that are produced in the endosomal compartment of eukaryotic cells.

Frontotemporal Lobar Degeneration (FTLD): Also known as Pick's disease, a group of brain disorders caused by degeneration of the frontal and anterior temporal lobes of the brain and characterized by

progressive decline in behavior (e.g. personality changes, apathy) or movement, speaking or language comprehension.

Huntingtin (HTT): a ubiquitously expressed protein most highly expressed in brain and serving varied roles in synaptic transmission, transport, and cell survival. Abnormal expansion of a glutamine stretch (polyQ) in mutant HTT causes Huntington's Disease.

Huntington disease (HD): a monogenic neurodegenerative disorder caused by the huntingtin gene, HTT, characterized by loss of striatal neurons, and resulting in motor, psychiatric and cognitive symptoms.

Induced pluripotent stem cells (iPSC): A type of stem cell derived from adult somatic cells which have been reprogrammed through inducing genes and factors to be pluripotent.

LC3 family members: Members of the ATG8 genes family that are classical markers for autophagosomes

Lysosomal Storage Disorder (LSD): a group of more than 50 mainly childhood disorders that are inborn errors of metabolism characterized by abnormal accumulation of substrates due to defective lysosomes and usually involving deficiency of a single lipid metabolizing enzyme.

Micropinocytosis: A process where macromolecules are engulfed by small vesicles from the plasma membrane.

Microvesicles: Diverse membrane-enclosed vesicles that are released from cells into the extracellular space.

Mitochondrial nucleoid: A structure comprising mitochondrial DNA (mtDNA) and numerous nucleoid-associated proteins that enables submitochondrial organization of mtDNA.

Neuronal Ceroid Lipofuscinosis (NCL): Also called Batten disease, NCL comprises a group of 14 inherited LSDs characterized by intracellular accumulation of autofluorescent lipopigment (ceroid and lipofuscin) and progressive neurodegeneration.

Parkinson Disease (PD): a chronic degenerative disorder targeting dopaminergic neural circuits and initially causing tremors, rigidity, and slowed movement and later additional intellectual functions, including dementia in a minor population of affected individuals.

Presenilin 1 (PSEN1): a multifunctional trans-membrane protein that, in loss-of-function mutant form, is one of three autosomal dominant causes of Alzheimer's Disease. Presenilin1 is one of four core proteins in the gamma secretase endoprotease complex that performs intramembrane cleavage of dozens of

integral membrane proteins, including the APP that sequentially generates βCTF and Aβ, two polypeptides implicated in AD pathogenesis.

Ryanodine Receptors (RyRs): Ion channels residing in the sarcoplasmic/endoplasmic reticulum membrane and responsible for Ca^{2+} release from intracellular stores in excitable tissues, like muscles and neurons.

Tau (MAPT): a group of six highly soluble protein isoforms produced by alternative splicing from MAPT that help stabilize the microtubule cytoskeleton of neurons *and compose* neurofibrillary tangles, a hallmark of Alzheimer disease.

TFEB, TFE3: both molecules are members of the MiT-TFE family of helix–loop–helix leucine zipper transcription factors that regulate expression of genes involved in the biogenesis and function of lysosomes and autophagosomes.

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TOC

The autophagy–lysosome pathway eliminates damaged organelles and aggregation-prone proteins, which is particularly important in neurons, where clearance of such substrates is restricted. Autophagy or lysosome deficiencies, often exacerbated by ageing, impact neuronal function and cause neurodegenerative diseases such as Alzheimer disease or Parkinson disease.