Endolysosomal Dysfunction in Parkinson's Disease: Recent Developments and Future Challenges

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ABSTRACT: Increasingly, genetic, cell biological, and in vivo work emphasizes the role of the endolysosomal system dysfunction in Parkinson's disease pathogenesis. Yet many questions remain about the mechanisms by which primary endolysosomal dysfunction causes PD as well as how the endolysosomal system interacts with α-synuclein-mediated neurotoxicity. We recently described a new mouse model of parkinsonism in which loss of the endolysosomal protein Atp13a2 causes behavioral, neuropathological, and biochemical changes similar to those present in human subjects with *ATP13A2* mutations. In this Scientific Perspectives, we revisit the evidence implicating the endolysosomal system in PD, current hypotheses of disease pathogenesis, and how recent studies refine these hypotheses and raise new questions for future research. © 2016 International Parkinson and Movement Disorder Society.

Key Words: Parkinson's disease; Atp13a2; Kufor-Rakeb syndrome; endolysosomal system

The cause of Parkinson's disease (PD) is multifactorial. An increasing number of genes have been implicated in both familial and sporadic forms of disease and may contribute up to 50% of the PD risk.[1] How these genes relate to one another and to what extent they participate in the pathogenesis of sporadic disease are among the central questions being explored by scientists engaged in PD research. This work has established 2 prevailing (yet not mutually exclusive) hypotheses regarding PD pathogenesis: (1) altered homeostasis, aggregation, and transmission of α-synuclein causes neurodegeneration; (2) a primary defect in mitochondrial function causes neurodegeneration secondary to bioenergetic failure and increased oxidative stress.[2-4] More recent genetic studies have suggested that endolysosomal dysfunction may be a primary defect, subsequently leading to abnormalities of α-synuclein homeostasis and mitochondrial function, but very likely also causing toxicity through less well-defined mechanisms. Indeed, mutations in several genes encoding endolysosomal proteins cause familial forms of PD, highlighting the importance of this pathway in disease pathogenesis (see Table 1 and Fig. 1).

Mutations in the same pathway and even in some of the same genes have also been implicated in other neurodegenerative diseases, including Alzheimer's disease (AD). In AD, endolysosomal dysfunction appears to increase disease risk through increases in amyloid-β levels. In early endosomes, γ-secretase cleaves amyloid precursor protein (APP) to initiate the amyloidogenic cascade.[5] Mutations that increase the early endosomal pool or enhance substrate time within early endosomes promote this cleavage and amyloidogenesis. For example, nearly all subjects with trisomy 21 eventually develop AD, and these subjects exhibit increases in synaptojanin 1 (encoded by *SYNJ1*), which increases the uptake of cell-surface proteins like APP and γ-secretase into the endosome.[6] Deletion of 1 Synj1 allele in mice rescues AD behavioral and synaptic phenotypes,[7] likely by altering the flux of APP away from the endosome and toward lysosomal degradation.[8] Conversely, retromer proteins are decreased in postmortem tissue from

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Relevant conflicts of interest/financial disclosures: The authors declare no competing financial interests.

Funding agencies: This work was supported by National Institute of Neurological Diseases and Stroke grants R01 NS061098 (to W.T.D.), F31 NS078817 (to L.R.K.), and T32 GM007863 (to L.R.K.).

Received: 15 March 2016; **Revised:** 8 August 2016; **Accepted:** 9 August 2016

Published online 13 September 2016 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.26797

TABLE 1. Genes linked to familial Parkinson disease (PD) and syndromes with prominent parkinsonism (PD plus)

Locus	Mode of inheritance	Gene	Protein product	Protein function	Clinical phenotype (age of onset)	Pathology	Citations (first reported)
PARK1/4	AD	*SNCA*	α-Synuclein	Synaptic protein	Early-/late-onset PD (30s triplication, 40s/50s duplication, 30s-60s missense)	LB+	Polymeropoulos (1997) Singleton (2003)
PARK8	AD	*LRRK2*	Leucine-rich repeat kinase 2	GTPase/kinase	Late-onset PD (50s)	Pleomorphic, most LB+	Paisan-Ruiz (2004) Zimprich (2004)
PARK17	AD	*VPS35*	Vacuolar protein sorting 35 homologue	Subunit of retromer complex	Late onset (40s-50s)	Unknown	Vilarino-Guell (2011) Zimprich (2011)
	AD	*DNAJC13*	Receptor-mediated endocytosis 8	Regulates clathrin coats on endosomes	Late onset (60s)	Unknown	Vilarino-Guell (2013)
	AD	*GBA*	Glucocerebrosidase	Lysosomal enzyme	Late-onset PD (late 50s), homozygosity associated with Gaucher's	LB+	Goker-Alpan (2004)
PARK2	AR	*PARKIN*	Parkin	E3 ligase	Early-onset PD (20s-30s)	Pleomorphic, most LB-	Kitada (1998)
PARK6	AR	*PINK-1*	Pten-induced kinase 1	Mitochondrial kinase	Early-onset PD	1 case — LB+	Valente (2004)
PARK7	AR	*DJ-1*	DJ-1	Peptidase, oxidative stress sensor	Early-onset PD	Unknown	Bonifati (2003)
PARK9	AR	*ATP13A2*	ATP13A2	Endolysosomal ATPase	Juvenile-onset PD plus — myoclonus, dementia, psychosis, gaze palsy (teens)	Unknown	Ramirez (2006)
PARK14	AR	*PLA2G6*	A2 phospholipase	Membrane remodeling	Early-onset dystonia-parkinsonism (20s). Commonly associated with NBIA	LB+, tau+, iron deposits	Paisan-Ruiz (2009)
PARK15	AR	*FBXO7*	F-box protein 7	Ubiquitin protein ligase complex — involved in mitophagy	Juvenile-onset PD with pallidopyramidal symptoms (late teens)	Unknown	Shojaee (2008)
	AR	*DNAJC6*	Auxilin	Endocytosis	Juvenile-onset PD (7-11 years)	Unknown	Edvardson (2013) Koroglu (2013)
	AR	*PANK2*	Pantothenate kinase 2	Coenzyme A synthesis	Early-onset PD (20s), commonly associated with NBIA	LB-, Ub+, iron deposits	Hayflick (2003)
	AR	*SYNJ1*	Synaptojanin1	Endocytosis	Early onset PD plus — seizures, dystonia, cognitive changes (20s)	Unknown	Krebs (2013) Quadri (2013)

AD autosomal dominant, *AR* autosomal recessive. *LB* Lewy Body, *NBIA* neurodegeneration with brain iron accumulation.

AD patients[9] and in experimental models cause increased retention of APP within the endosome and its subsequent cleavage into amyloid-β. It is appealing to think that a similar α-synuclein-centered model underlies PD pathogenesis, but several pieces of available evidence do not appear consistent with this view. How primary endolysosomal dysfunction drives PD pathogenesis remains a major, unanswered question. Specifically, it is unknown (1) how endolysosomal dysfunction interacts with α-synuclein accumulation and toxicity, (2) the extent of α-synuclein-independent contributions of endolysosomal impairment to neurodegenerative processes, and (3) how endolysosomal dysfunction leads to selective neurodegeneration in different patients, causing some to develop PD and others to develop AD.

We recently reported a new mouse model with deletion of the parkinsonism gene *ATP13A2*, which encodes an endolysosomal protein.[10] This model is one of the first to show behavioral, neuropathological, and biochemical phenotypes consistent with the human disease (Kufor-Rakeb syndrome [KRS]) and raises new questions about the consequences of endolysosomal dysfunction in PD. In this Scientific Perspective, we revisit the genetics implicating the endolysosomal pathway, review several proposed pathogenic mechanisms and explain how new evidence from cellular and animal models refines our understanding of the disease.

Background

Genetics

In 1997 mutations in the gene encoding α-synuclein (*SNCA*) were linked to a familial form of early-onset PD.[11] Subsequently, familial early-onset PD has been

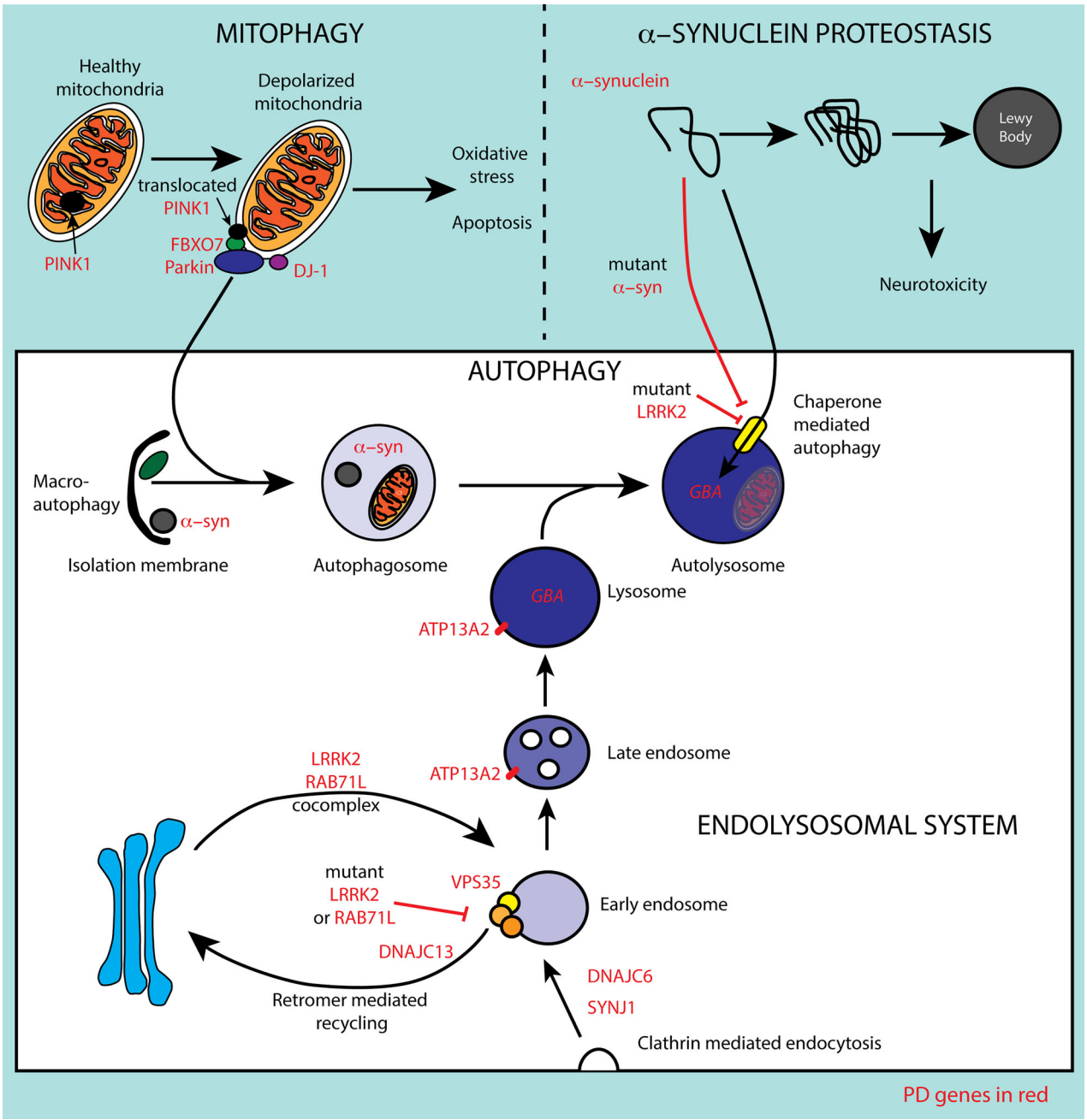

FIG. 1. PD-related genes converge on the endolysosomal and autophagic pathways. Genes important in familial and sporadic PD (shown in red) encode many proteins involved in the degradation of damaged mitochondria (or mitophagy), α-synuclein proteostasis, or the endolysosomal system.

linked to duplication and triplication events involving the *SNCA* gene,[12-19] with comparable increases in mRNA and insoluble protein levels.[20] α-Synuclein is a synaptic protein that is highly abundant in Lewy bodies, the cytoplasmic, proteinaceous inclusions that are the neuropathological hallmark of idiopathic PD.[21,22] These genetic and postmortem studies identify elevated levels of α-synuclein as a direct cause of PD. Consequently, considerable work on PD pathogenesis has focused on pathways that both promote α-synuclein aggregation and disrupt its clearance from the cell.

In the last 15 years, mutations in 13 additional genes have been linked to familial forms of parkinsonism (Table 1), identifying new pathogenic cascades. Genome-wide association studies have identified additional low-risk genetic variants associated with sporadic disease. We direct the readers to many excellent reviews on PD genetics,[1,23] including a recent one focused on these genes in autophagy and protein trafficking,[24] and will briefly summarize some of the more recently discovered genes within the endolysosomal system.

A great deal of attention has focused recently on mutations in the glucocerebrosidase gene (*GBA*). In 2004, *GBA* mutations were recognized as the most common genetic risk factor for PD (imparting up to a 6-fold risk) and the related dementia with Lewy bodies.[25-28] Loss of glucocerebrosidase, a lysosomal enzyme, has been implicated in a positive-feedback loop with α-synuclein.[29] Mutations in another late endosomal/lysosomal protein, ATP13A2, were linked to KRS, an early-onset autosomal-recessive disorder characterized by parkinsonism with additional neurological features.[30] Additional *ATP13A2* mutations have been identified in patients with early-onset PD,[31] parkinsonism plus dystonia,[32] and neuronal ceroid lipofuscinosis.[33] Whole-exome sequencing of families with PD have identified mutations in additional endolysosomal proteins (encoded by *DNAJC13, DNAJC6*, and *SYNJ1*).[34-38] Genome-wide association studies identified a novel locus, PARK16, as a modifier of disease risk,[39] which further study refined to the gene encoding Rab7L1, a protein involved in the trafficking of proteins between the trans-Golgi network and endosomes.[40] Taken together, these genetic studies suggest that mutations in multiple proteins throughout the endolysosomal system participate in PD pathogenesis (Fig. 1). However, despite increased understanding of the genetic mutations underlying PD risk, PD-related research has suffered from the lack of genetic mouse models that display the hallmarks of disease: age-related motor abnormalities, progressive neurodegeneration, SNpC cell loss, and α-synuclein aggregation.[64] Without reliable animal models, it is difficult to reconstruct the precise sequence of events leading to neurodegeneration or identify novel candidate approaches to halt early events in pathogenesis. Most research has focused on the role of these proteins in autophagy, with more recent studies highlighting the roles of these proteins in related pathways.

Autophagic-Lysosomal Pathway

Autophagy is 1 of 2 major degradation pathways in the cell and is reviewed extensively elsewhere.[41] Autophagic pathways encompass a set of processes that identify and deliver cytosolic components to the lysosome for degradation and recycling. Macroautophagy (Fig. 1) occurs when a double-membrane vesicle sequesters a large portion of the cytosol and then fuses with a lysosome or late endosome to mediate the degradation of its contents.[42] Microautophagy involves a small invagination of the cytosol directly into the lysosome.[43] Chaperone-mediated autophagy (CMA) is a process whereby proteins with the amino acid sequence KFERQ are bound to Hsc70 and translocated across the lysosomal membrane by the lysosomal membrane protein LAMP2A.[44]

Autophagy was first implicated in PD pathogenesis in studies of α-synuclein proteostasis. Duplication and triplication in the α-synuclein gene increase the risk of early-onset PD[19] identifying increased levels of α-synuclein as neurotoxic. As a result, research has focused on processes that degrade α-synuclein, including autophagy. The ubiquitin-proteasome system, macroautophagy, and CMA all can degrade α-synuclein, but the relative proportion of α-synuclein degraded by these pathways changes depending on the folding state, cellular localization, or oligomeric state of α-synuclein and the presence of cellular stressors.[45-47] Mutant forms of α-synuclein can inhibit both macroautophagy[48] and CMA,[49] blocking the degradation of itself and other proteins. Inhibition of autophagy upregulates the ubiquitin-proteasome and vice versa, providing cross talk between the various intracellular pathways.[46] One unifying theory, widely embraced, is that upstream events either promote α-synuclein aggregation or inhibit autophagy, creating a positive feedback loop between autophagic deficits and α-synuclein, aggregation ultimately leading to cell death.

One prediction from this theory is that other PD-related proteins could initiate this feedback loop by disrupting autophagic function. In vitro, LRRK2 has been found on the membranes of multivesicular bodies and autophagic vacuoles, with a PD-mutant LRRK2 causing incomplete autophagic degradation.[50] The *Drosophila* LRRK2 homologue physically interacts with Rab7 to position lysosomes,[51] and several Rab proteins have been identified as LRRK2 substrates.[52] LRRK2-null mice develop age-related impairment of autophagy in the kidney, resulting in α-synuclein aggregation.[53,54] It remains unclear from this work, however, whether LRRK2 serves a primary function in autophagy or if the autophagic changes are secondary to other cellular defects.

In studies of glucocerebrosidase, Mazzulli et al (2011) provided evidence for a positive feedback loop between autophagy disruption and α-synuclein aggregation. Loss of glucocerebrosidase function disrupted lysosomal function in vitro and in vivo, causing α-synuclein accumulation and neurotoxicity. Aggregated α-synuclein caused further impairment of lysosomal function, completing the loop.[29]

Considering its localization to the lysosome, several studies have examined how ATP13A2 affects autophagy and α-synuclein aggregation. ATP13A2 is a type 5 P-type ATPase that localizes to the late endosome/lysosomal membrane.[30,55] Its function is still unknown, but it appears to function as either a zinc transporter[55-58] or lipid flippase.[59] There is controversy regarding the effect of loss of or mutant Atp13a2 on α-synuclein levels. Several in vitro reports have suggested a modest increase in α-synuclein levels[58,60,61] or aggregation[61] in cells following ATP13A2 knockdown or mutation, whereas

no significant effects on levels of α-synuclein were seen following the depletion of Atp13a2 from SH-SY5Y cells[62] or Medaka fish.[63]

Generating a Model of Endolysosomal Dysfunction in Parkinson's Disease

To explore the function of Atp13a2 in vivo including further exploring the role of this protein in α-synuclein homeostasis, we generated a novel mouse model in which loss of Atp13a2 causes age-related lysosomal abnormalities, protein aggregation, gliosis, and ultimately motor deficits.[10] This model is among the first to show an age-related motor abnormality. The mutant mice have decreased movements and by 18 months develop abnormal motor behaviors. Furthermore, similar to PD, in which up to 50% of dopaminergic neurons degenerate prior to the onset of symptoms, the neuropathological changes in Atp13a2-null mice precede behavioral abnormalities by many months. Consequently, future studies will need to focus on elucidating the abnormalities that occur during this time frame, which may represent therapeutic targets. There are several drawbacks to our model, however. Similar to other genetic mouse models of PD,[64] Atp13a2-null mice do not lose dopaminergic substantia nigra pars compacta (SNpc) neurons, potentially reflecting important differences in the susceptibility to selective neurodegeneration between mouse and human dopaminergic neurons. Instead, we see widespread neuropathological changes throughout the brain, including the cortex, hippocampus, and cerebellum. KRS, the human disease originally linked to *ATP13A2* mutations,[30] is a widespread neurological condition in which patients develop not only parkinsonism but also early dementia, ataxia, myoclonus, and other neurological symptoms. Therefore, Atp13a2-null mice may more accurately model these other aspects of KRS than pathogenic events in dopaminergic neurons.

Interaction of Atp13a2 With α-Synuclein

Having generated a mouse that shows not only behavioral, but also extensive neuropathological changes, we used these mice to explore in vivo how loss of Atp13a2 affected α-synuclein homeostasis. In contrast to previous work in vivo,[65] we find Atp13a2-null mice do not develop changes in α-synuclein levels, solubility, or degradation by lysosomes despite the presence of ubiquitinated protein aggregates and autophagic deficits. Schultheis et al described a similar Atp13a2-null mouse line, which likewise had widespread and early lipofuscinosis, with only a mild increase in α-synuclein deposition limited to the hippocampus in older mice.[65] These 2 reports may initially seem contradictory with respect to α-synuclein. However, an important and potentially central finding in both animal models is the widespread and early onset of lipofuscinosis and related neuropathological changes in the absence of significant α-synuclein deposition. These similarities suggest additional pathogenic mechanisms at work independent of α-synuclein. Our findings left open the possibility that soluble toxic forms of α-synuclein not detected by our methods contributed to the observed phenotypes. We rigorously tested this possibility by genetically altering α-synuclein levels by crossing Atp13a2-null mice with either mice lacking or overexpressing α-synuclein. In these double mutants, we observed no change in the onset or extent of neuropathology seen in Atp13a2-null mice. These findings dissociate alterations in α-synuclein homeostasis from the initiation and early progression of neuropathological and behavioral abnormalities in a model of endolysosomal dysfunction similar to that causing L-dopa-responsive parkinsonism in humans. Importantly, these results demonstrate that endolysosomal dysfunction can cause neuropathology and disrupt neural function via an α-synuclein-independent mechanism.

Although our genetic crosses to α-synuclein mutants demonstrate that additional mechanisms operate to contribute to the neuropathological changes seen in Atp13a2-null mice, they do not rule out a role for α-synuclein contributing to human KRS. In vitro studies have reported a modest increase in α-synuclein levels in cells following ATP13A2 knockdown or mutation,[60,61] and Schultheis et al saw a mild increase in α-synuclein levels in aged Atp13a2-null mice.[65] Because our double-mutant mice were examined at 1–9 months, it remains possible that α-synuclein has a role at older ages.

Endolysosomal Dysfunction in Atp13a2-Null Mice

To understand the mechanism underlying pathogenesis in Atp13a2-null mice, we undertook detailed studies of lysosomal function in vivo, especially with respect to autophagy. Loss of key autophagy genes in the SNpc causes accumulation of α-synuclein,[66,67] and mice lacking key lysosomal enzymes like glucocerebrosidase or cathepsin D develop α-synuclein aggregation[29,68] and mitochondrial abnormalities.[69] Loss of Atp13a2 did not cause α-synuclein aggregation, but did result in accumulation of other autophagy substrates including ubiquitin, p62, and lipofuscin. P62 and ubiquitin aggregation has been identified in virtually every neurodegenerative disease and likely represents a cellular stress response.[70,71] Lipofuscin deposits are similarly found in many neurodegenerative disorders, most notably the neuronal ceroid lipofuscinoses,[72] but also AD.[73] Despite having prominent

deposits throughout the cortex, detailed study of cortical lysosomal function revealed abnormalities limited to cathepsin D maturation. Assays performed on Atp13a2-null cortical lysosomes indicated that proteolysis and chaperone-mediated autophagy were normal. However, the levels cathepsin D were decreased, indicating a deficit in its trafficking to the lysosome.

Future work will be required to unravel how Atp13a2 selectively affects cathepsin D processing and if cathepsin D loss contributes to the pathology of Atp13a2-null mice. Cathepsin D abnormalities occurred late in the disease process, after the onset of reactive astrocytosis and the accumulation of lysosomal proteins and lipids, suggesting that cathepsin D trafficking deficits may be a downstream effect of Atp13a2 loss of function. Yet, cathepsin D abnormalities occurred in a similar time frame to the onset of lipofuscinosis, p62, and ubiquitin aggregation, and prior to behavioral changes in Atp13a2-null mice. Furthermore, alterations to cathepsin D are increasingly identified in genetic forms of PD. Cathepsin D is known to degrade α-synuclein.[68,74-76] Complete loss of cathepsin D, as seen in cathepsin D–null mice, results in much earlier onset of lipofuscin accumulation,[77] glial activation, and neurotoxicity,[78] potentially linking cathepsin D loss to the pathological changes observed in Atp13a2-null mice. Although cathepsin D–null mice show increased levels of insoluble α-synuclein,[68] heterozygous mice do not,[79] consistent with our finding that α-synuclein does not change in the presence of partial loss of lysosomal cathepsin D. Such alterations in lysosomal cathepsin D could contribute to selective neurodegeneration, which may occur independently of α-synuclein.

It is also possible that the selective deficits in cathepsin D processing reflect a fundamental upstream alteration of endolysosomal vesicle trafficking. Follett et al showed that the D620N mutation in *VPS35* leads to defects in cathepsin D trafficking to the lysosome, presumably through impairment in the trafficking of the cation-independent mannose-6-phosphate receptor (CI-MPR).[80] Endosome maturation and distribution within the cell were also impaired. Improper retrograde trafficking of the CI-MPR in VPS35 D620N cells led to decreased delivery of CI-MPR substrates to the lysosomes.[80] Similar trafficking defects of CI-MPR occurred with loss or mutation of *LRRK2* or *Rab7L1*.[40] Accumulation of lysosomal proteins and lipids preceded altered cathepsin D processing, indicating that lysosomal upregulation may not be a response to improper degradation of autophagy substrates, but instead a compensatory response to the abnormal movement of vesicles along microtubules or improper maturation of late endosomes/lysosomes. Although many studies have identified interactions between LRRK2 and microtubules or microtubule-related proteins[51,81-84], additional study of the effects of PD-related proteins on vesicle movement and the trafficking of specific cargo proteins is required to better evaluate the significance of this mechanism.

Beyond Autophagy: Alternative Sites of Disruption in the Endolysosomal System

As multiple endolysosomal proteins are linked to PD pathogenesis, it is tempting to look for similar patterns, especially with respect to α-synuclein. However, PD mutations cluster in several distinct parts of the endolysosomal system: the lysosome (eg, *GBA, ATP13A2*), clathrin-mediated endocytosis (eg, *SYNJ1, DNAJC6*), and retromer trafficking (eg, *RAB7L1, LRRK2, VPS35*). It is not immediately clear what effect most of these mutations have on these various compartments, and there is not yet a clear locus at which pathogenic events converge. As with mutations in glucocerebrosidase, some of these mutants likely operate in a feed-forward loop with α-synuclein through autophagy,[29] but our data and findings from others suggest that additional mechanisms are co-occurring. The endolysosomal trafficking system is a critical cellular pathway that interacts with every core cellular function. Disruption of such a crucial intracellular pathway is bound to have myriad effects in the cell, any number of which could cause toxicity. Furthermore, α-synuclein is known to interact physically with vesicular proteins at many different stages of the endolysosomal system, including many of the Rab proteins that govern vesicle movement.[85-87] It seems highly likely, therefore, that a key mechanism of α-synuclein toxicity stems from its disruption of the endolysosomal pathway itself (Fig. 2).

Retromer-Mediated Recycling

The retromer protein complex is a site of endolysosomal dysfunction implicated in multiple neurodegenerative diseases. This protein complex controls recycling of transmembrane proteins from the endosome back to the trans-Golgi network (TGN). The strongest genetic evidence for retromer dysfunction in PD comes from the identification of mutations in *VPS35*, which cause autosomal-dominant, late-onset PD.[38,88] VPS35 is a component of the retromer. Reduced levels of VPS35 in AD patients cause increase retention of APP in the endosomal compartment, leading to excess APP cleavage and production of toxic amyloid-→.[5] In PD, cell biological studies have indicated that *VPS35* mutations impair the recycling of important receptors linked to mitochondrial fusion/fission,[89,90] synaptic function,[91] and chaperone-mediated autophagy.[92] All these studies emphasize that PD-related

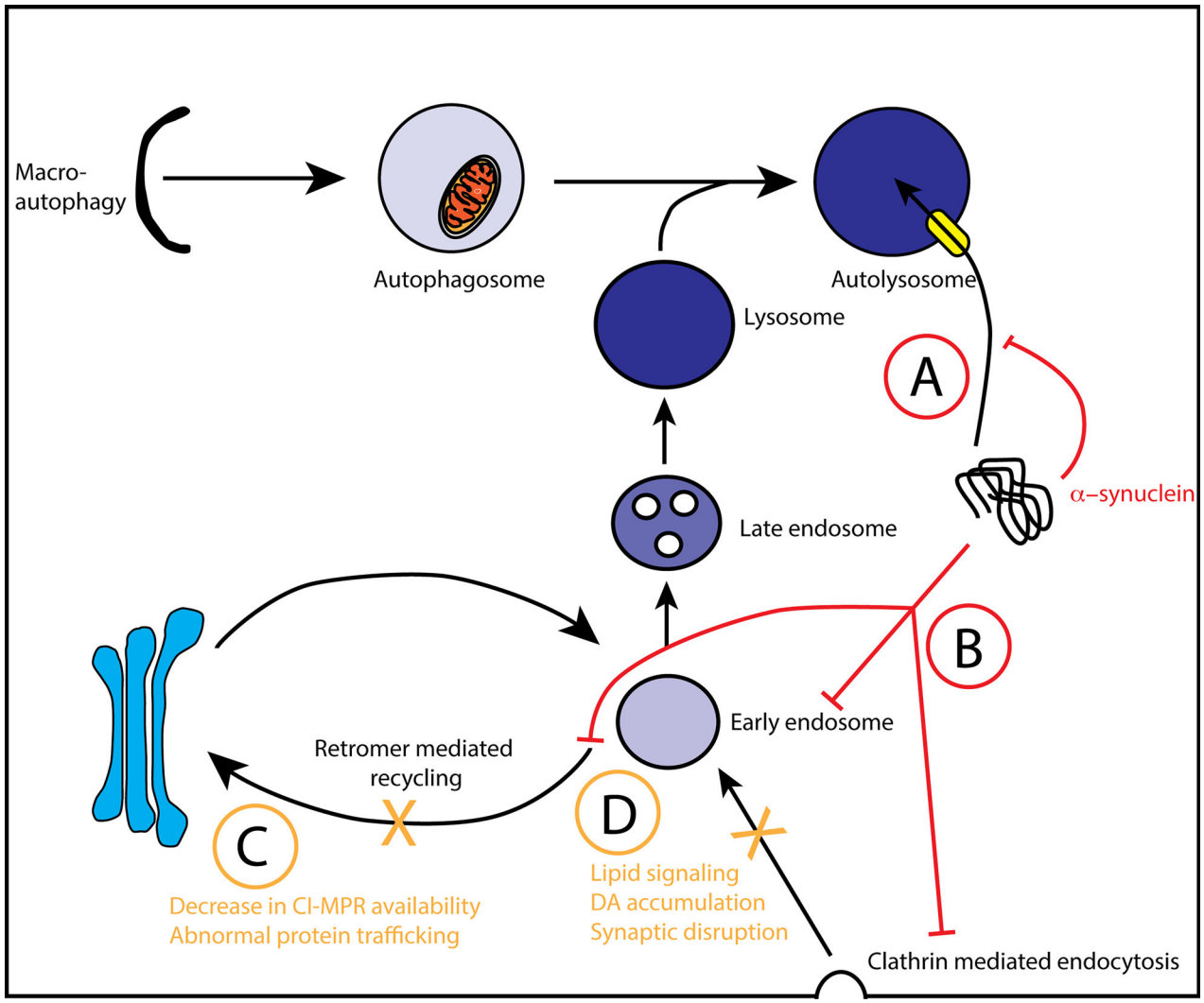

FIG. 2. α-Synuclein's interactions with the endolysosomal system and α-synuclein-independent mechanisms of endolysosomal dysfunction. In addition to inhibiting its own degradation via CMA and macroautophagy (A), α-synuclein interacts with Rab GTPases on multiple vesicles including endocytic vesicles, early endosomes, and recycling endosomes (B). α-Synuclein-independent mechanisms cluster around retromer-mediated recycling (C) and clathrin-mediated endocytosis (D). It is unknown how disruption of retromer-mediated recycling causes neurodegeneration, but inhibition of receptor recycling (including of the cation-independent mannose-6-phosphate receptor) between endosomes and the trans-Golgi network likely prevents the subsequent delivery of necessary proteins to lysosomes, synapses, and cell membranes. Similarly, disruption of clathrin-mediated endocytosis likely has unspecified effects on lipid signaling, synaptic proteins, and/or extra-vesicular dopamine accumulation.

mutations in VPS35 cause an abnormal flux of key proteins between the endosomes and TGN.

Another protein that appears to function in this pathway is the Rab GTPase Rab7L1. Two independent studies exploring LRRK2-interacting pathways identified Rab7L1, whose gene resides within the PARK16 locus. MacLeod et al found that variation in the *RAB7L1* gene interacts with *LRRK2* to influence risk of sporadic PD.[40] Rab7L1 overexpression rescues mutant *LRRK2* neurodegenerative phenotypes in both primary neurons and *Drosophila*. To understand the cellular function of these 2 proteins, MacLeod et al showed that LRRK2 and Rab7L1 function in a common pathway, in which loss of either protein causes lysosomal swelling and decreased recycling of CI-MPR to the TGN. Loss of LRRK2 or Rab7L1 caused decreased levels of the retromer proteins VPS35 and VPS26, potentially accounting for the decreased recycling of CI-MPR.

In a complementary study, Beilina et al identified a LRRK2 complex consisting of Rab7L1, Cyclin-G-associated kinase (GAK), and Bcl2-associated athanogene (BAG5) using an unbiased protein–protein interaction array.[93] In overexpression studies in vitro, they demonstrated that this complex mediates the clearance of proteins from the trans-Golgi network via autophagy, suggesting that the Rab7L1 complex functions to transport proteins from TGN to the endosomes. This result initially appears the inverse of MacLeod et al; however, experimental differences make direct

comparisons difficult. That 2 independent groups using differing methodology both identified Rab7L1 is powerful evidence of its involvement in LRRK2 biology, and it may work in concert with GAK, BAG5, and/or retromer to coordinate the movement of vesicles between endosomes and TGN. Disruption of this complex results in a relative increase in flux from TGN to endosomes to lysosomes, but whether this increase occurs from enhancement of forward trafficking or inhibition of retrograde trafficking is unclear. Furthermore, how disruption of this trafficking leads to neurodegeneration remains unanswered.

Clathrin-Mediated Endocytosis

The identification of mutations in synaptojanin 1 (*SYNJ1*) and auxilin (*DNAJC6*) in families with early-onset parkinsonism implicates alterations in clathrin-mediated endocytosis in PD. Both proteins are necessary for effective endocytosis of clathrin-coated vesicles.[94] Auxilin is recruited to vesicles by phosphatidylinositol 4,5-bisphosphate (PIP_2) and works in concert with Hsc70 to uncoat vesicles.[95] Synaptojanin 1 is the main neuronal PIP_2 phosphatase, is enriched at synapses, and is similarly necessary in both the endocytosis and uncoating of vesicles.[96] Individuals with trisomy 21, who carry an extra chromosomal copy of *SYNJ1*, as well as trisomy 21 mouse models show enlarged endosomes, which are normalized following *SYNJ1* silencing.[6] It is unknown how mutations in *SYNJ1* and *DNAJC6* cause parkinsonism, but recessive inheritance and loss of phosphatase activity with the R58Q synaptojanin 1 mutation[36] suggest a loss of function mechanism.

There are several potential toxic events consequent to disrupted endocytic function that could contribute to neurodegeneration. Alterations in synaptic clathrin-mediated endocytosis may lead to decreased availability of vesicles for dopamine with corresponding increases in free cytosolic dopamine at the synapse. Increased dopamine within the synapse could react with reactive oxygen species to form toxic species. Endocytosis changes could also affect the membrane localization of other pre- or postsynaptic proteins at the nigrostriatal synapse[97,98] or affect the uptake of α-synuclein via exocytosis. Finally, the link with PIP_2, a phospholipid known to be important for vesicle fusion, suggests possible disruption of phospholipid signaling and/or lipid trafficking in PD pathogenesis. Lipid dysfunction is most directly linked to neurodegeneration in the rare genetic disease Niemann-Pick disease, type C (for review see reference[99], in which genetic disruption of cholesterol transporters in the endolysosomal system results in lipid accumulation and widespread organ dysfunction including early-onset neurodegeneration. Lipid signaling abnormalities are heavily implicated in AD as well,[100] in which the single biggest genetic risk factor is inheritance of the E4 allele of the lipid transport protein APOE.[101,102]

Conclusions

Converging research is highlighting the importance of the endolysosomal system in neurodegeneration in ways that are still unclear. One model in AD is that altering flux through the endolysosomal system causes retention of APP in the early endosomes, in which it is cleaved into amyloid-β. It is appealing to think that a similar α-synuclein-based model is functioning in PD. However, our understanding of the abnormalities of α-synuclein homeostasis consequent to PD genetic lesions remains at an early stage. The endolysosomal system is an appealing target for potential therapeutic development, and the convergence from many other disorders suggests cross-disease drug development. Furthermore, understanding the differential impact of endolysosomal impairment in different neurodegenerative disorders like PD and AD will be critical. It remains difficult to understand how mutations in the same pathway can lead to the development of AD, whereas others lead to PD (and still others cause additional neurodegenerative diseases). This disease heterogeneity suggests that much remains to be learned about the organization and function of the endolysosomal system. For example, although it is generally assumed that all lysosomes are the same, there are likely to be lysosomal subtypes that perform specific functions and localize to specific subcellular sites.[45,103,104] Disease mutations may differentially impact these lysosome subclasses, potentially providing some insight into the heterogeneous diseases linked to endolysosomal dysfunction.

The discovery of PD-related mutations in multiple endolysosomal proteins suggests several lines of inquiry important for future study. First, it will be important to understand the specific steps in the endolysosomal pathway that are affected by mutations in these proteins and delineate the subsequent sequence of pathogenic events. Vesicle trafficking has predominantly been examined in the context of α-synuclein accumulation and transmission. Cell biological and in vivo studies are needed to clarify if and how primary dysfunction of the lysosome, late endosome, retromer, or endocytic pathway causes neurodegeneration — including independent of (indeed, in the absence of) the effects of α-synuclein. Perhaps most pressing is to what extent and how these various pathways interact. Preliminary studies, primarily in cell culture, suggest overlap between α-synuclein homeostasis, mitochondrial dysfunction, and/or vesicle trafficking, but no work has demonstrated in vivo if these pathways converge on a common end point or operate in parallel to one another. Depending on the degree of

interaction, it will be important to identify early sites of dysfunction as points of maximal therapeutic intervention. For example, if lysosomal dysfunction is "upstream" of both α-synuclein aggregation and oxidative stress, then normalizing lysosomal function early in the disease course may limit later sequelae like spreading α-synuclein pathology or nonmotor symptoms. ■

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