Trends in Endocrinology & Metabolism

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REVIEWS

Review

Sphingolipids in the Pathogenesis of Parkinson's Disease and Parkinsonism

Guang Lin,[1,2] Liping Wang,[2,3] Paul C. Marcogliese,[1,2] and Hugo J. Bellen[1,2,3,4,*]

The pathogenic mechanisms underlying Parkinson's disease (PD)/parkinsonism affect mitochondrial and endolysosomal trafficking. The retromer is required to retrieve some proteins from endosomes to the Golgi and plasma membrane. Here, we discuss how retromer-dependent retrieval also affects ceramide metabolism. Compelling studies across PD models in *Drosophila* and mammalian neurons reveal a pathogenic cascade implicating retromer dysfunction and mitochondrial defects. We argue that ceramides may play a critical role in the pathobiology based on the studies of *PLA2G6* and *VPS35* in *Drosophila* mutants and human knock-down cells. In addition, pathogenic variants in many lysosomal storage disorder genes have recently been associated with PD, suggesting a potential overlap between the pathogenic mechanisms underlying these disorders. We propose that disruption of ceramide metabolism may affect endolysosomal and mitochondrial function, and plays an important role in PD/parkinsonism.

Highlights

Studies in the past two decades suggest that both mitochondrial and endolysosomal trafficking pathways are affected in PD/parkinsonism.

A pathogenic cascade of retromer dysfunction and endolysosomal defects leads to ceramide accumulation.

GBA, an LSD gene, is the most common PD risk factor. Additionally, many other LSD genes have recently been associated with PD, indicating a potential overlap in PD and LSD pathogenesis.

Disruptions in the sphingolipid metabolism pathway may connect a variety of phenotypes including both mitochondrial and endolysosomal defects, and may play a prominent role in PD/parkinsonism.

Introduction

Parkinson's disease (PD) is a progressive and incurable neurodegenerative disorder that affects ~1% of individuals older than 60 years. PD is primarily characterized by a constellation of motor signs known as parkinsonism, which includes tremor, increased muscle tone (rigidity), slowness of movement, and impaired gait and balance [1]. At autopsy, PD is defined by the presence of ubiquitin and α-synuclein (α-Syn) positive protein aggregates, Lewy bodies, and loss of dopaminergic neurons in the substantia nigra pars compacta. Atypical parkinsonism is a less common disorder that is also characterized by prominent primary motor symptoms along with other heterogeneous clinical features [2]. At autopsy, most of these atypical cases are characterized by nigral degeneration with or without Lewy body pathology.

Over the past two decades, both genetic and toxin-induced models of PD have been established. The recessive forms of familial PD (e.g., *PINK1*, *PRKN*, and *PARK7*) are associated with early onset of the disease and the implicated genes are involved in mitochondrial quality control [3]. Additionally, environmental toxins such as rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine are mitochondrial complex inhibitors that induce dopaminergic neuronal death [4]. Moreover, mitochondrial complex I deficiencies and mitochondrial electron transport chain defects have been reported in the nigra of PD patients [5]. Hence, there are multiple lines of evidence implicating mitochondrial dysfunction as a primary cause of PD.

In addition to mitochondrial dysfunction, recent evidence implicates alterations in the endolysosomal pathway in numerous forms of parkinsonism or PD [6–8]. This pathway requires many proteins for each step, including endocytosis, retromer function, as well as a sorting machinery for autolysosomal degradation and numerous proteins required for proper

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA
[2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX 77030, USA
[3]Program in Developmental Biology, Baylor College of Medicine, Houston, TX 77030, USA
[4]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, USA

*Correspondence:
hbellen@bcm.edu (H.J. Bellen).

lysosomal function. Membranes and membrane dynamics are required for these processes and an imbalance of lipids, including ceramide and sphingolipid intermediates, may contribute to mitochondrial and endolysosomal dysfunction that could lead to the demise of neurons. Here, we propose a model based on our recently published study of the atypical parkinsonism gene, *PLA2G6* [8], and extend these results to other models of PD arguing that lipid imbalance may contribute to many forms of parkinsonism and sporadic PD.

Mutations in a specific phospholipase, *PLA2G6,* which encodes phospholipase A2 (iPLA2-β), are known to cause dystonia–parkinsonism as well as infantile neuroaxonal dystrophy (INAD), and atypical NAD [9]. These diseases are different as INAD has early onset (1–2-year-old infants) and rapid progression, whereas parkinsonism has typical onset in the late teens and 20s and is slowly progressive. We created flies lacking the fly ortholog of *PLA2G6*, *iPLA2-VIA*. Loss of *iPLA2-VIA* does not affect phospholipids but binds and stabilizes retromer subunits, Vps26 and Vps35. This leads to retromer dysfunction and altered sphingolipid homeostasis, culminating in the accumulation of ceramides that results in neurotoxicity [8] (Figure 1). Pharmacological or genetic manipulations that either reduce ceramide levels or promote retromer function robustly suppress *iPLA2-VIA* neurodegenerative phenotypes.

Interestingly, genetic variants in two genes known to cause familial PD (*VPS35* and *SNCA*) also disrupt retromer function, cause endolysosomal dysfunction and accumulate ceramide in flies, and respond to retromer-promoting and ceramide-reducing drugs [8]. Moreover, recessive variants in *GBA*, which encodes β-glucosylceramidase (GCase), a key enzyme in sphingolipid metabolism, is known to cause Gaucher's disease (GD). GD is a lysosomal storage disease (LSD). Heterozygous carriers are at a significantly higher risk for PD [10]. Hence, *GBA* provides yet another link to sphingolipid metabolism, endolysosomal dysfunction, and PD. Note that *PLA2G6*, *VPS35*, and *GBA*, as well as a gain of *SNCA* [11] are all known to cause mitochondrial defects, implicating a crosstalk between endolysosomal dysfunction, sphingolipid metabolism, and mitochondrial dysfunction in PD/parkinsonism.

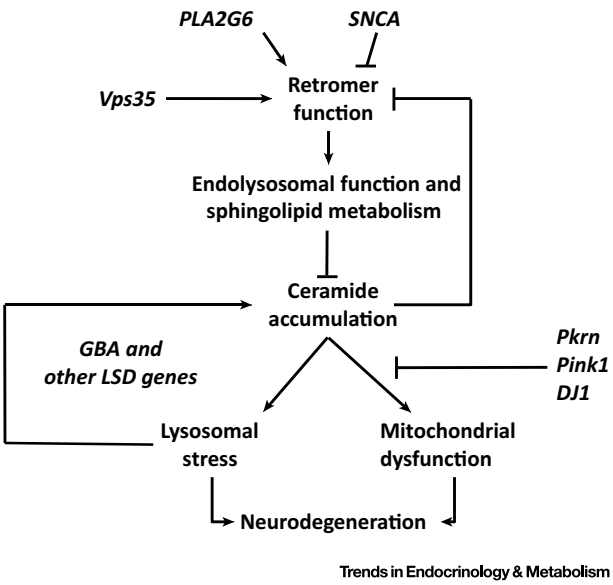

Figure 1. Membrane-Lipid-Metabolism-Mediated Neurodegeneration Caused by Disruption of PD/Parkinsonism Genes. Disruption of iPLA2-VIA, Vps35, and overexpression of α-Syn impairs retromer function, leading to disruption of sphingolipid metabolism and accumulation of ceramides. The latter further leads to a positive feedback loop that disrupts retromer function and accelerates ceramide accumulation in endolysosomal and mitochondrial compartments, leading to the demise of neurons. Abbreviation: α-Syn, α-synuclein.

Here, we review recent published data that bolster the hypothesis that impaired retromer/endolysosomal function and the resulting sphingolipid imbalance may be at the root of multiple forms of PD. In this review, we: (i) discuss recent human genetic data identifying genetic alterations in sphingolipid and endolysosomal pathways that are shared by PD and LSDs; (ii) review the current literature on the evidence of altered sphingolipid metabolism that may drive the pathogenesis and progression of PD/parkinsonism; (iii) unravel the link between PD genes and retromer function, lysosomes, and sphingolipids; and (iv) propose a model as to how sphingolipid metabolism may connect multiple pathways in PD.

Genetic Alterations in Sphingolipid and Endolysosomal Pathways May Underlie PD and LSDs

Based on recent discoveries in human genetics, PD and LSD share a significant amount of overlapping genetic burden. Indeed, substantial evidence implicates the role of the endolysosomal pathway in PD pathogenesis [12]. The vast majority of PD-linked genes have been implicated in the endolysosomal pathway (Table 1). Additionally, some common variants in LSD genes increase the risk of developing PD [10,13,14]. Many of these LSD genes are also involved in the endolysosomal pathway. A genome-wide association study identified 17 novel risk loci associated with PD [13]. Three loci: *CTSB* (cathepsin B), *ATP6V0A1* (ATPase H+ transporting V0 subunit a1), and *GALC* (galactosylceramidase) play a known role in the endolysosomal pathway. In two cohorts of PD and control cases, it has been shown that ~21% of the cases have multiple LSD causing variants and ~56% of PD patients have at least one LSD causative variant [14], indicating that the pathological mechanisms of LSD and PD are intertwined. In the same study, novel PD risk genes were uncovered including *CTSD* (cathepsin D) and *CTSB* (cathepsin B), lysosomal cysteine proteases. *In vitro* studies have shown that both proteins are essential for lysosomal degradation and clearance of α-Syn [15]. CTSD degrades α-Syn at the N terminus, whereas CTSB cleaves the amyloid region of α-Syn. Suppressing the levels of CTSB reduces α-Syn fibril-induced aggregation, indicating that CTSB regulates α-Syn fibril aggregation [16]. Hence, both lysosomal proteases promote the aggregation of α-Syn through the endolysosomal pathway.

Altered Sphingolipid Metabolism May Drive Pathogenesis and Progression of PD/Parkinsonism

Sphingolipids are a group of phospholipids that contain a backbone of sphingoid bases. Figure 2 illustrates the sphingolipid metabolic pathways. Several enzymes that regulate sphingolipid metabolism are localized to the lysosomes (enzymes highlighted in yellow in Figure 2). Defects in genes that encode these enzymes have been shown to cause LSD and are risk factors for PD [14]. Here, we highlight how a loss of sphingolipid metabolic enzymes may affect the physical properties of membranes and lead to defects in the endolysosomal and mitochondrial pathways to cause LSD and/or PD. We focus on four LSD genes that encode lysosomal enzymes: *GBA*, *ASAH1, SMPD1*, and *SCARB2*.

β-Glucocerebrosidase (GCase), encoded by *GBA*, is a lysosomal hydrolase that regulates sphingolipid metabolism by cleaving GlcCer to ceramide and glucose. Recessive mutations in *GBA* cause GD, while heterozygous *GBA* carriers have a fivefold increased risk of developing PD [10]. Reduced GCase activity and expression levels are observed in the nigra of PD patients [17]. In flies, expression of human *GBA* variants leads to dopaminergic neuronal loss and causes gradual locomotor defects [18]. In zebrafish, *gba1* knockdown causes sphingolipid accumulation in early development, impaired motor activity, and dopaminergic neuronal loss at later stages [19]. In mice, knocking out *Gba* leads to α-Syn accumulation and mitochondrial dysfunction in the central nervous system [20]. Altogether, these results definitively implicate *GBA* and sphingolipid metabolism in PD/parkinsonism.

Table 1. PD Causal Genes That Cause Defects in the Endolysosomal Pathway

Gene	Loci	Protein	Function	Role in endolysosomal pathway	Refs
Autosomal dominant PD					
SNCA	PARK1/4	α-Synuclein	Multiple functions including: Integrating synaptic signaling Releasing/transportation of dopamine	GoF leads to α-Syn oligomer accumulation and the formation of α-Syn aggregates	[50]
VPS35	PARK17	VPS35	Retrograde transportation from endosome to Golgi	LoF leads to impairment of retromer complex activity, enlargement of late-endosomes and α-Syn oligomer accumulation and the formation of α-Syn aggregates	[7,50]
LRRK2	PARK8	LRRK2	Ser/Thr kinase Vesicular trafficking?	GoF leads to reduced number and enlarged lysosomes, and decreased lysosomal pH and activity	[6]
Autosomal recessive PD					
PRKN	PARK2	Parkin	E3 ubiquitin ligase Key protein in mitophagy	LoF leads to decreased mitophagy and endosomal tubulation, diminished retromer function and mitochondrial-derived vesicle biogenesis	[7]
PINK1	PARK6	PINK1	Ser/Thr kinase Regulator of Parkin in mitophagy	LoF leads to diminished mitophagy and mitochondrial-derived vesicle biogenesis	[80]
PARK7	PARK7	DJ-1	Oxidative stress sensor	LoF leads to age-dependent accumulation of insoluble α-Syn; overexpression of *SNCA* in DJ-1 mutant background results in reduced lysosomal glucocerebrosidase activity	[81]
Atypical parkinsonism					
ATP13A2	PARK9	ATP13A2	Lysosomal P5-type transport ATPase	Elevated expression level in LRRK2 mutants	[6]
PLA2G6	PARK14	iPLA2-β	Phospholipid remodeling Regulator of Retromer	LoF leads to increasing number and enlarged lysosomes, and impairment of retromer complex activity	[8]
SYNJ1	PARK20	Synaptojanin-1	Phosphoinositide phosphatase	LoF leads to accumulation of large vesicular structures, enlargement of lysosomes, impairment of endosomes, and elevation of autophagosomes	[82]
Novel PD risk genes					
ATP6V0A1	–	VPP1	V-type proton ATPase that pumps protons into the luminal environment of the endolysosomal system	LoF leads to increased lysosomal pH and abolished lysosomes and phagosomes fusion	[83]
CTSD	–	Cathepsin D	Lysosomal aspartic acid protease that breaks down intracellular proteins	LoF leads to decreased lysosomal degradative activity and increased autophagic vesicles	[84]
CTSB	–	Cathepsin B	Lysosomal cysteine protease that degrades proteins intracellularly	LoF leads to accumulation of undegraded proteins in the lysosomes	[85]
GALC	–	Galactosylceramidase	Lysosomal enzyme that hydrolyzes galactosylceramide or galactosylsphingosine into galactose and ceramide	Lysosomal localization is required for the activation of the enzyme	[86]
GBA	–	Glucosylceramidase	Lysosomal enzyme that hydrolyzes glucosylceramide into glucose and ceramide	LoF leads to enlarged lysosomes and increased number of autophagosomes	[23]
SMPD1	–	Acid sphingomyelinase-1	Lysosomal enzyme that hydrolyzes sphingomyelin into ceramide and phosphatidylcholine	LoF leads to enlarged lysosomes, increased number of autophagosomes, and accumulation of ubiquitinated proteins	[31]

Abbreviations: GoF, gain of function; LoF, loss of function; PD, Parkinson's disease.

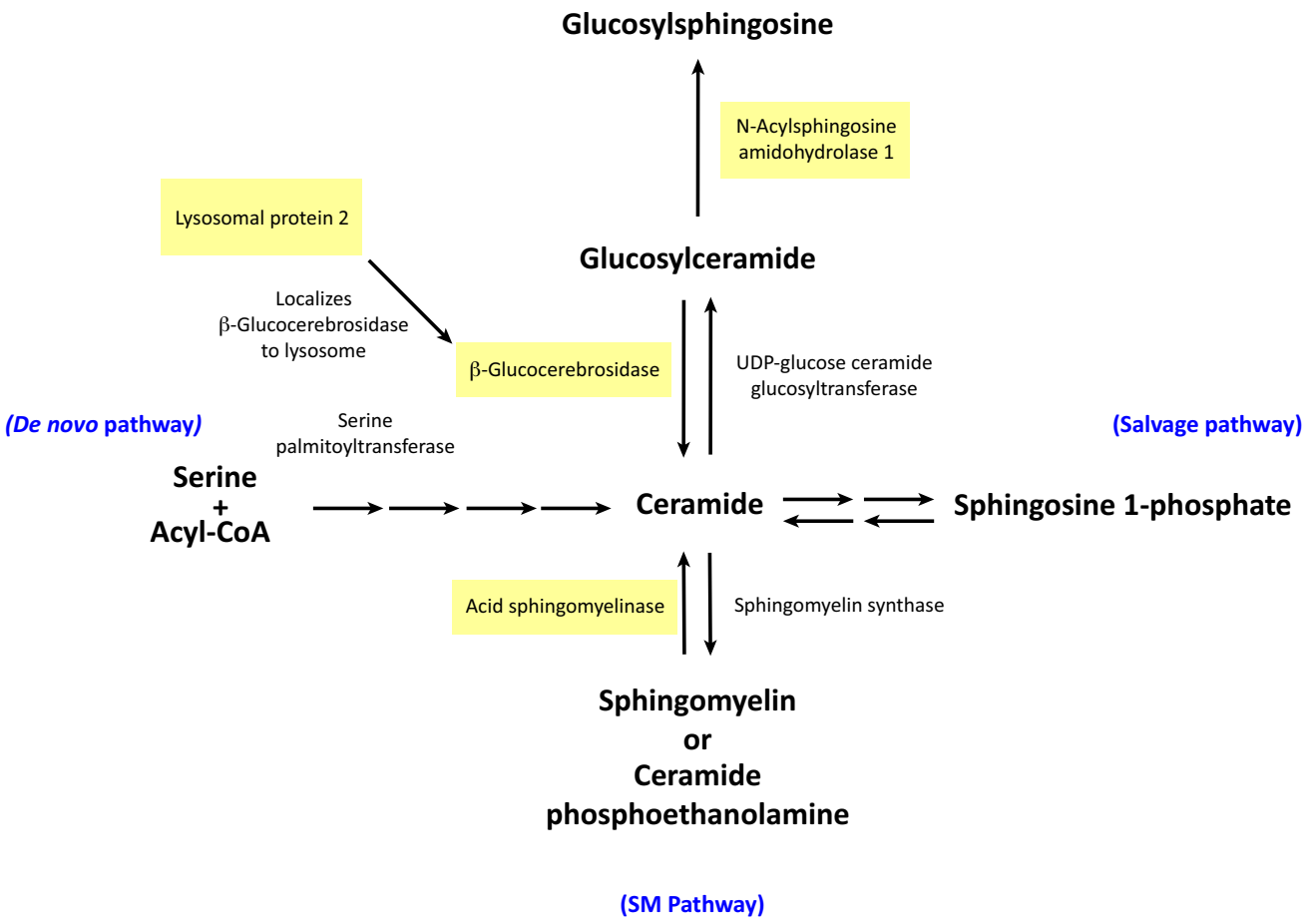

Figure 2. The Sphingolipid Metabolism Pathway. Serine palmitoyltransferase catalyzes the fusion of serine with acyl-CoA to generate 3-ketosphinganine, which is then converted to ceramide. Ceramide is a metabolic hub of the sphingolipid metabolism pathway. It is converted to sphingosine-1-phosphate, as part of the salvage pathway. Ceramide can be converted to ceramide phosphoethanolamine or sphingomyelin, the higher-order forms of sphingolipids, by sphingomyelin synthase. The lysosomal acidic sphingomyelinase breaks down ceramide phosphoethanolamine/sphingomyelin to ceramide. Finally, ceramide is glycosylated to form glucosylceramide and then glucosylsphingosine by UDP-glucose ceramide glucosyltransferase and N-acylsphingosine amidohydrolase-1. Another lysosomal enzyme, β-glucocerebrosidase, removes the sugar on GlcCer to form ceramide. Lysosomal enzymes implicated in Gaucher's disease and Parkinson's disease are highlighted in yellow.

The lysosome is the primary organelle for protein and lipid degradation. Lysosomal dysfunction leads to accumulation of proteins, sugars, and lipids in the lysosomes, causing stress, and sometimes cell death. An aberrant autophagy lysosomal pathway may also be involved in the pathogenesis of PD as it may affect the clearance of α-Syn [21]. Dopaminergic neurons derived from induced pluripotent stem cell lines from PD patients with the most common heterozygous *GBA* mutations p.N370S and p.L444P lead to misfolded and reduced GBA, an aberrant autophagy lysosomal pathway, as well as lysosomal expansion [22]. Similarly, loss of *dGba1*, the fly ortholog of *GBA*, causes lysosomal dysfunction, accumulation of GlcCer in the brain, progressive locomotor defects and a shorter lifespan due to impaired autophagy [23]. Moreover, *Gba* loss-of-function mutations in rats cause autophagic deficits leading to an accumulation of α-Syn [24]. Finally, substantial data suggest interplay between GBA and α-Syn (see Box 1 for a discussion). All these studies strongly suggest that *GBA* contributes to PD pathogenesis.

Box 1. GBA and α-Synuclein

The presence of α-Syn-positive aggregates is observed in the brains of GD patients [47]. In flies, inhibiting GCase in a $SNCA^{A53T}$ overexpression background enhances α-Syn aggregation [74]. Similarly, loss of *Gba* in rats leads to elevated α-Syn protein levels [24]. Chronic pharmacological inhibition of GCase leads to the accumulation of intracellular α-Syn oligomers in mice [75]. *GBA* knockout in SH-SY5Y cells also results in increased α-Syn monomers with decreased oligomeric structures [76]. Moreover, coexpression of a human GD variant, GBA^{D409H}, with $SNCA^{A53T}$ in mice enhances the formation of insoluble α-Syn aggregations that causes neurodegeneration and motor defects [77]. These data strongly suggest a connection between *GBA* and *SNCA* and highlight that PD and GD may share molecular mechanisms in driving their pathogenesis.

It has been shown that α-Syn forms a complex with GlcCer, the substrate of GCase. This stabilizes α-Syn oligomers and promotes the formation of α-Syn aggregates. An elevated GCase activity reduces GlcCer levels and suppresses α-Syn secretion from exosomes [75]. In transgenic flies that express α-Syn (wild-type, A30P or A53T), knocking down d*Gba1* promotes the formation of α-Syn aggregates, possibly through the elevated GlcCer which stabilizes α-Syn oligomers and promote α-Syn aggregation. Importantly, loss of dopaminergic neurons was observed in these flies [74]. Similarly, inhibiting GCase activity in cultured cells and mice also increases GlcCer levels, compromises lysosomal degradation and enhances α-Syn accumulation [48,78]. Altogether, these studies suggest that GCase activity can modulate the levels of GlcCer and thereby modulates the stability of α-Syn to promote the formation of α-Syn aggregates. α-Syn can inhibit the activity of lysosomal GCase in neurons and PD brain tissue, possibly through a direct interaction with GCase in the acidic environment of lysosomes [79]. This may lead to a positive feedback loop that further impairs GCase activity and in turn causes more α-Syn secretion and accumulation. The accumulated α-Syn builds up progressively leading to disruption of the lysosomal protein degradation machinery, further amplifying defects and resulting in neurodegeneration.

ASAH1 encodes a lysosomal acid ceramidase, which metabolizes GlcCer into glucosylsphingosine (GlcSph) [25] (Figure 2). Recessive variants associated with *ASAH1* cause a spectrum of *ASAH1*-related disorders, including Farber disease, an LSD [26]. Loss of *ASAH1* should lead to an accumulation of GlcSph. Excessive GlcSph is found in GD patients, suggesting a connection between *ASAH1* and *GBA*. So far, little is known about how GlcSph accumulates and how this is related to GD as well as PD pathology. A possible link is that the accumulation of GlcSph promotes α-Syn pathology [27]. Further studies are needed to determine the role of *ASAH1* and GlcSph in GD and PD.

The *SMPD1* gene encodes a lysosomal hydrolase or acidic sphingomyelinase that converts sphingomyelin into ceramide in the acidic environment of lysosomes. Recessive variants in *SMPD1* impair the enzymatic activity of acidic sphingomyelinase leading to an LSD, called Niemann–Pick disease types A and B [28]. Importantly, carriers of these variants are at an increased risk of developing PD [29], again illustrating a connection between LSD, PD, and ceramide metabolism.

Smpd1 knockout mice are healthy at birth and develop normally until ~4 months of age. These mice then start to develop neurological symptoms including ataxia, tremors, and loss of appetite [30]. Consistent with the enzymatic function of Smpd1, sphingomyelins accumulate in knockout mice and patients with Niemann–Pick disease types A and B [30]. Moreover, lysosomal damage and autophagy dysfunction are observed in the cerebellum of *Smpd1* knockout mice as well as fibroblasts from patients with Niemann–Pick disease types A and B [31]. Reducing sphingomyelin levels by suppressing the *de novo* sphingolipid synthesis pathway with fumonisin B1, restores the dysfunctional autophagy in patient fibroblasts. This suggests that the accumulated sphingomyelins promote lysosomal and autophagic defects to causes neurodegeneration.

SCARB2 encodes lysosomal protein 2, a mannose-6-phosphate-independent GBA receptor [32]. Recessive mutations in *SCARB2* are linked to action myoclonus–renal failure syndrome and cause reduced GCase activity [33]. Multiple association studies have identified heterozygous variants in *SCARB2* as a PD risk factor [32,34]. More studies are needed in animal models

to delineate the role of SCARB2 in PD. The immune cells derived from patients with action myoclonus–renal failure syndrome display an inability to process autophagosomes [35], suggesting that SCARB2 affects lysosomal function and may contribute to PD through the lysosomal–autophagic function and sphingolipid pathways.

In summary, at least four proteins that are associated with lysosomes and affect ceramide metabolism have been linked to PD and LSD. Given the previously described association of LSD causing genes and PD, there is potentially a mechanistic link between lysosomal dysfunction, aberrant ceramide metabolism, and the development of PD.

Unraveling the Link between PD Genes and Retromer Function, Lysosomes, and Sphingolipids

Based on the above data and our observations that ceramides accumulate upon a disruption of retromer function when *PLA2G6* and *VPS35* is lost or when α-Syn is overexpressed in *Drosophila* or vertebrate neurons, we propose that sphingolipid dysfunction leads to defects in the endolysosomal and mitochondrial pathways that contributes to the pathogenesis of PD. This model is consistent with recent reports that ceramide levels in the plasma of PD patients may serve as a biomarker for PD [36,37]. Here, we attempt to further link endolysosomal and mitochondrial pathways to sphingolipid homeostasis based on the cellular defects associated with dominant (*SNCA*, *LRRK2*, and *VPS35*) and recessive (*PRKN* and *PINK1*) as well as atypical (*PLA2G6* and *ATP13A2*) forms of PD/parkinsonism.

SNCA

SNCA encodes α-Syn, a presynaptic protein, which forms fibrillar aggregates that mediate neurotoxicity in PD [38]. Duplication or triplication of the *SNCA* locus is sufficient to cause early-onset PD, indicating a clear relationship between α-Syn levels and PD pathogenesis [38]. The ectopic expression of α-Syn in *Drosophila* neurons causes the accumulation of multivesicular or multilamellar bodies [39], suggesting a disruption in autophagy and endolysosomal flux. Similarly, overexpression of α-Syn induces lysosomal expansion in mammalian neurons [8] as well as in human midbrain dopaminergic neurons [40]. This dysfunctional endolysosomal pathway may be caused by the disruption of the endoplasmic reticulum to Golgi trafficking by α-Syn aggregates [41].

α-Syn contains a mitochondrial targeting signal [42], suggesting that it also plays a role in mitochondria. Indeed, fragmented mitochondria are frequently reported in α-Syn overexpression models including in cultured cells [43], human-embryonic-stem-cell-derived neurons [44], and dopamine neurons of nematodes [45] or zebrafish embryos [46]. Moreover, overexpression of α-Syn in human dopaminergic neurons causes the accumulation of α-Syn in the mitochondria, which reduces mitochondrial complex I activity and leads to the generation of reactive oxygen species [42]. Furthermore, mitochondrial electron transport chain defects and complex I deficiencies have been frequently reported in the nigra of PD patients [5]. These data strongly implicate a pathogenic role for α-Syn in mitochondrial function.

α-Syn-positive Lewy bodies are present in the brain tissue of GD patients [47]. Loss of *GBA* in primary cortical neurons or in human induced-pluripotent-stem-cell-derived dopamine neurons leads to the accumulation of α-Syn aggregates that impair lysosomal protein degradation and cause neuronal death *in vitro* [48] (Box 1). We observed that overexpression of α-Syn in mammalian neurons upregulates ceramides and causes lysosomal stress [8]. Consistent with our findings, suppressing GlcCer synthase activity in α-Syn-overexpressing neuroblastoma cells causes the accumulation of ceramides and promotes α-Syn-induced cytotoxicity [49]. Moreover,

myriocin, a potent serine palmitoyltransferase inhibitor that suppresses the *de novo* synthesis of ceramide and galactosylceramide, significantly suppresses neurodegenerative phenotypes associated with α-Syn overexpression [8]. These data suggest a connection between the levels of α-Syn and sphingolipid metabolism. Interestingly, reducing Vps35 levels exacerbates α-Syn-induced locomotor impairment and promotes eye defects in flies [50], suggesting that the retromer complex plays a role in modulating the interactions between α-Syn and sphingolipid metabolism. Given that sphingolipids are elevated in both loss of Vps35 and gain of α-Syn, the synergism between both indicates yet again a link between ceramides and PD.

LRRK2

Dominant mutations in *LRRK2* are the most common cause of familial PD and are responsible for 1–2% of sporadic PD [51]. The most common *LRRK2* mutation is p.G2019S, which acts as a hyperactive kinase [52]. The function of LRRK2 is enigmatic as it has been implicated in a variety of pathways including mitochondrial dynamics [53], but most studies have centered on its potential role in vesicular trafficking and the endolysosomal pathway [54]. Specifically, LRRK2 may regulate autophagy via vesicle sorting and lysosomal motility along microtubules via its interaction with multiple Rab proteins including: Rab5, Rab7L1, Rab7, and Rab32 [55]. Ceramide is increased and GCase activity is decreased in Lrrk2 null mouse brains [56]. However, it is unclear if ceramide is dysregulated in an LRRK2 gain-of-function model.

VPS35

Heterozygous loss-of-function mutations in *VPS35* cause PD [57]. *VPS35* encodes a component of a trimeric cargo recognition complex critical to retromer function [57]. Loss of *Vps35* in flies or primary cultured neurons, or expression of VPS35 harboring the PD-associated variant, p.D620N, induces endolysosomal dysfunction [58,59]. Neuronal expression of $VPS35^{D620N}$ also causes lysosomal dysfunction [59] and results in mitochondrial defects [60], suggesting a connection between the retromer regulated endolysosomal pathway and some mitochondrial functions. Similarly, loss of *Vps26*, another key component of the retromer, leads to endolysosomal dysfunction and neurodegeneration [58]. The retromer complex is an important regulator of sphingolipid metabolism [61] and loss of Vps26 or Vps35 causes an accumulation of ceramides in degenerating neurons [8], suggesting that the accumulation of ceramides may also contribute to neurodegenerative phenotypes in PD VPS35 patients. In *Drosophila*, overexpression of Vps35 reduces locomotor defects and prolongs lifespan in *LRRK2* and *Parkin* models [62,63], implicating a role for retromer function in these mutants and raising the possibility that ceramide may be a key link between endolysosomal and mitochondrial function.

Recessive Forms of PD (*PINK1* and *PRKN*)

The two main recessive forms of PD caused by the loss of function of *PRKN* and *PINK1* are associated with early-onset and juvenile PD. The role for *PRKN* and *PINK1* in sphingolipid metabolism is not well established; however, their role in the cell has been linked to mitochondrial quality control and mitophagy [64]. Mitophagy requires dynamic membrane rearrangements that depend on ceramide metabolism [65]. What remains unclear is the role of specific ceramide species in this process. Lipidomic analysis of fibroblasts from patients with *PRKN* mutations display alterations in levels of phospholipids and glycosphingolipids [66]. These data suggest a connection between *PRKN* and *PINK1* and sphingolipid metabolism in PD pathogenesis. However, additional studies that explore these links are warranted.

Atypical Parkinsonism (*PLA2G6* and *ATP13A2*)

Alterations in retromer and endolysosomal trafficking and sphingolipid metabolism are also observed in *PLA2G6*- and *ATP13A2*-linked parkinsonism. Recessive variants in

ATP13A2 cause an LSD, Kufor–Rakeb syndrome, which is characterized by juvenile-onset parkinsonism, pyramidal signs, and cognitive decline [67]. ATP13A2 is a transmembrane lysosomal type 5 P-type ATPase [67]. Its cellular function remains to be characterized but overexpression of *ATP13A2* suppresses α-Syn toxicity in yeast and rescues dopaminergic cell loss caused by α-Syn overexpression in nematodes and rats [68]. Moreover, depletion of ATP13A2 leads to α-Syn accumulation, lysosomal dysfunction, and enhanced α-Syn induced toxicity [69]. Although no link has been established between ATP13A2 and sphingolipid metabolism, the above data suggest that it is worthwhile to assess sphingolipid levels in models of *ATP13A2* knockdown and/or overexpression.

A neuropathological hallmark in the *PLA2G6*-associated parkinsonism patients is the formation of spheroids throughout the nervous system [9]. These spheroids are comprised of tubulovesicular structures that often contain α-Syn and ubiquitin [70]. We recently showed

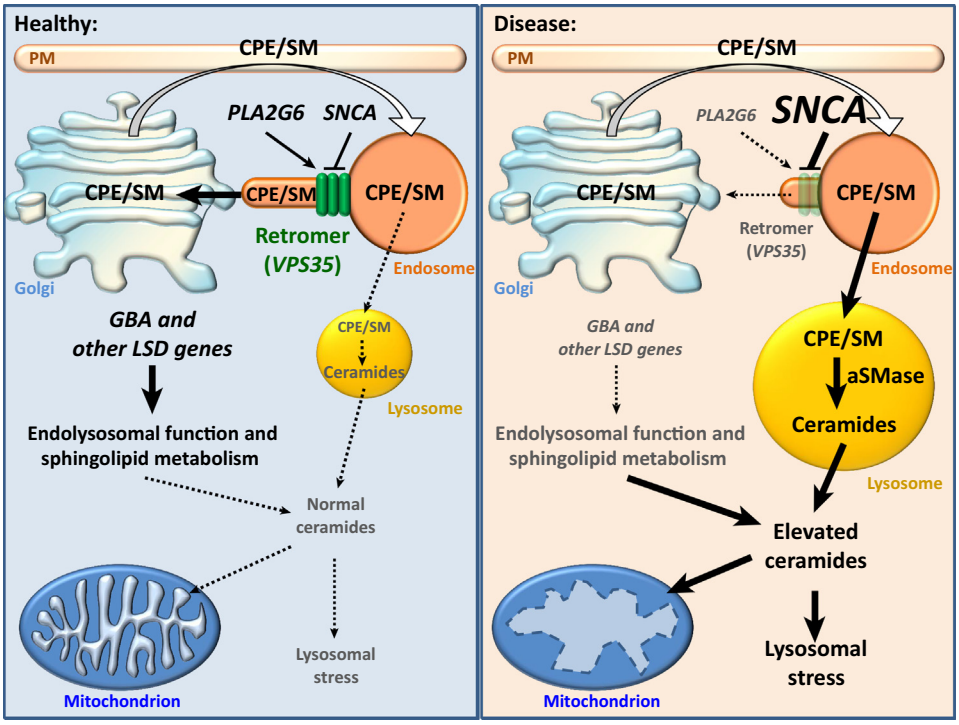

Figure 3. Proposed Model of Sphingolipid-Metabolism-Mediated Neurodegeneration Caused by Disruption of PD/Parkinsonism Genes. In the healthy condition, CPE/SM is endocytosed from the PM to endosomes and recycled through the retromer, which contains VPS35, to the Golgi. Disruption of PLA2G6, VPS35, or overexpression of α-synuclein reduces VPS35 levels, impairs retromer function and leads to disruption of recycling of CPE/SM. This in turn causes the transport of CPE/SM to the lysosomes where they are hydrolyzed to ceramide, which leads to its accumulation. The accumulated ceramide increases the stiffness of the membrane, causing broader effects to many organelles, including endolysosomes and mitochondria, resulting in neuronal demise. Many lysosomal storage disease genes, such as *GBA*, *SMPD1*, and *ASAH1* encode lysosomal proteins that regulate endolysosomal function and sphingolipid metabolism. Genetic variants associated with these genes have been recently identified as risk factors for PD. Hence, genetic defects of many PD/parkinsonism genes seem to affect a mechanism that converges on the sphingolipid metabolism pathway. Abbreviations: CPE, ceramide phosphoethanolamine; PD, Parkinson's disease; PM, plasma membrane; SM, sphingomyelin.

that loss of *PLA2G6* in flies or human cell lines causes neurodegeneration and cell death, respectively [8]. Loss of PLA2G6 leads to a defect in the stability of Vps35 and Vps26 and affects retromer function. The reduction in retromer function leads to impaired recycling of membrane sphingolipids during endocytosis, an overload of lysosomal function associated with a severe lysosomal expansion, and an accumulation of ceramides. We argue that the accumulated ceramides further impair the endolysosomal system by stiffening membranes and impairing retromer function even more. Indeed, pharmacologically, promoting retromer function with the retromer chaperone, R55 [71], potently alleviates the neurodegenerative phenotypes associated with *PLA2G6* loss [8]. Similarly, myriocin (a serine palmitoyltransferase inhibitor) or desipramine (an acidic sphingomyelinase inhibitor) also significantly ameliorates the neurodegenerative phenotypes observed upon loss of *PLA2G6*. These data highlight the possibility of targeting retromer-mediated sphingolipid metabolism pathways in PD/parkinsonism.

Finally, flies lacking *iPLA2-VIA* exhibit a progressive loss of mitochondrial function followed by severe morphological defects. These mutants exhibit a loss of complex I activity and a reduction of ATP levels [8,72]. Suppression of ceramide levels significantly restores complex I activity and ameliorates neurodegenerative phenotypes, suggesting that excess ceramide not only affects the endolysosomal pathway but also mitochondrial health. In summary, ceramide levels may serve as a convergence point for defects in endolysosomal and mitochondrial pathways.

Concluding Remarks

We argue that the retromer plays a critical role in the retrieval of lipids. In the absence of normal retrieval of membrane lipids from endosomes, too many lipids are shuttled to the lysosomes. This in turn leads to a dramatic expansion of the lysosomes and an increase in ceramides. We argue that elevated ceramides alter membrane stiffness, as documented in biophysical studies, further impairing retromer function. This leads to a positive feedback loop that exacerbates cellular defects (Figure 3). Our data dovetail with recent genetic studies indicating a strong association of LSD and PD/parkinsonism. Given that ceramide is widely distributed in cellular membranes, elevated ceramides may lead to a decrease in cardiolipins resulting in mitochondrial stress. To firmly establish whether sphingolipid metabolism is critical in PD pathogenesis, future studies will need to examine sphingolipid metabolism in multiple PD models (see Outstanding Questions). It is particularly important to determine the spatial (tissue/cell type) and temporal (preclinical/during disease) signature of sphingolipid changes. Additionally, drugs like myriocin and desipramine that can modulate this pathway should be tested across PD models. Finally, it is not obvious how our model fits with the selective vulnerability of nigral dopaminergic neurons seen in PD/parkinsonism. However, multiple studies have shown that these pacemaking neurons exhibit high mitochondrial energy requirements [73]. Could increased ceramide be limited to nigral neurons due to these intrinsic factors *in vivo*? In order to keep up with metabolic demands, lipid homeostasis, membrane dynamics, and trafficking may comprise a crucial pathway to keep dopaminergic mitochondria healthy, another hypothesis that remains to be tested.

Outstanding Questions

To what extent do the levels of sphingolipids correlate with the pathogenesis of PD/parkinsonism? This is a fundamental question remains to be answered. Accumulating evidence suggests that sphingolipid metabolism may play an important role in PD/parkinsonism. To answer this question, we need to systematically measure the levels of sphingolipids in patient derived samples (i.e., serum, fibroblasts) and across animal models for PD/parkinsonism.

Do defects in the endolysosomal pathway affect mitochondrial function? Dysfunction of both mitochondrial and endolysosomal pathways are commonly affected in PD/parkinsonism. However, the interaction between these pathways is not well established. Given that loss of retromer components in mice causes mitochondrial defects, a cross talk between retromer mediated endolysosomal pathway and mitochondrial dysfunction is likely. Moreover, do sphingolipids and their metabolites participate?

What are the other components of endolysosomal and mitochondrial signaling that regulate sphingolipid metabolism and do they contribute to the pathogenesis of PD/parkinsonism?

How do elevated levels of ceramides affect lysosomal and mitochondrial functions? Elevated ceramide has been detected in both patients and animal models of PD/parkinsonism. We propose that increased ceramide stiffens membranes, impairing the flexibility of the membrane and membrane bending. This argument is supported by *in vitro* experiments using reconstituted membranes with different concentrations of sphingolipids including ceramide and sphingomyelin. However, the concentration of sphingolipids in membranes should be established during the course of the disease.

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