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Lysosomal trafficking defects link Parkinson's disease with Gaucher's disease

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Abstract

Lysosomal dysfunction has been implicated in multiple diseases including lysosomal storage disorders such as Gaucher's disease, in which loss-of-function mutations in the *GBA1* gene encoding the lysosomal hydrolase β-glucocerebrosidase (GCase) result in lipid substrate accumulation. In Parkinson's disease, α-synuclein accumulates in Lewy bodies and neurites contributing to neuronal death. Previous clinical and genetic evidence has demonstrated an important link between Parkinson's and Gaucher's disease, as *GBA1* mutations and variants increase the risk of Parkinson's, and Parkinson's patients exhibit decreased GCase activity. Using human midbrain neuron cultures, we have found that loss of GCase activity promotes α-synuclein accumulation and toxicity, while α-synuclein accumulation further contributes to decreased lysosomal GCase activity by disrupting GCase trafficking to lysosomes. Moreover, α-synuclein accumulation disrupts trafficking of additional lysosomal hydrolases, further contributing to lysosomal dysfunction and neuronal dyshomeostasis. Importantly, promoting GCase activity reduces α-synuclein accumulation and rescues lysosomal and neuronal dysfunction, suggesting that GCase may be an important therapeutic target for advancing drug discovery in synucleinopathies including Parkinson's disease.

Keywords

Parkinson's disease; Gaucher's disease; α-synuclein; β-glucocerebrosidase; LIMP-2

Introduction

Studying the cellular mechanisms and pathways disrupted in disease is critical for identifying therapeutic targets to prevent disease onset or slow disease progression. Unfortunately, in multiple neurodegenerative diseases including Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), Huntington's disease and Parkinson's disease, our understanding of the cellular pathways which lead to neuronal dysfunction and death is limited, resulting in a lack of validated therapeutic targets for drug discovery.

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Recent clinical and genetic evidence has demonstrated an important epidemiological link between Parkinson's disease and Gaucher's disease. In addition, results from our lab and others have identified important mechanistic interplays between α-synuclein in Parkinson's disease and β-glucocerebrosidase (GCase) in Gaucher's disease, identifying GCase as a potentially effective therapeutic target for Parkinson's disease drug discovery.

In this Scientific Perspective, we discuss recent work demonstrating the cellular interplay between α-synuclein and GCase in the context of lysosomal trafficking. We first summarize the epidemiological link between Parkinson's and Gaucher's disease and discuss previous work on vesicle trafficking defects associated with α-synuclein toxicity. We then discuss work from our lab demonstrating an important bidirectional loop between α-synuclein and the lysosomal hydrolase GCase: namely, that 1) loss of GCase activity promotes α-synuclein accumulation via accumulation of its lipid substrate GlcCer, and 2) α-synuclein accumulation further contributes to decreased GCase activity by disrupting GCase trafficking to lysosomes. Next, we summarize our recent findings that α-synuclein accumulation additionally disrupts trafficking of other lysosomal hydrolases further contributing to overall lysosomal dysfunction. Finally, we address the use of GCase activation as a potential therapeutic target for synucleinopathies including Parkinson's disease.

The Epidemiological Link: Parkinson's and Gaucher's disease

α-Synuclein in Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), resulting in the onset of clinical parkinsonian symptoms including bradykinesia, resting tremors, muscular rigidity, and postural instability[1]. Although the majority of patients do not have a family history of PD, genetic screening of familial PD cases has provided important insight into the pathophysiology of PD.

α-synuclein (*SNCA*) was the first gene linked to familial PD[2], with subsequent studies identifying additional point mutations in the N-terminus of α-synuclein as causative for familial autosomal dominant PD[3–7] (Fig. 1A). α-synuclein was also found to accumulate in Lewy bodies and neurites in the brain, spinal cord and peripheral nervous system of sporadic PD patients[8], demonstrating a critical role for α-synuclein in both sporadic and familial PD. Both *SNCA* locus duplication and triplication also cause familial PD[9, 10], and importantly, patients with *SNCA* triplication display earlier PD onset by several decades as compared to patients with *SNCA* duplication or sporadic PD[1], revealing a dose-dependent toxicity from increased α-synuclein levels. Moreover, increased α-synuclein expression due to a risk variant in a non-coding distal enhancer element in *SNCA* was recently found to be associated with PD, further establishing a role for increased α-synuclein levels in PD pathogenesis[11].

Subsequent studies have identified other genes linked to PD, including LRRK2[12, 13], VPS35[14, 15] and the recently identified TMEM230[16] linked to autosomal dominant PD, while parkin[17], DJ-1[18], PINK1[19] and PARK9/ATP13A2[20] have been linked to autosomal

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recessive PD. In addition, various genome-wide associate studies (GWAS) have identified additional risk variants for PD in at least 24 different loci[21, 22], including tau (*MAPT*)[23] and α-synuclein (*SNCA*)[24]. Interestingly, α-synuclein also accumulates in other synucleinopathies including multiple system atrophy (MSA) and dementia with Lewy bodies (DLB)[25, 26].

GCase in Gaucher's disease

Gaucher's disease is an autosomal recessive genetic lysosomal storage disorder (LSD) caused by homozygous loss-of-function mutations in *GBA1* encoding β-glucocerebrosidase (GCase) (Fig. 1B). More than 300 mutations in *GBA1* have now been identified in Gaucher's disease, with the N370S as the most common mutation within the Ashkenazi Jewish population[27]. Gaucher's disease is a systemic disease which commonly results in thrombocytopenia, anemia, hepatosplenomegaly, and bone pain with disease onset at adulthood. However, in some cases, it presents as a more severe, predominantly neuronopathic form involving seizures, cognitive impairment, and oculomotor problems, with death in early childhood[27].

GCase is a lysosomal hydrolase which converts glucosylceramide (GlcCer) into ceramide and glucose by cleaving the β-glucosyl linkage of GlcCer[28] (Fig. 1C). GCase targeting to lysosomes is mediated via the lysosomal integral membrane protein type-2 (LIMP-2), independently of mannose-6 phosphate[29]. Although GlcCer accumulates in all forms of Gaucher's disease, it is still unclear what causes the variability across patients with different forms of Gaucher's disease.

Epidemiological Links Between Parkinson's and Gaucher's Disease

The link between PD and Gaucher's disease was initially observed when a subset of Gaucher's patients were found to have parkinsonian symptoms and α-synuclein-positive Lewy body pathology typical of synucleinopathies[30–33]. In addition, Gaucher patients with parkinsonism often had relatives with parkinsonism who were heterozygous for *GBA1* mutations (Gaucher carriers)[34]. Subsequent genetic studies in large cohorts found that PD patients with *GBA1* mutations presented earlier with the disease, and were more likely to have relatives affected with parkinsonism[35, 36]. *GBA1* mutations are now considered one of the most common risk factors for PD, with recent GWAS studies of PD patients further confirming the identification of risk variants within the *GBA1* loci[21]. In addition, mutations in *GBA1* have also been linked to patients with the synucleinopathy DLB[37] and *GBA1* mutations may play an even greater role in DLB etiology than PD[38], further suggesting an important link between α-synuclein toxicity and loss of GCase function. Interestingly, variants in the LIMP-2 gene (*SCARB2*) have also been associated with increased risk for PD[39], suggesting that defective lysosomal trafficking of GCase may contribute to PD pathogenesis. More recently, reduced wildtype GCase activity has been observed along with accumulation of GCase substrates in blood and postmortem brains of sporadic PD patients[40–42], raising the possibility that α-synuclein accumulation in sporadic PD can also influence GCase lysosomal activity.

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Lysosomal Trafficking Defects: Linking α-Synuclein with GCase

Background: Vesicle Trafficking Defects in α -Synuclein Toxicity

α-synuclein was first found to disrupt vesicle trafficking in yeast, as overexpression of either wildtype or the PD-linked mutant A53T α-synuclein perturbed vesicle distribution[43]. Subsequently, overexpression of wildtype α-synuclein was observed to cause cell death, increase endoplasmic reticulum (ER) stress and impair ER-associated degradation, as well as inhibit ER to Golgi trafficking of a soluble misfolded substrate (CPY) and alkaline phosphatase (ALP)[44]. A genome-wide screen in yeast to identify suppressors and enhancer of α-synuclein toxicity found that the most effective class of suppressors were involved in vesicle-mediated membrane trafficking including the Rab GTPase Ypt1p/Rab1. Importantly, overexpression of Rab1 was able to rescue dopaminergic neuron death in *Drosophila* expressing either wildtype or A53T α-synuclein, as well as reduce neurodegeneration in a *C. elegans* model expressing α-synuclein in dopaminergic neurons[44]. In addition, expression of Rab1 in rat primary midbrain neurons expressing A53T α-synuclein improved neuronal viability in culture[44], demonstrating that rescuing transport of ER-derived vesicles through Rab1 suppressed α-synuclein toxicity.

Subsequent work using a reconstituted cell-free assay demonstrated that addition of wildtype or A53T α-synuclein inhibited ER to Golgi transport, and showed that α-synuclein expression led to a time and dosage-dependent accumulation of transport vesicles by electron microscopy (EM) and fluorescence microscopy[45]. Expression of GFP-tagged α-synuclein showed that it localized to the plasma membrane at early time points, and subsequently localized to cytoplasmic inclusions which were abolished upon Ypt1 overexpression[45]. In addition, α-synuclein foci were found to colocalize with various Rabs including Ypt1[45]. Indeed, Rab3A and Rab8A overexpression also protected against dopaminergic cell loss in a *C. elegans* model expressing wildtype α-synuclein in dopaminergic neurons and in rat primary midbrain neurons expressing A53T α-synuclein[45], suggesting that multiple Rabs may be involved in α-synuclein toxicity.

Additional studies have found that α-synuclein expression disrupts the ER to Golgi trafficking of other cargo including the dopamine transport (DAT), leading to a reduction in the function and cell surface expression of DAT, and a reduced immobile DAT fraction[46]. α-synuclein overexpression also disrupts the ER to Golgi trafficking of the autophagy protein Atg9 which may contribute to defective autophagy, and which is rescued by Rab1a overexpression[47]. One mechanism through which α-synuclein may disrupt ER to Golgi transport is through disruption of ER/Golgi SNAREs, as α-synuclein has previously been shown to interact with the V-SNARE VAMP2 to regulate SNARE complex formation on synaptic vesicles[48]. Indeed, overexpression of wildtype or A53T α-synuclein disrupted protein trafficking, which was rescued by co-overexpression of ER/Golgi R-SNAREs[49]. In particular, expression of the ER/Golgi SNARE Ykt6 effectively rescued protein trafficking, while purified A53T α-synuclein inhibited COPII vesicle docking and fusion, and was able to directly bind ER/Golgi SNAREs to inhibit SNARE complex assembly[49].

Further screens to identify modifiers of α-synuclein toxicity also identified the compound NAB2 (N-Aryl Benzimidazole) which acts on the E3 ubiquitin ligase Rsp/Nedd4 to promote

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ubiquitin-mediated endosomal transport[50]. In yeast, NAB2 treatment rescued α-synuclein's disruption of bulk endosomal transport from the plasma membrane to the vacuole. Furthermore, NAB2 treatment in cortical neurons generated from induced pluripotent stem (iPS) cells from PD patients harboring α-synuclein mutations prevented defective ER to Golgi trafficking of multiple substrates[51], suggesting that defects in vesicle trafficking are a key component of α-synuclein cellular dyshomeostasis, and that targeting vesicle trafficking may be beneficial in PD.

Part 1: GCase Deficiency Promotes α-Synuclein Toxicity

As loss-of-function GCase mutations are associated with α-synuclein accumulation in a subset of Gaucher disease patient brains, and ER to Golgi vesicle trafficking defects have been implicated in α-synuclein toxicity, our lab investigated the cellular interplay between α-synuclein and GCase and whether defective vesicle trafficking due to α-synuclein toxicity might play a role in their cellular interaction (Fig. 2A).

We began by examining the role of GCase depletion on α-synuclein homeostasis, and found that GCase depletion in neurons significantly decreased the rate of overall cellular proteolysis, which was not further inhibited by lysosomal inhibitors, demonstrating that GCase depletion affected a lysosomal-mediated pathway[52]. Moreover, GCase depletion increased the steady-state protein levels of α-synuclein, but did not affect α-synuclein mRNA levels. These results were further validated in dopaminergic neurons generated from iPS cells derived from skin fibroblasts of a Gaucher's disease patient harboring a GCase (N370S/84GG insertion) mutation which showed reduced GCase protein levels and activity, concomitant with a dramatic increase in α-synuclein protein levels[52]. Importantly, huntingtin and tau protein levels showed little or no change, demonstrating that loss of GCase activity from endogenous Gaucher's mutations affected lysosomal proteolysis resulting in the preferential accumulation of α-synuclein.

Moreover, GCase depletion reduced neuronal viability in neurons expressing human wildtype α-synuclein or mutant A53T α-synuclein[52]. In contrast, no toxicity was observed upon expression of an artificial fibrilization-incompetent mutant (71–82 α-synuclein)[53], suggesting that GCase depletion promoted α-synuclein neurotoxicity through a fibrilization-dependent manner. GCase depletion additionally resulted in the formation of high-molecular-weight (HMW) α-synuclein assemblies with a molecular radius of 64–95 Å. Importantly, lysosomal inhibition with leupeptin did not enhance α-synuclein-mediated neurotoxicity or generate soluble HMW α-synuclein species[52], demonstrating that alterations in the GCase metabolic pathway, rather than a general lysosomal inhibition, influenced α-synuclein toxicity and stabilization of soluble HMW species.

Inhibition of GCase activity is known to result in the accumulation of its lipid substrate GlcCer. We found that while GlcCer accumulation had no effect on α-synuclein *in vitro* fibril formation at physiological conditions, increasing the amount of GlcCer to 75% while keeping the total lipid amount constant (PC25/GlcCer75) altered the kinetic profile of α-synuclein fibril formation under acidic conditions which mimicked lysosomal conditions[52]. Under these conditions, the levels of HMW oligomeric α-synuclein and aggregation-prone conformational α-synuclein intermediates increased, suggesting that GlcCer altered the

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conformation of α-synuclein to increase solvent-exposed hydrophobic regions. Importantly, other sphingolipids did not alter the levels of soluble α-synuclein oligomers, indicating a specific effect by GlcCer on α-synuclein fibrilization. Misfolded α-synuclein was also observed to localize to GlcCer tubular structures by immuno-EM[52]. Thus, these results suggested that GlcCer stabilized the formation of a soluble assembly-competent intermediate α-synuclein species during the lag phase of the fibril formation reaction.

The effects of GCase depletion on α-synuclein accumulation and toxicity were further confirmed in multiple *in vivo* Gaucher's disease models. In a well-established Gaucher's mouse model (4L/PS-NA)[54], eosinophilic spheroids were observed in multiple brain regions including the substantia nigra and cortex, suggesting the presence of degenerating neurons, as compared to in wildtype mice[52]. These degenerative changes occurred concomitantly with increased levels of α-synuclein in these regions. Moreover, α-synuclein accumulated in the form of punctate structures in Gaucher's mice, while wildtype mice showed a normal neuropil α-synuclein staining pattern. Of note, α-synuclein accumulations were also observed in other neural regions including cerebellum, hippocampus, and brainstem in Gaucher's mice[52]. Increased levels of Triton-soluble, putative oligomeric forms and aggregated species of α-synuclein were also observed in 4L/PS-NA mice compared to wildtype mice. In a second well-characterized Gaucher's mouse model with a GCase D409H loss-of-function mutation[55], similar increases in α-synuclein punctate structures were observed in addition to higher levels of soluble oligomers and insoluble α-synuclein species. Finally, in a well-established *C. elegans* model of GCase depletion, α-synuclein was also observed to accumulate, demonstrating that GCase depletion promoted the formation of soluble oligomeric and insoluble α-syn *in vivo*[52].

These observations were further replicated in human brain samples. Brain lysate from a Gaucher's patient with atypical parkinsonism[56] demonstrated a dramatic increase in α-synuclein levels[52]. In addition, elevated levels of α-synuclein oligomers were observed in patients that were homozygous or heterozygous for GCase mutations with a diagnosis of DLB, and in infants diagnosed with neuronopathic GD, concomitant with lower GCase protein and activity levels[52]. Finally, both homozygote and heterozygote carriers of *GBA1* mutations with a neuronopathic phenotype contained significantly higher levels of pathological α-synuclein oligomers[57] compared to controls[52], further demonstrating that toxic oligomeric α-synuclein was elevated in patients harboring *GBA1* mutations and was preferentially associated with neuronopathic forms of Gaucher's disease. Thus, these initial observations demonstrated *in vitro, in vivo*, and in patient brain tissue that loss of GCase activity resulted in the accumulation and stabilization of oligomeric α-synuclein via GlcCer substrate accumulation.

Part 2: α-Synuclein Accumulation Disrupts Trafficking of GCase

We subsequently investigated the effect of α-synuclein accumulation on GCase activity as α-synuclein toxicity has been previously linked to defective ER to Golgi trafficking of various cargo. Indeed, we found that overexpression of human wildtype and A53T mutant α-synuclein in primary cortical neurons disrupted GCase trafficking from the ER, resulting in an altered post-ER/ER GCase ratio with an accumulation of the immature ER form and a

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decrease in the post-ER forms[52]. In contrast, the fibrilization-incompetent mutant 71–82 α-synuclein did not affect GCase trafficking. Moreover, in lysosomal fractions from primary neuronal cultures, expression of both wildtype and A53T α-synuclein resulted in a significant decrease in GCase lysosomal activity and a concomitant increase in the microsome-enriched fraction activity, while expression of 71–82 α-synuclein did not affect GCase lysosomal activity[52].

These findings were further confirmed using human brain tissue from elderly healthy controls without common *GBA1* mutations. While all samples appeared to have similar levels of post-ER GCase, samples with lower α-synuclein level contained much less of the ER form, while microsome-enriched fractions from samples with higher α-synuclein levels showed increased GCase activity[52], suggesting that normal variation of α-synuclein protein levels was able to modulate lysosomal maturation and activity of GCase *in vivo*. In addition, both total GCase protein levels in Triton-soluble lysates and lysosomal GCase activity from the cingulate cortex of PD brain were dramatically decreased compared to age- and postmortem time-matched controls[52], further demonstrating that elevated levels of α-synuclein in PD led to decreased lysosomal activity of normal GCase, which could in turn contribute to further propagation and stabilization of oligomeric α-syn. Thus, our results demonstrate that elevated levels of toxic α-synuclein species lead to depletion of lysosomal GCase and this could be further exacerbated by the stabilization of α-synuclein oligomers by GlcCer accumulation upon GCase depletion (Fig. 2A). Importantly, these results suggest that targeting GCase may be an effective therapeutic target to modulate α-synuclein oligomerization and toxicity.

Part 3: α-Synuclein Disrupts Lysosomal Function via Hydrolase Trafficking Dysfunction

Lysosomal dysfunction has been implicated in multiple diseases including lysosomal storage disorders such as Gaucher's disease. Our recent data demonstrates that α-synuclein accumulation in synucleinopathies also contributes to lysosomal dysfunction by impairing vesicular trafficking of lysosomal hydrolase during the early secretory pathway, resulting in reduced lysosomal function[58] (Fig. 2A). To examine the interaction between α-synuclein, lysosomes, and trafficking components, human midbrain synucleinopathy dopaminergic neurons generated by lentiviral overexpression of α-synuclein in healthy control iPS neuronal lines, or through the generation of patient lines harboring PD-causing α-synuclein (*SNCA*) triplication were used. Importantly, this stable neuronal model allowed us to culture patient neurons for several hundred days and to validate pathogenic changes in the lysosomal system in the context of naturally occurring mutations leading to α-synuclein accumulation[58].

We found that α-synuclein was localized to synapses in control and patient neurons, but began accumulating at the cell body of *SNCA* triplication neurons at day 60[58]. Moreover, amyloidogenic α-synuclein inclusions were observed in cell bodies and neurites by day 90, and soluble oligomeric and insoluble α-synuclein species accumulated which persisted till day 330[58], demonstrating that this neuronal model recapitulated key pathological features found in the PD brain. When neurons were examined for changes in lysosomal proteolysis, lenti-wildtype-α-synuclein–infected midbrain dopaminergic neurons showed a decline in

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long-lived proteolysis rates compared to control neurons expressing the fibrilization-incompetent α-synuclein 71–82 mutant[58], demonstrating a defect in the lysosomal system. In addition, proteolysis rates were decreased in *SNCA* triplication midbrain neurons at day 180 compared to control lines, while midbrain neurons from Gaucher patients began to show reduced proteolysis at an even earlier stage (day 110). Consistent with lysosomal dysfunction, increased lysosomal mass was observed in both PD and Gaucher patient neurons, and was partially rescued by lenti-shRNA knockdown of α-synuclein in both PD and Gaucher lines[58], demonstrating that α-synuclein accumulation contributed to lysosomal dysfunction, but could be partially reversed upon α-synuclein reduction.

Interestingly, the activity of multiple lysosomal hydrolases were defective in both PD and Gaucher patient neurons as compared to control neurons, including lysosomal cathepsin B activity, but were rescued upon reduction of α-synuclein by shRNA knockdown[58]. In addition, other non-protein-degrading lysosomal enzymes, including GCase, β-galactosidase (β-gal), and hexosaminidase (Hex) showed decreased lysosomal activity in PD and GD neurons, while their total cellular activity were not changed. Idiopathic PD neurons also demonstrated a reduction in GCase activity specifically within acidic subcellular compartments, while neurons expressing 71–82 mutant α-synuclein or aggregation-prone poly-Q-huntingtin had a minimal effect on lysosomal GCase activity. Gaucher lines also exhibited reduced GCase activity as expected, but additionally demonstrated decreased activity of other lysosomal hydrolases, including β-gal, Hex, and sulfatase[58].

Importantly, the decline in lysosomal enzymatic activity was sufficient to induce substrate accumulation in patient neurons[58]. Quantitative analysis of GCase substrates, including total hexosylceramide and hexosylsphingosine species revealed a dramatic increase in both PD *SNCA* triplication and Gaucher neurons. In addition, both glucosyl and galactosylceramide were significantly elevated in PD neurons, whereas ceramide levels were decreased. In contrast, levels of dihydroceramide, a nonlysosomal lipid species, were not altered[58], demonstrating that chronic lysosomal hydrolase deficiency in PD midbrain neurons induced lipid accumulation due to α-synuclein accumulation.

This deficiency in lysosomal function was subsequently found to be due to dysfunctional trafficking of lysosomal enzymes from the ER. Control midbrain neurons infected with lenti-WT α-synuclein showed reduced ratios of mature to immature hydrolases including Hex A and B, demonstrating a decline in enzyme maturation[58]. In addition, lysosomal enzymes accumulated in pre-Golgi COPII endoplasmic reticulum (ER)-to-Golgi transport vesicles in cells expressing high levels of α-synuclein, which was reversible upon reduction of α-synuclein expression. Moreover, maturation of GCase, β-gal, iduronate-2-sulfatase, and Hex A were reduced in extracts from patient neurons which was similarly reversible upon lenti-mediated knockdown of α-synuclein. Importantly, hydrolase maturation was not altered by treatment with the lysosomal inhibitor Bafilomycin-A1, indicating a specific trafficking defect induced by α-synuclein[58]. In addition, patient neurons which showed α-synuclein accumulation in the cell body demonstrated aberrant colocalization of α-synuclein with vesicle-tethering factor GM130 within fragmented, vesicular Golgi structures, which was not observed in control neuron[58], suggesting that α-synuclein disrupted hydrolase trafficking at the cis-Golgi level.

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The GTPase Rab1a which regulates ER to Golgi trafficking has previously been shown to rescue α-synuclein–induced neurodegeneration[44]. Although Rab1a levels were not altered between control and patient neurons and α-synuclein did not interact with Rab1a, α-synuclein accumulation in patient lines altered Rab1a localization from its normal perinuclear ER-Golgi localization to a more diffuse pattern[58]. In addition, lenti-Rab1a expression restored Golgi structure and improved lysosomal hydrolase maturation and activity in both lenti-α-synuclein infected control lines and patient lines at early time points. Furthermore, at later time points, Rab1a overexpression reduced α-synuclein accumulation within cell bodies of *SNCA* triplication neurons due to lysosomal enhancement, and successfully improved neuronal viability in neurons derived from three distinct synucleinopathy patients (*SNCA* triplication, Gaucher's disease, and idiopathic PD)[58]. Thus, these results demonstrate that α-synuclein accumulation disrupts overall lysosomal function by perturbing lysosomal hydrolase trafficking at the early secretory pathway in multiple patient neuronal models of synucleinopathies, further contributing to neuronal dyshomeostasis (Fig. 2A).

As Rab1a promotes vesicle tethering at the cis-Golgi, Rab1a overexpression may rescue neuronal viability by increasing vesicle-fusing opportunities at acceptor membranes, thus contributing to enhanced lysosomal hydrolase trafficking in patient neurons. In addition, this increased trafficking of lysosomal enzymes to lysosomes may help restore lysosomal function, and further help to reduce α-synuclein accumulation through chaperone mediated autophagy or macroautophagy-mediated degradation of α-synuclein[59–61]. Moreover, Rab1a may help decrease GlcCer substrate levels by restoring GCase activity, resulting in the reduced ability for GlcCer to stabilize α-synuclein oligomers[52]. Importantly, therapies which enhance the trafficking machinery of lysosomal hydrolases may thus prove beneficial across synucleinopathies in which α-synuclein accumulates.

Therapeutic Implications: Targeting GCase Activity in Synucleinopathies

Multiple studies from our group and others have now demonstrated that α-synuclein accumulation contributes to decreased lysosomal activity and trafficking of wildtype GCase[40, 51, 52, 58, 62]. Indeed, midbrain neurons from PD *SNCA* triplication patients expressing wildtype *GBA1* show reduced lysosomal GCase activity, which is reversible upon shRNA-mediated α-synuclein knock-down[58]. Moreover, wildtype GCase activity is decreased in blood samples and postmortem brains of sporadic PD patients[40, 41] and GCase substrates accumulate in certain regions of synucleinopathy brain expressing wildtype *GBA1*[42]. Thus, GCase substrates can accumulate in neurons with either mutant or wildtype GCase, and reducing these substrates may be beneficial to both idiopathic and familial PD patients regardless of whether they harbor *GBA1* mutations.

In addition, numerous studies have convincingly demonstrated that GCase depletion or loss-of-function *GBA1* mutations lead to α-synuclein accumulation[52, 58, 63–67], although toxic gain-of-function mechanisms may additionally contribute to α-synuclein toxicity[68]. Of note, reducing GCase trafficking by loss of LIMP-2 also results in α-synuclein accumulation and neurotoxicity in dopaminergic neurons[69], while LIMP-2 derived peptides which activate endogenous lysosomal GCase are able to reduce α-synuclein levels[70]. Various studies in

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both cell and animal models have also shown that reducing GCase activity promotes α-synuclein aggregation[63, 67, 71–74]. Most importantly, multiple epidemiological studies have now demonstrated a link between loss-of-function *GBA1* mutations and PD and DLB[75].

Thus, promoting GCase lysosomal activity offers an obvious therapeutic target for decreasing α-synuclein accumulation and toxicity across synucleinopathies (Fig. 2B). Indeed, recent studies in synucleinopathy mouse models have demonstrated that increasing GCase activity by virus-mediated overexpression of GCase reduces α-synuclein levels, improves cognitive behavior and is protective from loss of striatal dopaminergic markers[76–79].

We recently demonstrated that a non-inhibitory small molecule modulator of GCase NCGC00188758 (758)[80] was capable of increasing GCase activity and decreasing α-synuclein accumulation and toxicity in human midbrain neuron cultures[81]. Treatment with 758 preferentially activated GCase lysosomal activity, but had no effect on its non-lysosomal activity in human synucleinopathy midbrain dopaminergic neurons from patients with *GBA1* gene mutations or PD *SNCA* triplication[81]*.* Moreover, 758 treatment was able to reduce lysosomal GluCer levels and whole-cell hexosylsphingosine levels in *SNCA* triplication neurons. Importantly, treatment with 758 reduced the accumulation of α-synuclein across multiple synucleinopathy lines, including *SNCA* triplication lines, *GBA1* mutant lines, idiopathic PD lines, a PD-linked *PARK9/ATP13A2* mutant line[82] and a familial PD A53T α-synuclein mutant line[83]. Treatment with 758 also reduced the accumulation of amyloidogenic Thioflavin S-positive α-synuclein in cell bodies and neurites, and rescued α-synuclein's proper synaptic localization in human dopaminergic neurons[81].

Finally, as α-synuclein disrupts lysosomal function due to defective lysosomal hydrolase trafficking[58], 758 treatment was also able to improve hydrolase trafficking of both GCase and lysosomal Hex B, and restored the lysosomal activity of Hex and β-gal in synucleinopathy patient neurons, but had no effect in control neurons[81]. Importantly, 758 treatment improved proteolysis and rescued neuronal viability in *SNCA* triplication neurons[81], further demonstrating that activation of GCase by non-inhibitory small molecules can rescue pathological α-synuclein toxicity in human neurons. Indeed, another non-inhibitory GCase chaperone NCGC607 also restores GCase activity and reduces α-synuclein levels in human iPS neurons[84]. Ultimately, the work from our lab and others strongly suggests that activating GCase in human patients will be an effective therapeutic target for decreasing α-synuclein toxicity and may be greatly beneficial for accelerating drug discoveries in synucleinopathies including Parkinson's disease.

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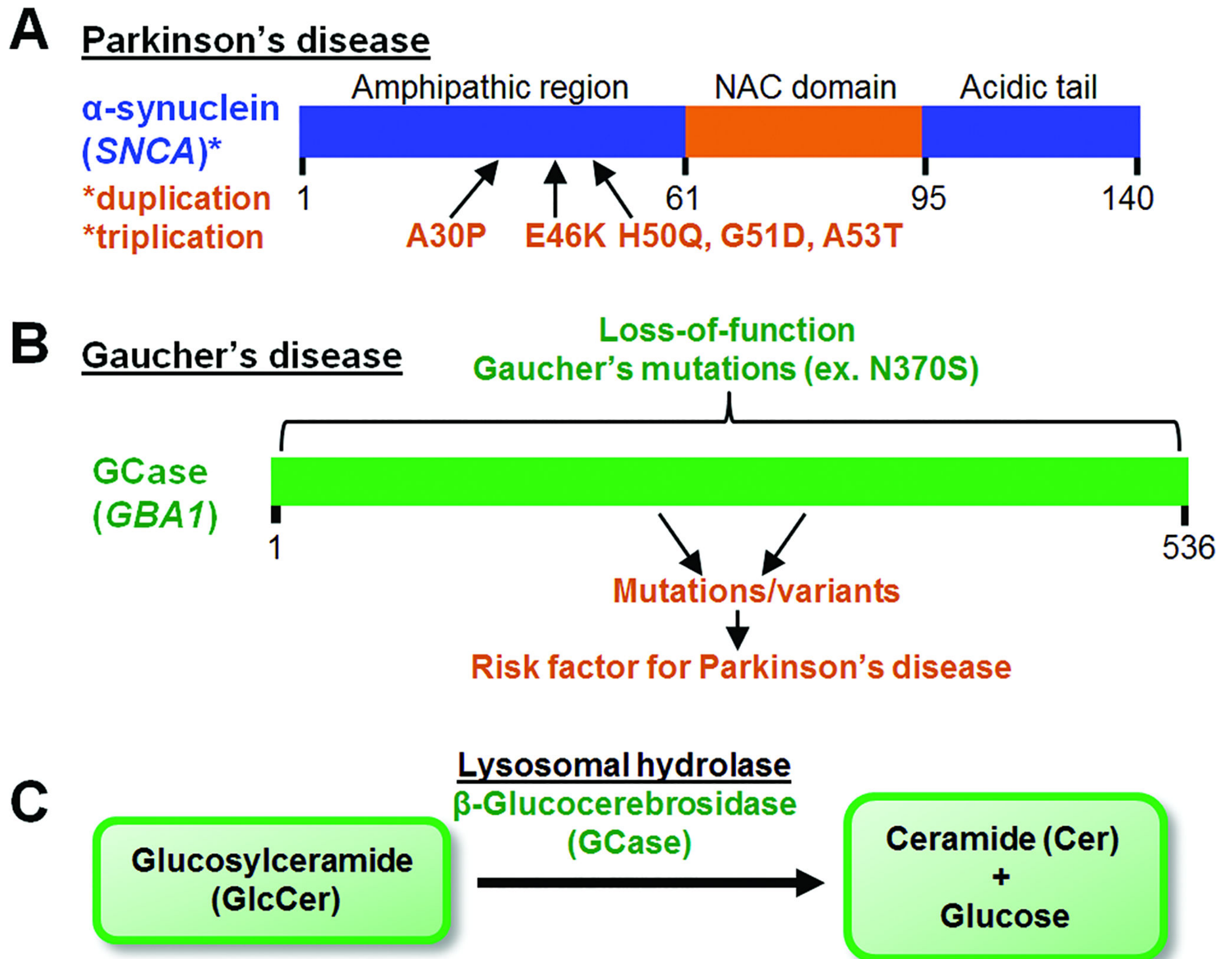

Fig. 1. α-synuclein in Parkinson's and GCase in Gaucher's disease

(A) α-synuclein accumulates in both sporadic and familial forms of Parkinson's disease. N-terminal mutations in α-synuclein (*SNCA*) and *SNCA* duplication/triplication lead to familial PD. The NAC domain of α-synuclein mediates its fibrilization. **(B)** Loss-of-function mutations in the *GBA1* gene encoding β-glucocerebrosidase (GCase) lead to Gaucher's disease. Variants in *GBA1* are one of the most common risk factors for Parkinson's disease. **(C)** GCase is a lysosomal hydrolase which converts glucosylceramide (GlcCer) into ceramide and glucose by cleaving the β-glucosyl linkage of GlcCer. Loss of GCase activity results in GlcCer substrate accumulation.

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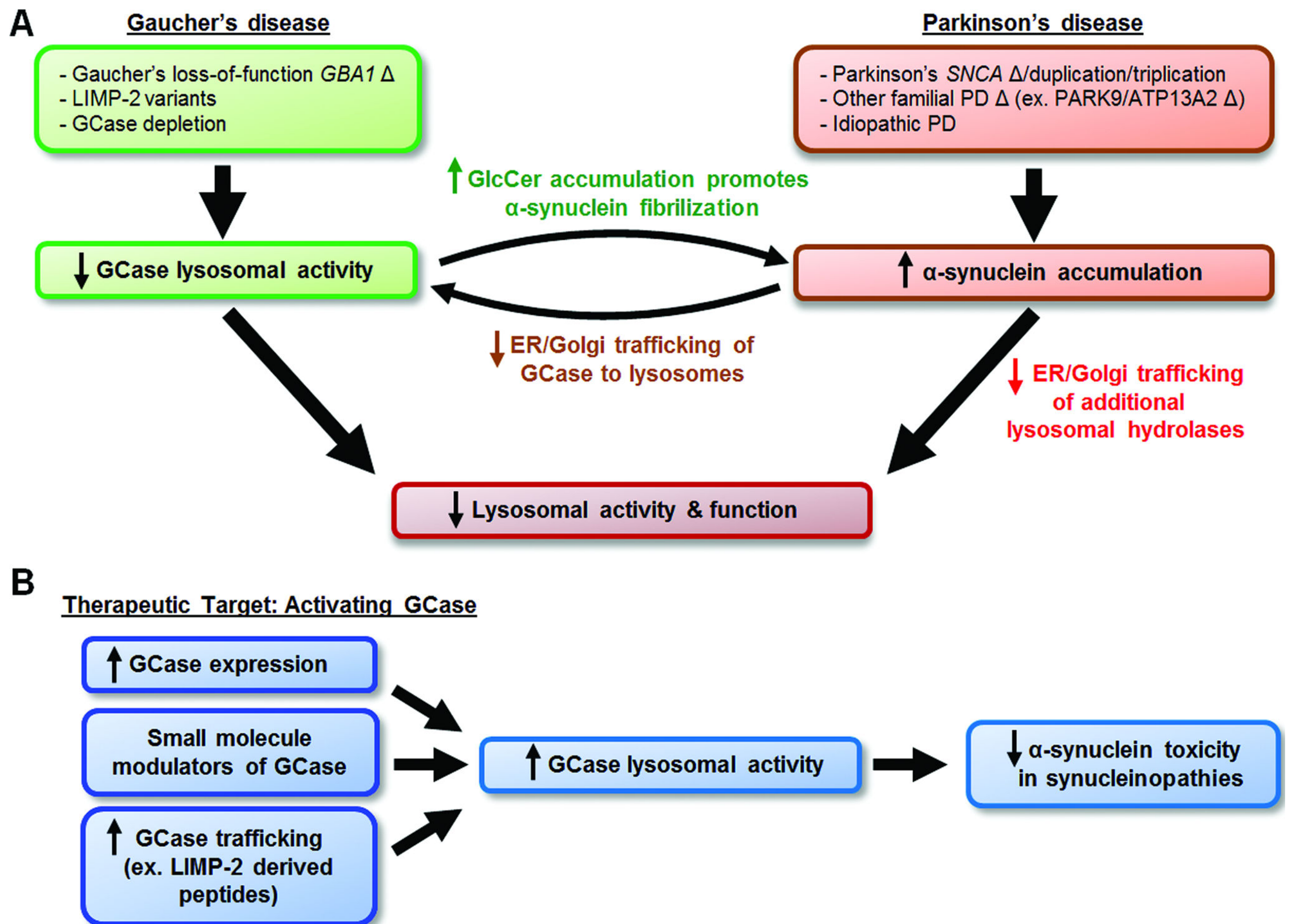

Fig. 2. Lysosomal dysfunction links Parkinson's and Gaucher's disease

(A) Gaucher's disease and Parkinson's disease are linked via a bidirectional loop between α-synuclein and the lysosomal hydrolase GCase, in which 1) loss of GCase activity disrupts lysosomal function and also promotes α-synuclein accumulation and fibrilization via accumulation of its lipid substrate GlcCer, while 2) α-synuclein accumulation further contributes to decreased GCase lysosomal activity by disrupting GCase trafficking to lysosomes. α-synuclein accumulation further disrupts the ER/Golgi trafficking of additional lysosomal hydrolases including cathepsin B, β-galactosidase, and hexosaminidase, resulting in reduced lysosomal enzymatic activity and overall lysosomal dysfunction. Additional factors including loss-of-function Gaucher mutations in *GBA1* contribute to decreased GCase lysosomal function, while multiple factors including familial Parkinson's mutations contribute to α-synuclein accumulation and toxicity. **(B)** Activation of lysosomal GCase activity may be an effective therapeutic for decreasing α-synuclein accumulation and toxicity across synucleinopathies including Parkinson's disease.