

Lysosomal Impairment in Parkinson's Disease

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ABSTRACT: Impairment of autophagy-lysosomal pathways (ALPs) is increasingly regarded as a major pathogenic event in neurodegenerative diseases, including Parkinson's disease (PD). ALP alterations are observed in sporadic PD brains and in toxic and genetic rodent models of PD-related neurodegeneration. In addition, PD-linked mutations and post-translational modifications of α -synuclein impair its own lysosomal-mediated degradation, thereby contributing to its accumulation and aggregation. Furthermore, other PD-related genes, such as leucine-rich repeat kinase-2 (*LRRK2*), *parkin*, and phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (*PINK1*), have been mechanistically linked to alterations in ALPs. Conversely, mutations in lysosomal-related genes, such as glucocerebrosidase (*GBA*) and lysosomal type 5 P-type ATPase (*ATP13A2*), have

been linked to PD. New data offer mechanistic molecular evidence for such a connection, unraveling a causal link between lysosomal impairment, α -synuclein accumulation, and neurotoxicity. First, PD-related *GBA* deficiency/mutations initiate a positive feedback loop in which reduced lysosomal function leads to α -synuclein accumulation, which, in turn, further decreases lysosomal *GBA* activity by impairing the trafficking of *GBA* from the endoplasmic reticulum-Golgi to lysosomes, leading to neurodegeneration. Second, PD-related mutations/deficiency in the *ATP13A2* gene lead to a general lysosomal impairment characterized by lysosomal membrane instability, impaired lysosomal acidification, decreased processing of lysosomal enzymes, reduced degradation of lysosomal substrates, and diminished clearance of autophagosomes, collectively contributing to α -synuclein accumulation

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and cell death. According to these new findings, primary lysosomal defects could potentially account for Lewy body formation and neurodegeneration in PD, laying the groundwork for the prospective development of new neuroprotective/disease-modifying therapeutic strategies aimed at restoring lysosomal levels and function. © 2013 *Movement Disorder Society*

Key Words: Parkinson's disease; ATP13A2; glucocerebrosidase; lysosome; neurodegeneration; Lewy body

Lysosomes are dynamic acidic organelles that contain hydrolytic enzymes capable of degrading intracellular components through several degradation pathways, including endocytosis, phagocytosis, and autophagy^{1,2} (Fig. 1). Lysosomes are responsible for the clearance of long-lived proteins, such as aggregate-prone α -synuclein among others, and for the removal of old or damaged organelles, such as mitochondria. Both α -synuclein aggregation and mitochondrial dysfunction are considered major pathogenic events in Parkinson's disease (PD).³⁻⁵ Increasing evidence indicates that impairment of lysosomal function may contribute to the pathogenesis of several neurodegenerative diseases, including PD.⁶ Here, we review recent data, mostly derived from genetic alterations in lysosomal-related genes, supporting a potential pathogenic role for lysosomal dysfunction in PD.

Dysregulation of the Autophagy-Lysosome System in PD

Neurons are particularly sensitive to alterations in protein degradation pathways. Constitutive autophagy is essential for neuronal survival, because its genetic inactivation selectively in neurons leads to the formation of ubiquitinated intracellular inclusions and cell loss in mutant mice.⁷⁻⁹ Implicating an impairment of lysosomal activity in PD, a reduced number of intraneuronal lysosomes, decreased levels of lysosomal-associated proteins (cathepsin D, lysosomal-associated membrane protein 1 [LAMP-1], LAMP-2a, and heat shock cognate 71 kDa protein [Hsc70]) and accumulation of undegraded autophagosomes (APs) have been observed in postmortem brain samples from patients with idiopathic PD and toxin and genetic rodent models of PD.¹⁰⁻¹⁴

In addition, impaired lysosomal-mediated clearance of APs has been reported in cultured dopaminergic neurons generated from reprogrammed induced pluripotent stem cells (iPSCs) derived from skin fibroblasts of sporadic and genetic PD patients.¹⁵ Mechanistic studies in 1-methyl-4-phenyl-11.2.3.6-tetrahydropyridine (MPTP)-treated mice revealed that PD-linked lysosomal deficiency preceded cell death and was instrumental in the impairment of autophagy and in overall dopaminergic neurodegeneration.¹⁶ In these animals, pharmacologic reactivation of autophagy-lysosomal pathways (ALPs) with rapamycin resulted

in an increased number of functional lysosomes, reversed AP accumulation, and attenuated dopaminergic cell death.^{13,17} Further demonstrating a deleterious role of impaired lysosomal/autophagic degradation in relation to PD, directed genetic deletion of an essential autophagy gene, autophagy related 7 (*Atg7*), within catecholaminergic neurons in mice resulted in decreased striatal dopamine; abnormal presynaptic neurotransmission; and age-dependent axonal morphologic alterations, motor deficits, and neurodegeneration.¹⁸⁻²¹ Remarkably, these animals also developed presynaptic α -synuclein accumulations, suggesting that macroautophagy may play a critical role in axons, whereas other degradative pathways (such as chaperone-mediated autophagy [CMA] or the ubiquitin-proteasome system [UPS]) may have a more prominent role in cell bodies. α -Synuclein is a major constituent of Lewy bodies (LBs) and Lewy neurites and is believed to play a significant pathogenic role in both familial and idiopathic forms of PD. Although it was originally thought that α -synuclein was exclusively degraded by the UPS, we now know that this protein can also be degraded inside lysosomes, through CMA, or through endocytosis²²⁻²⁷ (Fig. 2). The signals responsible for sending α -synuclein to either 1 or another degradation pathway are not yet fully understood, particularly in neurons, but depend on several factors intrinsic to the status of the protein, such as: (1) its folding state (unfolded, properly folded, or misfolded), (2) its localization (cytosolic, associated to membranes, or even extracellular), (3) the presence of post-translational modifications (unmodified, ubiquitinated, phosphorylated, nitrated, oxidized, or dopamine-modified), and (4) its oligomeric state (monomeric, oligomeric, protofibrillar, fibrillar, or aggregated).^{25,28} All these factors, together with possible interactions with different chaperones and co-chaperones, determine the degradation destiny of α -synuclein. Although macroautophagy is able to degrade different forms of α -synuclein, it has been recently reported that α -synuclein, in turn, can directly impair macroautophagy both in vitro and in vivo.²⁹⁻³¹ Furthermore, PD-linked pathologic α -synuclein (ie, mutated, post-translationally modified, or oligomeric/aggregated) can directly impair UPS and lysosomal functions, resulting in defective clearance and subsequent accumulation of abnormal α -synuclein species

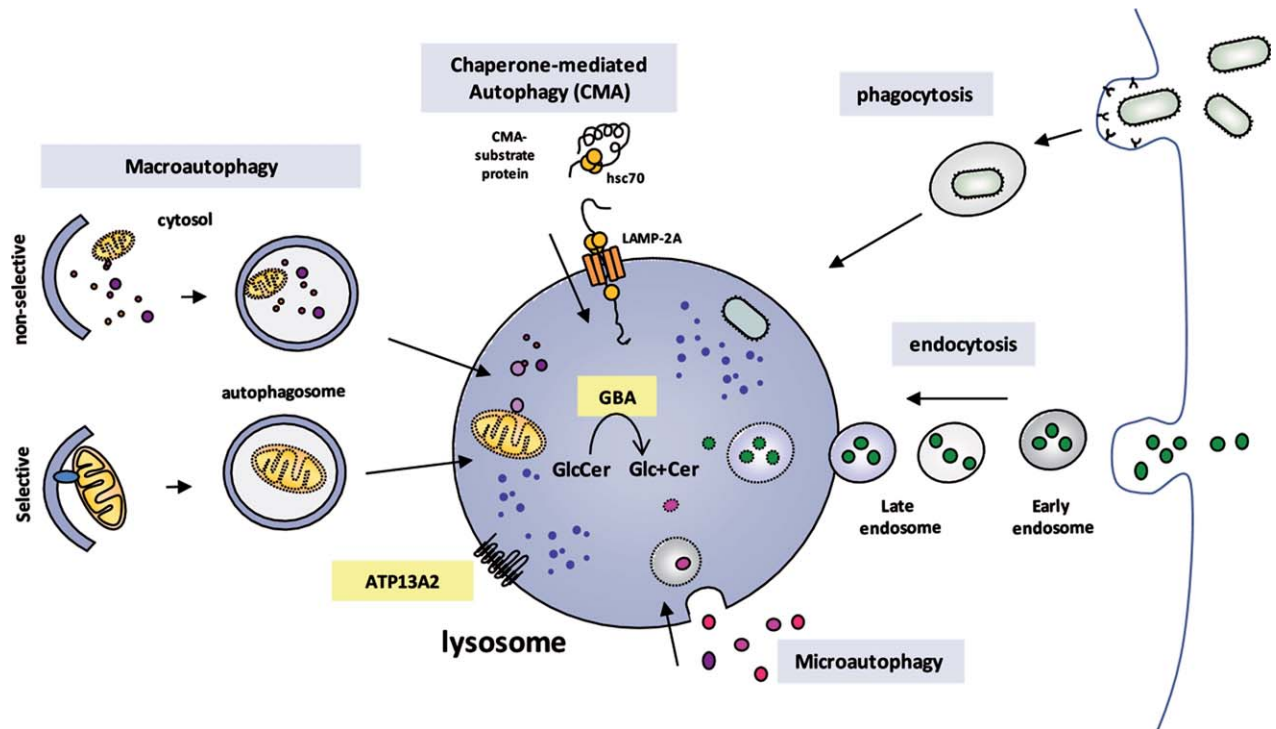


FIG. 1. Lysosomal degradation pathways are illustrated. Several degradation pathways, including endocytosis, phagocytosis, and autophagy, converge at the lysosome as a final destination. Autophagy is a tightly regulated process by which certain intracellular components are recycled inside lysosomes. Various types of autophagy, including microautophagy and macroautophagy and chaperone-mediated autophagy (CMA), differ in their mechanisms and functions. Microautophagy involves the sequestration and degradation of complete regions of the cytosol (including proteins and organelles) through invaginations and tubulations of the lysosomal membrane. In macroautophagy, intracellular components are enclosed in a double membrane vesicle called the autophagosome. This vesicle is then fused with lysosomes, wherein hydrolytic enzymes complete the degradation of the sequestered material. Macroautophagy can exert a nonselective degradative effect by engulfing in-bulk portions of the cytosol and can constitute a more selective mechanism whereby specific substrates are recognized by distinct autophagic adaptors and selectively degraded. In CMA, specific cytosolic proteins that contain a KFERQ-like consensus sequence directly cross the lysosomal membrane via a specific membrane receptor, LAMP-2A, assisted by chaperones, including Hsc70. Glucocerebrosidase (GBA) is an intralysosomal enzyme that catalyzes the conversion of the glycolipid glucosylceramide into glucose and ceramide inside lysosomes. ATP13A2 is a transmembrane type 5 P-type adenosine triphosphatase (ATPase) protein present in lysosomal membrane.

and other UPS/lysosomal substrates.^{23,32,33} Hence, α -synuclein accumulation in PD may represent both a cause and a consequence of impaired proteolytic activity in this disease. It is noteworthy that the lysosomal enzyme cathepsin-D, the most active protease in degradation of α -synuclein, is neuroprotective against α -synuclein-induced dopaminergic neurodegeneration in a *Caenorhabditis elegans* model, and genetic ablation of this enzyme in mutant mice leads to α -synuclein accumulation.^{34,35} In addition to α -synuclein, other PD-related genes recently have been linked to ALP alterations (Fig. 2). For instance, PD-linked mutations in leucine-rich repeat kinase-2 (*LRRK2*) have been associated with impaired autophagy by an as yet unknown mechanism. In addition, PD-linked mutations in the phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (*PINK1*) and *parkin* genes have been shown to disrupt the coordinated normal regulatory role of these molecules at promoting autophagic degradation of dysfunctional mitochondria, thereby leading to the deleterious consequences of defective mitophagy. Taken together, these observations

strongly support the concept that the ALP may be impaired in PD.

Lysosomal-Related Genetic Alterations and PD

Although the above-reported data associate lysosomal insufficiency with PD, genetic analyses also indicate that lysosomal impairment may play a primary pathogenic role in this disease. In particular, mutations in 2 genes that encode lysosomal proteins, including the enzyme glucocerebrosidase (*GBA*) and lysosomal type 5 P-type ATPase (*ATP13A2*), have been linked to PD—the former as an important risk factor for PD through a multicenter genetic analysis,³⁶ and the latter through linkage in rare families with prominent parkinsonism.^{37–39} Recent data offer mechanistic molecular evidence for such a connection (Fig. 2).

1. *GBA*

Loss-of-function mutations in the gene encoding *GBA* cause Gaucher disease (GD), the most common

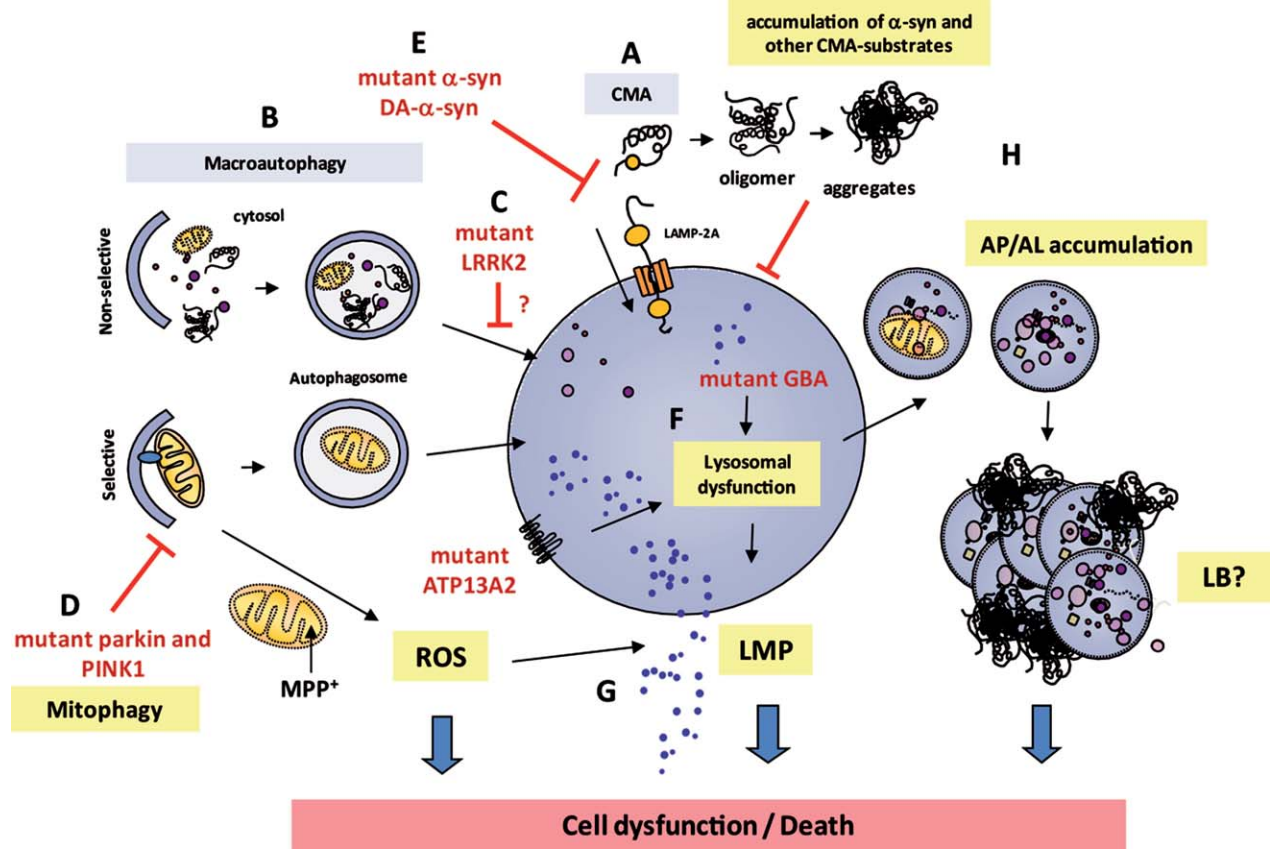


FIG. 2. Lysosomal deficiency is illustrated in Parkinson’s disease (PD). α -Synuclein can be degraded by various proteolytic pathways within the cell, including autophagy and the ubiquitin proteasome system. Lysosomes can degrade different types of α -synuclein species by means of different pathways, including macroautophagy, chaperone-mediated autophagy (CMA), and endocytosis. (A) Soluble or wild-type α -synuclein are preferentially degraded in the lysosome by CMA, whereas (B) macroautophagy can degrade both soluble and large protein complexes that contain modified or oligomeric forms of α -synuclein. (C) PD-linked mutations in leucine-rich repeat kinase-2 (*LRRK2*) have been associated with impaired autophagy by an as yet unknown mechanism. (D) In addition, PD-linked mutations in phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (*PINK1*) and *Parkin* have been shown to disrupt the coordinated normal regulatory role of these molecules at promoting autophagic degradation of dysfunctional mitochondria, thereby leading to defective mitophagy. (E) PD-linked A30P or A53T α -synuclein mutants and dopamine-modified wild-type (WT) α -synuclein (DA- α -syn) block CMA activity, resulting in insufficient lysosomal clearance of α -synuclein and other CMA-substrates. (F) Mutations in lysosomal-associated genes (glucocerebrosidase [*GBA*], *ATP13A2*) directly cause lysosomal impairment and can be associated with PD-like neurodegeneration. (G) Enhanced reactive-oxygen species (ROS) production caused by mitochondrial neurotoxin 1-methyl-4-phenylpyridine (MPP)-positive or α -synuclein oligomers induces abnormal lysosomal membrane permeabilization (LMP) and disruption of lysosomal membrane integrity. (H) Overall, lysosomal dysfunction leads to the accumulation of toxic/aggregated α -synuclein, dysfunctional organelles, and undegraded/partially degraded autophagosomes (AP) and autophagolysosomes (AL), all of which may result in Lewy body (LB) formation and/or neurodegeneration.

lysosomal storage disorder. GBA catalyzes the conversion of the glycolipid glucosylceramide into glucose and ceramide inside lysosomes. Conditional knock-out mice of GBA in the central nervous system develop neuronal loss associated with microgliosis, indicating a critical role of GBA in neuronal survival.⁴⁰ Glucosylceramide levels are increased in the brains of these animals. Carrier status of a single mutant GBA allele is a significant risk factor for PD^{36,41} and for dementia with LBs.⁴² Conversely, patients with GD, although clinically different from PD, not infrequently exhibit parkinsonism, α -synuclein-immunoreactive LBs, and loss of melanized dopaminergic neurons.^{41,43} Intralysosomal accumulation of glucosylceramide has been proposed as the most likely pathogenic mechanism linked to GBA loss-of-function homozygous

mutations.⁴⁴ However, GBA mutations linked to an increased risk of PD are usually present only in the heterozygous state (ie, patients who carry 1 wild-type GBA allele and, thus, have at least 50% of normal enzyme function).⁴⁵ In addition, it has been reported that GBA is a component of LBs.⁴⁶

Recent mechanistic studies indicate that GBA can influence α -synuclein processing through both gain-of-function and loss-of-function mechanisms. Loss of GBA activity in mouse primary cortical neurons and in human neurons derived from iPSCs from a patient with GD resulted in glucosylceramide accumulation, decreased lysosomal degradation, and subsequent accumulation of α -synuclein, promoting α -synuclein oligomer formation and neurotoxicity.⁴⁷ α -Synuclein accumulations, in turn, impair the trafficking of GBA

from the endoplasmic reticulum-Golgi to lysosomes, thereby resulting in further decreased lysosomal GBA activity.⁴⁷ Thus, loss of GBA creates a positive feedback loop of reduced lysosomal function and α -synuclein accumulation that ultimately leads to neurodegeneration.⁴⁸ In another study, overexpression of several GBA mutants in cultured cell lines did not alter GBA activity but also resulted in α -synuclein accumulations, which were reversed by inducing autophagy with rapamycin or by promoting GBA translocation into lysosomes with the GBA chaperone isofagomine.⁴⁹ Taken together, these results indicate that both GBA gain of function and loss of function can promote pathologic α -synuclein accumulations and that restoring/enhancing normal GBA activity may hold promise as a potential therapeutic strategy for PD and other synucleinopathies. In this regard, it has been demonstrated that adeno-associated, virus-mediated expression of exogenous GBA attenuates α -synuclein pathology and cognitive deficits in a genetic mouse model of GD.⁵⁰ However, it is worth noting that, although individuals with GD and parkinsonism exhibit synucleinopathy, the majority of patients with GD (either homozygous or heterozygous) do not develop either synucleinopathies or parkinsonism.^{43,46}

2. ATP13A2

Mutations in the *ATP13A2/PARK9* gene have been linked to autosomal recessive, levodopa-responsive parkinsonism with nigrostriatal-pallidal pyramidal neurodegeneration (Kufor-Rakeb syndrome [KRS]).^{37,51} However, there is wide phenotypic heterogeneity in patients with KRS, depending on the type of *ATP13A2* mutation, thus indicating a high level of complexity of this disorder. To date, no brain histopathology data from *ATP13A2*-mutant patients have been reported, thereby precluding the assessment of α -synuclein pathology in these patients.

The *ATP13A2* gene encodes a lysosomal ATPase involved in selective active transport of cations across diverse biologic membranes.^{52,53} Genetic studies in yeast suggest that *ATP13A2* yeast ortholog is involved in protecting cells against manganese toxicity and, more broadly, heavy metals.⁵⁴ Conversely, *ATP13A2* also confers protection against α -synuclein misfolding in mammalian cells and attenuates α -synuclein toxicity in *Caenorhabditis elegans* and in primary dopaminergic cell cultures.⁵⁵ Thus, these results suggest a potential link between these 2 PD-associated pathogenic pathways.

Likewise, a general protective role for *ATP13A2* against a wide variety of cellular stresses, such as mitochondrial complex I impairment, oxidative stress, and proteasomal stress, has been demonstrated.⁵⁶ It is hypothesized that missense or truncation mutations in the *ATP13A2* gene exert their pathogenic effect by

causing loss of *ATP13A2* function due to impaired targeting of *ATP13A2* to lysosomes.^{39,57,58} Studies in KRS patient-derived fibroblasts and *ATP13A2*-deficient cell lines revealed a general lysosomal impairment characterized by instability of the lysosomal membrane, impaired lysosomal acidification, decreased proteolytic processing of lysosomal enzymes, reduced degradation of lysosomal substrates, and diminished lysosomal-mediated clearance of AP, all of which were associated with cell death. All these effects were rescued by restoring the expression of wild-type *ATP13A2* in *ATP13A2*-depleted cells.^{59–61} In both *ATP13A2*-mutant or *ATP13A2*-defective cells, impaired lysosomal proteolysis resulted in a marked accumulation of α -synuclein.^{59,60} Silencing of endogenous α -synuclein attenuated toxicity in *ATP13A2*-depleted neurons.⁶⁰ Conversely, cell death induced by *ATP13A2* knockdown was greatly enhanced by α -synuclein overexpression.⁵⁹ Relevant to PD, lentiviral vector-mediated *ATP13A2* knockdown in primary mesencephalic dopaminergic neurons resulted in selective dopaminergic, but not GABAergic, neurodegeneration.⁵⁹ In addition, *ATP13A2* levels were decreased in postmortem PD nigral samples in which 90% of LBs exhibited a positive signal for *ATP13A2* in their core and were surrounded by more peripherally located α -synuclein.⁵⁹

Overall, these results indicate a pathogenic role of *ATP13A2* deficiency in lysosomal function and cell viability. In addition, other studies have indicated that loss of *ATP13A2* function may also induce mitochondrial defects, likely because of decreased mitochondrial turnover secondary to impaired mitophagy.^{62,63} *ATP13A2* and some of its interacting partners have been identified as modifiers of α -synuclein toxicity in yeast 2-hybrid systems and RNA interference screens in worms^{64,65} and, thus, may represent potential therapeutic targets for the development of new strategies aimed at modulating *ATP13A2*-related pathways in PD.

Concluding Remarks and Future Directions

Increasing evidence indicates that impaired lysosomal function, which is essential to maintain proper protein and organelle quantity and quality within cells, may play an important role in the pathogenesis of PD. Lysosomal defects could potentially account not only for dopaminergic cell dysfunction/death but also for the presence of α -synuclein-containing LBs. The identification of AP/lysosomal markers as components of LBs in patients with sporadic PD, including LC3,^{13,14} LAMP-1,¹² LAMP-2a,¹⁴ cathepsin-D,¹² VPS35,⁶⁶ GBA,⁴⁶ and *ATP13A2*,⁵⁹ raises the possibility that LBs, the origin and significance of which remain unknown, may seed around impaired lysosomes and/or undegraded APs and grow in size by the

continuous deposition of lysosomal/AP-derived, undegraded material as the disease progresses. Consistent with this, (1) LBs contain abnormal mitochondria, autophagy-related molecules, lysosomes, and vacuolar structures^{12,13,67}; (2) patients with GD can exhibit α -synuclein-immunoreactive LBs similar to those found in PD^{46,68}; (3) specific environments inside membranous and vesicular structures, such as a molecularly crowded milieu, are more prone to α -synuclein aggregation^{67,69,70}; and (4) ubiquitin, which has been identified as 1 of the main components of LBs, was originally associated with the proteasome degradation pathway, but we now know that ubiquitin is a tag that can also target intracellular components for its degradation by some forms of selective autophagy.⁷¹

Although lysosomal impairment represents only 1 aspect of the many potential facets of PD pathogenesis, the results reviewed here raise the possibility that enhancement/restoration of lysosomal-mediated degradation may prove beneficial for PD. It is important to note, however, that, based on the current results, strategies/drugs aimed at activating autophagy solely by increasing AP formation without concomitant increases in lysosomal function could result in further cellular damage, rather than benefit, in the context of PD. Instead, therapeutic modulation of autophagy in PD should be aimed at the late steps of the ALP (ie, improving the efficiency of AP maturation and substrate digestion) by boosting AP maturation, fusion with lysosome, and lysosomal biogenesis, trafficking, and function.^{72,73} In this regard, autophagy induction with the mammalian target of rapamycin (mTOR)-inhibiting drug rapamycin or with mTOR-independent autophagy enhancers, such as lithium and trehalose, provide neuroprotection in several *in vitro* and *in vivo* genetic and toxic models of PD^{17,74–77} and have been shown to exert part of their proautophagy actions by enhancing lysosomal activation and AP clearance, and not solely by increasing new AP formation.^{13,17,78} Similarly, viral-vector-mediated expression of autophagy regulators, such as beclin-1, has been shown to reduce α -synuclein accumulations and synaptic pathology in α -synuclein transgenic mice by enhancing autophagic activity.⁷⁹ Overexpression of transcription factor EB (TFEB), a master activator of the ALP,^{80,81} also reportedly protects cultured cells against parkinsonian neurotoxins.¹³ Overall, those studies lay the groundwork for the potential development of novel therapeutic strategies aimed at restoring lysosomal-mediated degradation in PD.

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