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Dysregulation of the autophagic-lysosomal pathway in Gaucher and Parkinson's disease

Caleb Pitcairn[1], **Willayat Yousuf Wani**[1], and **Joseph R. Mazzulli**[1,*]

[1]The Ken and Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, Chicago IL 60611 USA

Abstract

The finding that mutations in the Gaucher's Disease (GD) gene *GBA1* are a strong risk factor for Parkinson's Disease (PD) has allowed for unique insights into pathophysiology centered on disruption of the autophagic-lysosomal pathway. Protein aggregations in the form of Lewy bodies and the effects of canonical PD mutations that converge on the lysosomal degradation system suggest that neurodegeneration in PD is mediated by dysregulation of protein homeostasis. The well-characterized clinical and pathological relationship between PD and the lysosomal storage disorder GD emphasizes the importance of dysregulated protein metabolism in neurodegeneration, and one intriguing piece of this relationship is a shared phenotype of autophagic-lysosomal dysfunction in both diseases. Translational application of these findings may be accelerated by the use of midbrain dopamine neuronal models derived from induced pluripotent stem cells (iPSCs) that recapitulate several pathological features of GD and PD. In this review, we discuss evidence linking autophagic dysfunction to the pathophysiology of GD and *GBA1*-linked parkinsonism and focus more specifically on studies performed recently in iPSC-derived neurons.

Gaucher's Disease: Clinical Description and Pathophysiology

GD is an autosomal recessive lysosomal storage disorder and is caused by mutations in *GBA1*, a gene encoding the lysosomal hydrolase β-glucocerebrosidase (GCase) (Sidransky, 2012). GCase hydrolyzes glucosylceramide (GluCer) to ceramide and glucose, and its deficiency causes GluCer to accumulate within lysosomes (Beutler, 1992; Murray and Jin, 1995; Willemsen et al., 1988). Peripheral accumulation of GluCer chronically activates inflammatory pathways as GluCer cannot be digested by mutant macrophages, ultimately leading to variable symptoms of hematopoietic imbalance such as hepatosplenomegaly, pancytopenia, and bone abnormalities (Dandana et al., 2016; Pandey et al., 2017). These

*Correspondence Joseph R. Mazzulli, PhD, The Ken and Ruth Davee Department of Neurology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave, Ward 12-369, Chicago IL 60611, Phone: 312-503-3933, Fax: 312-503-3950, jmazzulli@northwestern.edu.

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features reflect the classical presentation of GD, but variants have been further classified according to disease severity, age-at-onset, and involvement of the nervous system. Adult-onset non-neuronopathic GD is the most common presentation and is classified as Type I. Types II and III are characterized by severe early childhood neurodegeneration and later onset ataxia with myoclonic seizures, respectively (Westbroek et al., 2011). GluCer accumulates in affected tissues in each type of GD, but cellular and environmental factors affecting phenotypic variation are not yet fully understood. While specific mutations do not predict many phenotypic features, they have been correlated broadly with GD classification. For example, the most common mutation, N370S, has been associated with Type I GD, while the severely destabilizing L444P mutation is strongly associated with development of Types II and III. This association could be due to the severity of each mutation related to protein destabilizing effects or catalytic deficiency, where more damaging variants are associated with early pediatric onset and severe neurodegeneration (Sidransky, 2012). While there are over 300 distinct *GBA1* mutations known, they all result in loss of enzymatic function and protein through either premature stop codons or premature degradation through cellular quality control mechanisms (Grabowski, 2008).

Lysosomal proteins including GCase are synthesized in the endoplasmic reticulum (ER). For proper folding to occur, the nascent peptide must be modified by N-glycosylation which aids in the recognition by molecular chaperones including heat shock protein (Hsp) homologues, ER lectins such as calnexin, and thiol oxidoreductases (Bendikov-Bar and Horowitz, 2012). If the protein is not correctly folded, it is re-glycosylated to be folded by chaperones again, and mutated GCase is misfolded and retained in the ER by these quality control mechanisms. Following repeated cycles of glycosylation and attempted folding by chaperones, the protein is exported from the ER and finally degraded by the ubiquitin-proteasome pathway rather than maturing to lysosomes (Ron, 2005). In the most severe GCase mutations, such as L444P, this results in near-complete depletion of lysosomal GCase, resulting in lysosomal GluCer accumulation.

Given that ER processing and enzymatic activity of GCase play an essential role in the pathogenesis of GD, the factors necessary for its maturation and optimal activity have been the subject of many studies. Previous studies have shown that an ER localized Hsp40 co-chaperone, ERdj3, interacts with GCase to enhance its degradation (Tan et al., 2014). Reducing the interaction between ERdj3 and GCase increased lysosomal GCase activity by promoting proper folding and trafficking through the calnexin pathway, but these effects were notably observed only in the case of mutated GCase. Similarly, Hsp90 has been found to preferentially recognize and bind mutant GCase, enhancing its degradation when Hsp90 is deacetylated but allowing stabilization of the nascent peptide and increased GCase activity when acetylated (Yang et al., 2013). Degradation is mediated through recruitment of Hsp27 to the Hsp90 complex, targeting mutant forms to the VCP/26S proteasome pathway (Yang et al., 2015). GCase trafficking beyond the ER is similarly relevant to GD pathogenesis. While GCase is synthesized in the ER like other lysosomal enzymes, its transport from the ER to lysosomes is uniquely mediated by lysosomal integral membrane protein type-2 (LIMP-2) rather than the mannose-6-phosphate receptors that are responsible for the trafficking of nearly all other lysosomal hydrolases (Blanz et al., 2015; Reczek et al., 2007; Rothaug et al., 2014). GCase and LIMP-2 bind in a complex mediated by interactions between hydrophobic

helical domains of both proteins in the neutral pH of the ER, and this complex is trafficked to the Golgi and endosomes before dissociating in the acidic environment of the lysosome (Reczek et al., 2007; Zunke et al., 2016). The activity of distinct phosphatidylinositol 4-kinases (PI4Ks) is required for these trafficking steps to occur, as deficiency in PI4KIIα prevents exit from the Golgi and deficiency in PI4KIIIβ produces accumulation in endosomes (Jovic et al., 2012). Other factors such as the GCase-LIMP-2 co-chaperone progranulin have also been connected with abnormal GCase maturation in GD (Jian et al., 2016).

In addition to proper targeting to the lysosomal compartment, co-factors and post-translational modifications influence catalytic activity of GCase. For instance, GCase requires specific patterns of glycosylation to form a catalytically active site (Grace and Grabowski, 1990). GCase is N-glycosylated at 4 of 5 available asparagine residues, and mutation at the first residue, asparagine N19, prevents catalytic activity (Berg-Fussman et al., 1993). Moreover, optimal GCase activity within lysosomes requires the presence of negatively charged lipids such as phosphatidylserine and acidic pH, likely because these conditions promote the proper conformation needed for hydrolysis (Basu et al., 1986; Qi and Grabowski, 1998). A protein cofactor, saposin C (sap C), is also required for GCase hydrolysis of GluCer both *in vitro* and *in vivo*, most clearly illustrated by the finding that mutations in sap C are associated with a rare form of GD (Blanz and Saftig, 2016; Tamargo et al., 2012). Sap C localizes with both GCase and lipid membranes, and it remains unclear whether this cofactor increases GCase activity directly by forming a complex with the enzyme or indirectly by promoting GCase association with GluCer secondary to changes in the phospholipid bilayer (Blanz and Saftig, 2016). The overall effect of sap C and the lysosomal environment is thus to promote association of GCase with GluCer in catalytically active conformations. In summary, physiologic GCase maturation and activity requires the concerted action of folding machinery, trafficking proteins, and cofactors. Disruption of any of these individual steps may contribute to the pathology and severity of GD.

Since GCase mutations lead to loss-of-function, current therapies have been focused on restoring enzyme in lysosomes and thereby reducing substrate storage. Unfortunately, currently approved therapies are only effective for treating visceral GD symptoms because they largely fail to cross the blood brain barrier (Bennett and Mohan, 2013). There are two broad approaches to treatment: 1) enzyme replacement therapy (ERT), which uses recombinant GCase such as imiglucerase and velaglucerase alfa to increase catalytic activity, and 2) substrate reduction therapy (SRT), which uses inhibitors of glucosylceramide synthase such as miglustat and eliglustat to reduce production of GluCer (Shemesh et al., 2015). Current goals of treatment are thus focused on reducing visceral symptoms, maintaining quality of life, and preventing irreversible organ damage (Pastores et al., 2004). However, given the growing understanding of ER stress and trafficking dysregulation in GD pathogenesis, recent preclinical work has led to the development of small-molecule chaperones and other substrate reducing agents capable of crossing the blood-brain barrier which may be promising candidates as future neuronopathic GD therapies, discussed in the therapeutics section below.

The Relationship between Gaucher's Disease, GBA1-linked Parkinsonism, and idiopathic Parkinson's Disease

PD is the second most common age-related neurodegenerative disorder, and is classically associated with symptoms of bradykinesia, postural instability, tremor, and rigidity due to the progressive neurodegeneration of midbrain dopaminergic (DA) neurons (Li et al., 2015). While many details of PD pathophysiology remain unclear, its hallmark pathology is the accumulation of intracellular aggregates called Lewy bodies, composed largely of the presynaptic protein alpha-synuclein (a-syn) (Spillantini et al., 1997). A-syn is normally a soluble protein, but converts into insoluble amyloid fibrils in PD and other synucleinopathies through potentially neurotoxic intermediate oligomeric species (Forman et al., 2004). The majority of PD is idiopathic although rare familial forms of the disease have been described and represent about 10% of cases. The study of familial PD has provided critical clues into the disease etiology and has implicated multiple cellular pathways that converge on vesicular trafficking, protein clearance, and mitochondrial functions. In addition to the presence of Lewy bodies, strong evidence linking a-syn accumulation to disease etiology is derived from rare familial forms of PD caused by mutations in the *SNCA* gene that encodes a-syn (Hernandez et al., 2016). Nearly all of the *SNCA* mutations linked to familial PD increase the dose, stability, or aggregation of a-syn and are autosomal dominant, suggesting that a-syn accumulation causes PD through a toxic gain of function. In addition to familial PD, genetic studies have revealed several risk factors for idiopathic PD (iPD). Remarkably, the majority of variants associated with iPD appear to converge on protein homeostasis pathways, and in particular, the autophagic-lysosomal system (Chang et al., 2017; Robak et al., 2017). While the contribution of several of these variants to PD pathogenesis has yet to be determined, disruptions in the lysosomal system are expected to result in a-syn accumulation since lysosomes are important for the degradation of physiological a-syn (Cuervo et al., 2004). It is likely that predisposing genetic variants interact with each other, or environmental factors, to influence iPD onset and severity.

The connection between GD and PD was first suggested by the observations of a significant proportion of GD patients experiencing parkinsonian symptoms and a high incidence of PD in heterozygous carriers of *GBA1* mutations (Halperin et al., 2006; Neudorfer et al., 1996; Tayebi et al., 2001). Since that time, multiple genetic studies have demonstrated a strong association between mutations in *GBA1* and an increased risk for PD with aging, and mutations at this locus are now understood to be one of the most common genetic risk factors for developing disease (Clark et al., 2007; Goker-Alpan et al., 2006; Goker-Alpan et al., 2004; Sidransky et al., 2009). *GBA1* mutations are commonly analyzed on genetic screenings, and patients with PD and *GBA1* polymorphisms can be subcategorized as having "GBA1-linked parkinsonism" (GBA1-PD). GBA1-PD produces neurodegeneration in a pattern similar to iPD, but has been associated with an earlier age of onset and an increased incidence of neuropsychiatric symptoms (Migdalska-Richards and Schapira, 2016). Studies of human brain pathology have corroborated and extended this connection. Lewy bodies identical to those observed in iPD have been documented in patients with neuronopathic GD (Wong et al., 2004), suggesting that a-syn aggregation is involved in neurotoxicity of GD. Studies of iPD brain have shown that GCase expression and activity are both decreased in

the substantia nigra (SNc), and reduced GCase is associated with inclusion-bearing regions compared with unaffected brain regions (Alcalay et al., 2015; Gegg et al., 2012; Murphy et al., 2014; Murphy and Halliday, 2014). Together, this demonstrates the similarity of PD and neuronopathic GD at a pathological level, evidenced through the presence of a-syn inclusions as well as reduced GCase activity in synucleinopathy patients expressing wild-type GCase.

While the mechanisms of *GBA1*-induced neurotoxicity are not completely understood, two main hypotheses have been advanced that are related to either toxic gain of function induced by *GBA1* mutant expression, or loss-of-function leading to lysosomal dysfunction and substrate accumulation. The toxic gain of function hypothesis suggests that misfolded mutant GCase aberrantly interacts and interferes with cellular pathways involved in protein homeostasis, such as quality control machinery in the endoplasmic reticulum (ER). This may result in chronic ER stress which, in combination with other factors such as oxidation and aging, could lead to widespread cellular dysfunction and cell death. A recent study using GBA1-PD patient-derived iPS neurons showed that accumulation of mutated GCase in the ER is associated with markers of ER stress (Fernandes et al., 2016). Mutant GCase may also aberrantly interact with a-syn to influence aggregate formation, since GCase has been detected within Lewy bodies of PD brain (Goker-Alpan et al., 2010). While these studies suggest that mutant GCase may induce toxicity through toxic gain of function, it is difficult to gauge the contribution of this mechanism since all of the mutant GCase variants likewise exhibit a loss-of-catalytic function producing compromised lysosomal activity.

The loss-of-function hypothesis proposes that reduced GCase activity and GluCer substrate accumulation leads to cell death. Our previous studies have shown that GCase depletion reduces lysosomal proteolysis and stabilizes oligomeric intermediates of a-syn in mouse neuronal culture models and patient-derived iPS neurons (Mazzulli et al., 2011). The effect on a-syn oligomers was specific to GCase depletion, since non-specific lysosomal inhibition with leupeptin increased overall a-syn without stabilizing its oligomeric form, indicating that the lysosomal dysfunction alone was not sufficient to produce the same pathology as GCase depletion. Levels of another aggregation-prone protein and lysosomal substrate, tau, only mildly changed by GCase deficiency, further indicating the specificity of this effect at the initial stages of GCase depletion. However, it is possible that prolonged GluCer accumulation may result in secondary pathologies such as tau and amyloid-beta inclusions, that have been observed in GD mouse models (Sardi et al., 2013; Xu et al., 2014). Using cell-free *in vitro* assays, GluCer was found to directly interact and stabilize soluble oligomeric intermediates of a-syn preferentially under acidic conditions similar to the lysosome (Mazzulli et al., 2011). Other studies supporting the loss-of-function view show that targeted homozygous *GBA1* knockout in mice produces a-syn oligomer aggregation, neuronopathic GD symptoms, and neurodegeneration in brain regions similar to PD (Farfel-Becker et al., 2014; Osellame et al., 2013). Similarly, chemical inhibition of wild-type GCase activity using the inhibitor conduritol-b-epoxide (CBE) increases a-syn accumulation and causes neurotoxicity in SH-SY5Y, mouse primary cortical neurons, and *in vitro* mouse models (Cleeter et al., 2013; Magalhaes et al., 2016; Manning-Boğ et al., 2009). Loss of activity has also been shown to induce pathology in iPSC models (Schöndorf et al., 2014; Woodard et al., 2014). In summary, there is evidence that both gain of function and loss of

function mechanisms may contribute to GD neurotoxicity, and it should be noted that these pathological mechanisms are not mutually exclusive, although one may be found to predominate.

In addition to mutated GCase leading to loss-of-function, a-syn accumulation alone in the context of wild-type GCase, can also reduce GCase activity. Our previous studies indicated that a-syn overexpression decreases maturation of wild-type GCase through the early secretory pathway, leading to a decline of activity in lysosomal enriched fractions of iPD brain (Mazzulli et al., 2011). This effect is consistent with previous observations demonstrating that a-syn inhibits protein trafficking between the ER and Golgi (Cooper et al., 2006). Aggregation of a-syn potentiates this process, as dysfunction was observed following overexpression of A53T and WT a-syn, but not an aggregation-incompetent form of a-syn (Mazzulli et al., 2011). This suggests that GCase deficiency not only causes an increase in a-syn oligomers, but that a-syn aggregates also inhibit the trafficking of GCase to lysosomes, exacerbating the primary dysfunction. Subsequent studies have likewise verified reduced GCase activity in synucleinopathy animal models and other iPS models (Mazzulli et al., 2016a; Sardi et al., 2013).

Other studies found that a-syn did not selectively affect GCase, but also decreased the maturation and activity of multiple lysosomal hydrolases including cathepsin B, β-galactosidase, and hexosaminidase by inhibiting ER-Golgi trafficking in patient iPSC-derived neurons (Mazzulli et al., 2016a). Immature (ER) forms of these enzymes were found to accumulate while a-syn aberrantly co-localized with vesicle tethering factor GM130 at fragmented Golgi structures, suggesting that a-syn decreases lysosomal activity by disrupting COPII vesicle fusion early in the secretory pathway. Significantly, accumulation of a-syn altered the staining pattern of the ER trafficking regulator Rab1a, causing it to move from its normal ER-Golgi location into a diffuse cytosolic distribution. Rab1a overexpression was sufficient to completely reverse Golgi pathology, further demonstrating that a-syn-induced lysosomal dysfunction occurs through disrupted trafficking. Overall, this suggests a-syn causes lysosomal hydrolase dysfunction by specifically disrupting trafficking through Rab1a and ER-Golgi pathways. a-Syn-induced trafficking and lysosomal dysfunction may interact with other genetic variants associated with iPD, including *GBA1* mutations, to amplify protein homeostasis imbalance and ultimately lead to Lewy body pathology observed in PD brain.

The involvement of autophagic dysregulation in PD

Interplay between alpha-synuclein and autophagy

Studies have indicated that a-syn accumulation can be induced by disruptions in autophagic-lysosomal pathways. Conditional deletion of Atg7, an essential factor in autophagosome formation, increased pathogenic a-syn protein levels in neuronal processes in mice (Friedman et al., 2012). In a distinct model, deletion of Atg7 specifically within midbrain DA neurons resulted in neurodegeneration with movement abnormalities reminiscent of PD (Savitt et al., 2012). Other studies in mice have shown that depletion of PD-linked VPS35, a retromer component involved in intracellular trafficking, leads to a-syn accumulation and midbrain neurodegeneration through interfering with the trafficking of LAMP-2A, the

receptor for substrates degraded through chaperone mediated autophagy CMA (Tang et al., 2015b). As a-syn has been shown to be degraded through CMA *in vitro* (Cuervo et al., 2004), it is possible that VPS35-induced a-syn accumulation could occur through LAMP-2A deficiency. However, VPS35 depletion likely influences multiple trafficking steps, and a-syn accumulation may occur through LAMP-2A independent mechanisms. Studies in the human brain have further suggested a connection between CMA and a-syn accumulation in PD. The levels of CMA markers are decreased in idiopathic PD brain, an effect that correlates with a-syn accumulation (Alvarez-Erviti et al., 2010; Murphy and Halliday, 2014). These findings suggest that the autophagic-lysosomal system plays an important role in the degradation of a-syn, and disruptions in this pathway may lead to synucleinopathies.

Pathologic a-syn itself has been shown to dysregulate both autophagic-lysosomal activity. For example familial-linked mutant forms of a-syn, A53T and A30P, bind with high affinity to LAMP-2A on lysosomes, but fail to translocate, competitively inhibiting a portion of CMA machinery (Cuervo et al., 2004). These a-syn mutants are also degraded at a slower rate compared to the wild-type protein (Li et al., 2004), and mutating the CMA targeting domain of mutant a-syn also impedes a-syn clearance (Xilouri et al., 2009). Dopamine-modified a-syn oligomers generated from the wild-type protein can also impede substrate uptake through blocking LAMP-2A, implicating this process in idiopathic PD (Martinez-Vicente et al., 2008). A-syn may also interfere with autophagy through impeding the function of rab1a and Atg9, resulting in decreased autophagosome formation (Winslow et al., 2010). In a synucleinopathy mouse model, conditional overexpression of A53T in dopaminergic neurons using the dopamine transporter promoter (DAT) demonstrated mitochondrial inclusions that colocalized with the autophagic marker LC3 and adapter protein p62 (Chen et al., 2015). This pathology notably appears prior to neurodegeneration of DA neurons, suggesting a causative role for neurotoxicity in this model. These studies indicate that a-syn can impede protein degradation through autophagy. Since the autophagic process is particularly critical for neuronal health (Komatsu et al., 2006), this indicates a potential toxic mechanism for a-syn-induced neurotoxicity.

Other canonical PD mutations related to autophagic-lysosomal dysfunction

Further suggesting a contribution of autophagic-lysosomal dysfunction to PD pathophysiology, several other canonical mutations associated with PD are within autophagic pathways. Mutations in *LRRK2* encoding leucine-rich repeat kinase 2 have been shown to cause familial PD in an autosomal dominant manner and are also common genetic risk factors for iPD (Zhang et al., 2015). One of the most common LRRK2 mutations, *G2019S* (LRRK2^{G2019S}), has been shown to inhibit CMA in similar manner as mutant a-syn, through impeding the uptake of substrates by LAMP-2A receptor blockade (Orenstein et al., 2013). Interestingly, LRRK2 has recently been shown to phosphorylate trafficking mediator rab10 (Steger et al., 2016), which may explain LRRK2-mediated effects on vesicular trafficking and autophagic flux (Roosen and Cookson, 2016). While rab10 is known to mediate trafficking between the trans-Golgi network and the plasma membrane, recent studies have also linked its function to autophagic degradation of lipids (Kinghorn et al., 2016). Studies using patient-derived iPS neurons found an increase in LC3 positive puncta and p62 expression by immunofluorescence in both LRRK2-PD (*LRRK2*^{G2019S}) and

iPD lines compared to controls. This finding was reinforced by electron microscopy showing an increase in autophagic vacuoles, lipid accumulation, and dilated ER in PD-derived neurons (Sanchez-Danes et al., 2012).

In addition to LRRK2, other canonical mutations have been tied to autophagic dysfunction through different mechanisms. Autosomal recessive forms of PD caused by loss-of-function PINK1 or Parkin mutations are known to impede mitochondrial turnover through mitophagy (Pickrell and Youle, 2015). Studies in iPSC-derived midbrain neurons carrying the PINK1 or Parkin mutations show abnormal mitochondria and elevated oxidant stress, consistent with disrupted mitophagy (Chung et al., 2016). While much less common, several other familial PD mutations are more directly implicated in the autophagic-lysosomal pathway. ATP13A2/PARK9 is a lysosomal ATPase whose loss-of-function causes Kufor-Rakeb syndrome, characterized by early onset parkinsonism (Park et al., 2011; Ramirez et al., 2006). Deficiency in ATP13A2 observed in patient fibroblasts and primary cortical neurons produce an accumulation of enlarged lysosomes, reduced proteolysis, and decreased LC3 clearance, ultimately increasing a-syn aggregation and toxicity (Usenovic et al., 2012). Autosomal dominant parkinsonism is also associated with mutation of vacuolar protein sorting 35 (Vps35), a component of the retromer system that participates in mediating retrograde transport of endosomes and recycling of cargoes related to the autophagy-lysosomal function, including LAMP-2A and soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) (Pan and Yue, 2014; Tang et al., 2015b). While its contribution to PD pathogenesis remains incompletely understood, Vps35 mutation has been shown to produce DA neurodegeneration with deficits in mitochondrial function and turnover that may relate to disrupted mitophagy (Tang et al., 2015a; Wang et al., 2016). In addition to familial PD, recent large-scale genetic studies have implicated disrupted autophagic-lysosomal pathways in iPD. Variants in lysosomal hydrolases, transmembrane proteins, and trafficking regulators show a significant association with iPD (Chang et al., 2017; Robak et al., 2017). While these variants may not be capable of inducing pathogenesis individually, a combination of predisposing factors may culminate in autophagic-lysosomal dysfunction in PD, leading to the formation of Lewy bodies.

The majority of evidence indicates that a-syn and other PD mutants can inhibit autophagy, however it should be noted that some studies have shown that PD-linked proteins may aberrantly elevate the autophagic process leading to cell death. For example, A53T expression in primary neurons can increase mitophagy in a parkin-dependent manner, while inhibiting autophagic activity can partially block cell death (Choubey et al., 2011; Xilouri et al., 2009)). Such findings suggest that autophagic activity may be increased through a compensatory response to neurotoxic a-syn conformations, potentially leading to over-digestion of necessary cytosolic proteins and healthy organelles (Xilouri et al., 2016). Similarly, $LRRK2^{G2019S}$ expressing SH-SY5Y cells and iPSC -derived DA neurons exhibited increases in autophagic vacuoles with neurite shortening that was reversed by knockdown of autophagy related genes or prevention of aberrant mitochondrial fission (Plowey et al., 2008; Su and Qi, 2013). These studies indicate that while autophagic activity may provide benefit by reducing protein accumulation, over-activation of this system in an uncontrollable manner can also lead to deleterious effects resulting in the elimination of essential cellular components and toxicity.

Autophagic dysregulation characterizes GD and GBA1-PD

Animal models of GD demonstrate autophagic dysfunction

Autophagy is the major cargo delivery system for the transport of damaged proteins, organelles, and other macromolecules into lysosomes for degradation. Therefore, it is not unexpected that autophagic dysfunction is a prominent feature of several lysosomal storage disorders. In the case of GD, multiple studies in animal models have demonstrated autophagic dysfunction can be induced by loss-of-function *GBA1* mutations and by complete GCase knockout. Initial studies in a neuronopathic mouse model expressing a homozygote V394L *GBA1* mutation combined with saposin C haploinsuffiency (4L/PS-NA) demonstrated storage of both GluCer, glucosylsphingosine and accumulation of membranous vesicles reminiscent of dysfunctional autophagosomes (Sun et al., 2010). The intraneuronal accumulation of p62 was noted in affected regions of the nervous system, occasionally associated with inflammatory markers. As p62 is a well-established autophagy adaptor protein responsible for cargo delivery of ubiquitinated substrates (Komatsu et al., 2007), this suggested that neurotoxicity in this model could be due to disruption of substrate delivery or fusion with lysosomes. In a distinct neuronopathic GD mouse model where GCase was knocked-out specifically within neurons, ultrastructural analysis demonstrated accumulation of autophagosomes that occurred concomitantly with substrate storage, within degenerating regions of the nervous system. Similar phenotypes in the 4L/PS-NA and GCase knock-out mice indicate that the autophagic phenotype and associated neurodegeneration likely occurs through loss-of-enzymatic activity and substrate accumulation.

Other studies have shown that complete knock-out of GCase in mice can specifically influence the turnover of mitochondria, likely through disrupting their autophagic degradation via mitophagy. Using primary neuronal cultures isolated from these mice, reduced autophagic flux was documented through measuring the response of LC3-II levels to the lysosomal inhibitor, bafilomycin A1 (Osellame et al., 2013). The number and size of mitochondria were increased in $GBA^{-/-}$ neurons observed by confocal microscopy, an effect that was accompanied by functional respiratory chain defects. Mitochondria in $GBA^{-/-}$ neurons demonstrated decreased co-localization with LC3, and there were no changes in mitochondrial expression at the mRNA level, suggesting that this represents the disruption of mitochondrial turnover through mitophagy (Osellame et al., 2013). Similar to the genetic GD models, pharmacologic inhibition of wild-type GCase by injection of CBE in mice caused concomitant increases in GluCer substrate with LAMP-2A, LC3-II, and a-syn accumulation, suggesting that reduction of enzymatic activity alone can influence autophagic activity and turnover of a-syn (Rocha et al., 2015). These studies provide evidence that GCase deficiency in mice likely disrupts the autophagic-lysosomal system, resulting in the accumulation of protein inclusions, a-syn, and neurodegeneration. These deficits may contribute to neurodegeneration that occurs in neuronopathic GD as well as GBA1-PD.

Studies in non-mammalian models have also demonstrated an association between loss of GCase and autophagy disruption. Knock-out of the *GBA1* ortholog in Drosophila produces a reduction in lifespan, behavioral deficits, neurodegeneration, and increased ubiquitinated

protein aggregates (Davis et al., 2016). A concomitant increase in Ref(2)P, the Drosophila homolog of p62, without changes in the activity of lysosomal enzymes Cathepsin D and hexosaminidase suggests that disrupted autophagic flux may contribute to increased aggregation. A second more extensive study of *GBA1* knock-out flies reproduced features of neuronopathic GD, including GluCer accumulation, lysosomal dysfunction, and motor abnormalities. Measures of autophagic function in this model showed the accumulation of LC3II and only a minor response to starvation, suggesting dysfunctional autophagic-lysosomal turnover (Kinghorn et al., 2016). Similar to the phenotype observed in *GBA1* knock-out mice, the fly model also demonstrated signs of mitochondrial dysfunction and vulnerability to oxidative stress, as well as increased levels of p62 and ubiquitin. Collectively, these data indicate that GCase deficiency and substrate accumulation lead to autophagic lysosomal dysfunction *in vivo*. Further studies are will be required to delineate the precise mechanistic relationship between substrate accumulation, disrupted cellular clearance, and neurodegeneration.

Autophagic dysfunction in GD and GBA1-PD iPSC-derived neurons

Early studies of iPSCs that were reprogrammed from GD patients and differentiated into midbrain dopamine neurons demonstrated a disruption in long-lived protein degradation, consistent with dysfunction in the autophagic-lysosomal system (Mazzulli et al., 2011). iPSC-derived GD midbrain cultures recapitulated several features of neuronopathic GD brain including substrate accumulation, enlarged lysosomes, and the accumulation of a-syn (Mazzulli et al., 2011; Mazzulli et al., 2016a). Subsequent studies using iPSCs derived from GD and GBA1-PD heterozygote carriers demonstrated substrate accumulation, elevated a-syn, and deficient autophagic flux (Schöndorf et al., 2014). Importantly, these studies used genome editing techniques to generate isogenic corrected controls and methods to isolate pure populations of midbrain dopamine neurons, which is critical for the identification of potentially subtle biochemical changes that may occur in heterozygote *GBA1* carriers. This study also identified critical baseline differences in the glycosphingolipid profile of patient fibroblasts compared to fully differentiated dopamine neurons, emphasizing the importance of using relevant neuronal culture models for the study of *GBA1*-induced neurotoxicity.

Other studies using iPS neurons derived from GBA1-PD patients demonstrated elevated levels of ER chaperones BiP, and calreticulin as well as markers commonly associated with ER stress including PDI, IRE1, and calnexin, compared to healthy control individuals (Fernandes et al., 2016). The ER stress phenotype was accompanied by disturbances in the autophagic-lysosomal system, as indicated by decrease autophagic flux, enlarged lysosomes, and p62 accumulation, perhaps suggesting a disruption in the ability to engulf or deliver autophagic cargo to lysosomes for degradation. Although intracellular a-syn aggregates were not observed in this study, a-syn was found to be increased in the media, suggesting that autophagic dysfunction may cause the release and the cell-to-cell propagation of pathology, consistent with other studies (Bae et al., 2014). Consistent with disturbances in the ER of cells expressing *GBA1* mutations, other studies using both iPS neuronal cultures and mouse models have found elevated cytosolic Ca2+ levels (Liou et al., 2016; Schöndorf et al., 2014). Inhibiting ryanodine receptor-mediated Ca2+ release from the ER through dantrolene could mitigate phenotypes of neuropathic GD including improved motor behavior and reduced

inflammatory response in the brain (Liou et al., 2016). Interestingly, ER mediated Ca2+ release could also be improved by reducing the synthesis of GluCer, indicating that substrate accumulation may be responsible for these effects. These studies indicate that in addition to autophagic-lysosomal dysfunction, perturbations in the ER, either through stress associated with expressing a misfolded protein or through accumulation of GluCer substrates, can lead to cellular dysfunction in GBA1-PD patient neurons.

A unique study utilized iPSCs from a set of monozygotic twins that harbored a heterozygote N370S *GBA1* mutation but were discordant for PD (Woodard et al., 2014). Interestingly, both twins had the same 50% reduction in GCase activity and overall elevated a-syn levels, however the affected twin demonstrated elevated a-syn specifically within neurites. The affected twin also showed differences in dopamine metabolism through elevated levels of monoamine oxidase, the enzyme responsible for degrading dopamine, which may be responsible for the Parkinsonism phenotype in this patient. Another study compared iPS neurons from GD patients that exhibited Parkinsonism to those patients without PD (Aflaki et al., 2016a). While similar reductions in GCase activity were observed between lines, those exhibiting a neuronopathic or PD phenotype demonstrated elevated a-syn, which may underlie the neurodegeneration that occurs in these patients. Collectively, these studies suggest that heterozygote *GBA1* mutations alone are insufficient to cause a neuronopathic phenotype or aberrant a-syn accumulation. This is consistent with genetic studies of GBA1-PD, which indicate that *GBA1* mutations only predispose one to develop PD, while many *GBA1* carriers will never develop the disease. This indicates that other environmental factors, epigenetic modifications, or additional damaging genetic variants likely combine with *GBA1* mutations to result in neurodegeneration. These studies emphasize a critical advantage of using iPSCs for disease modeling by enabling the ability to isolate and study neurons from patients with similar predisposing genetic variants but with distinct, individual phenotypes. The study of isogenic corrected lines of *GBA1* carriers should help to uncover the contribution of other factors that lead to disease onset and neuronopathic phenotypes by refining controls and reducing unanticipated variability. While these findings represent an important first step in deciphering the complexity of idiopathic PD, further mechanistic studies will be required to uncover the cause of disease heterogeneity in GD and *GBA1* carriers. Accelerating our understanding of specific disease processes through the study of individualized patient-derived iPS models may lead to the development of future personalized therapies.

Advantages and challenges of modeling GD and GBA1-PD in iPSC-derived neurons

While animal models and cell lines have provided useful tools for the study of GD pathogenesis, artificial genetic manipulations that are required to simulate the pathogenic effect of the disease-causing mutations limits their utility and relevance to human disease. In the case of loss-of-function mutations such as those that cause GD, knock-out models may faithfully recapitulate some features of the disease, however there is potential for compensation or alterations in other cellular pathways that are not involved in the human disease. Using patient-derived material that harbors naturally occurring mutations provides the advantage of studying the pathobiological functions of these mutations in a context that is closer to its natural state. Since the reprogramming of somatic cells into induced

pluripotent stem cells (iPSCs) has now become standardized (Shi et al., 2017), the study of disease-causing mutations has been dramatically facilitated. In the case of modeling synucleinopathies, previous cell and mouse models relied on dramatic overexpression of the wild-type or mutant proteins to induce pathology driven by artificial promotors. Although the study of these models has proven valuable for certain disease processes, it is possible that such a dramatic overexpression and rapid formation of pathological a-syn may lead to phenotypes that are not associated with PD and therefore have little human relevance. The study of iPSn from familial PD, such as *SNCA* triplication and A53T mutant carriers, has demonstrated that pathological aggregation can occur as neurons mature in culture. Similarly, models of GD have shown GluCer and a-syn accumulation occurs through naturally expressed *GBA1* mutations. Using patient-derived iPSCs also facilitates the study of PD variants that occur within non-coding regions, that would otherwise be very difficult to generate in cell lines or primary cultures, such as *SNCA* intron variants that lead to increased a-syn expression (Soldner et al., 2016).

Recent advancements in our understanding of the factors that drive the development and differentiation of embryonic stem cells and iPSCs into neuronal phenotypes has offered more accurate models to study neurodegeneration. In the case of PD, pioneering studies outlining the development of midbrain dopamine neurons in animals (Joksimovic et al., 2009) have provided the foundation to develop standardized protocols for the differentiation of human iPSCs into homogeneous populations of patient-derived midbrain neurons at a scalable level required for biochemical and cellular-based studies (Kriks et al., 2011). Employing these methods to differentiate iPSCs into dopamine neurons has shown that they exhibit many properties of brain-derived neurons, including co-expression of the midbrain markers tyrosine hydroxylase, FOXA2, and LMX1A (Kriks et al., 2011). These neurons exhibit electrical activity, and can synthesize, store, and release dopamine similar to brain-derived neurons. In addition, they also show synaptic a-syn that colocalizes with synapsin and other markers of nerve terminals as cultures mature in vitro (Mazzulli et al., 2016a). While current protocols can achieve ca. 80% midbrain-type dopamine neurons from well-characterized iPSCs, some studies have employed flow cytometry to further purify the culture population (Schöndorf et al., 2014). These models facilitate methods that involve large-scale biochemical analysis required for the study or purification of proteins from human cells in a disease-relevant background. iPSC models also permit the discovery of cell-type specific phenotypes that would otherwise be difficult to detect in primary culture midbrain models, where most of the cells are non-dopaminergic, or brain tissues, which contain mixtures of many cell types.

While there are clear advantages of using iPSC derived midbrain models for the study of certain pathogenic pathways such as protein aggregation, their utility may be limited for other pathways. Advanced age is a primary risk factor for developing PD, and important age-related changes may be lost by reprogramming patient cells to a naïve state. Protocols have been developed to maintain and mature iPSC-derived neurons in long term cultures (Mazzulli et al., 2016a; Sanchez-Danes et al., 2012) and to induce aging phenotypes through expression of the truncated nuclear envelope protein progerin, mutated in the premature aging disorder Hutchinson-Gilford progeria syndrome (Miller et al., 2013). The contribution of environmental influences, particularly minor exposures that compound dysfunction over

time, can be difficult to address in iPSC models. However some iPSC studies have tested additive effects of mitochondrial toxins with genetic mutations to identify and rescue pathogenic pathways (Chung et al., 2016; Cooper et al., 2012; Ryan et al., 2013). Another concern involves changes that may occur during prolonged culturing of embryonic or iPSCs, such as epigenetic modifications that can alter disease phenotypes (Mekhoubad et al., 2012). Aberrant DNA and histone methylation can be observed in iPSC-derived cultures due to early errors that are maintained through differentiation (Lister et al., 2011), and hypermethylation has been connected to differential expression of transcription factors in DA neurons from iPSCs of LRRK2-PD and iPD patients (Fernández-Santiago et al., 2015). Current protocols address aberrant epigenetic modification by analyzing multiple clones of each line, but the importance of epigenetic changes in PD remains largely unknown. Finally, variable differentiation efficiency across different pluripotent cell lines may produce significant differences in neuronal properties. For example, the use of identical differentiation protocols on two distinct embryonic stem cell lines produced different types of neurons *in vitro*, suggesting that cell lines may be preprogrammed or biased towards developing certain types of neurons (Wu et al., 2007).

Additional challenges associated with pluripotent cell-based modeling involve the study of age-related pathogenic processes of multiple cell types, such as inflammation between microglia and neurons, which has proven difficult to model using co-cultured iPSCs due to the aggressive nature of microglia. Phenotypes that require interactions between multiple neuronal types or networks, such as pace-making properties exhibited by midbrain dopamine neurons, may be difficult to recapitulate using iPSC-derived neurons. Some of these challenges can be overcome by newly developed models, such as the development neurospheres or midbrain organoids that exhibit similar features to human midbrain including multicellular composition, electrical activity, and the formation of neuromelanin (Jo et al., 2016). Furthermore, the development of 3-demensional culture systems have enabled the detection of extracellular pathology, such as amyloid-beta plaques that occur in Alzheimer's disease brain (Choi et al., 2014). These technical advances have provided novel ways to study the mechanism of human-specific disease phenotypes, and allow for the development of novel therapies that can be translated into treatments for PD and GD.

Therapeutic developments for the treatment of GD and GBA1-PD

Therapeutic efforts for GD and GBA1-PD have been focused on restoring enzyme activity and reducing glycosphingolipid substrates. In addition to those potential therapies that aim to enhance GCase function and / or reduce lipids, general enhancement of the autophagic system may also provide benefit in GD and GBA1-PD through reducing protein and substrate accumulation. Previous studies have shown that autophagy enhancers such as rapamycin (an inhibitor of the mTOR pathway), trehalose, or metformin can reduce protein accumulation that occurs in several neurodegenerative disease models (Menzies et al., 2017). Studies of other proteinopathies have shown that rapamycin can reverse nuclear blebbing and an early senescence phenotype of patient fibroblasts derived from Hutchinson-Gilford progeria syndrome, a disease characterized by lamin aggregates and an accelerated aging phenotype (Cao et al., 2011). Rapamycin was found to clear lamin accumulations through enhancing autophagic flux and the binding of autophagy adaptors p62 and Alfy (Cao et al.,

2011), two proteins that are putatively involved in the selective removal of aggregates (aggrephagy) (Yamamoto and Yue, 2014). Selective activation of aggrephagy may provide therapeutic benefit in PD and other protein aggregation disorders by reducing pathological inclusions, but without the complications of general over-activation of macroautophagy.

Considering the growing understanding of ER stress and disrupted lysosomal component trafficking in GD and PD pathogenesis, recent preclinical work has led to the development of small molecule chaperones and other substrate reducing agents capable of crossing the blood-brain barrier, which may be promising candidates as future therapies for synucleinopathies. As proof of principle, mutant GCase lysosomal localization and activity in patient derived fibroblasts can be increased by treatment with the iminosugar, N-nonyl-1-deoxynojirimycin (NN-DNJ) or by altering ER environment to promote GCase stability (Sawkar et al., 2002; Sawkar et al., 2006). These chaperones exhibit high specificity and desirable blood-brain penetrance, however they are active-site binders that inhibit enzyme activity and rely on wash-out once translocated to the lysosomes. These inhibitory strategies complicate treatment by requiring a finely tuned balance between enzyme stabilization and competitive inhibition of endogenous substrate (Jung et al., 2016). The iminosugar isofagomine (IFG) is one such inhibitory chaperone shown to facilitate GCase folding, lysosomal transport, and activity in both N370S and L444P fibroblasts (Khanna et al., 2010; Steet et al., 2006). Oral administration of IFG to L444P mice reduced hepatosplenomegaly and increased GCase activity in affected tissues, including the brain (Khanna et al., 2010). A following study testing IFG in the neuronopathic V394L/V394L + saposin C−/− Gaucher mouse model found similar increases in peripheral and central GCase activity, a ~34% increase in lifespan, and a reduction in proinflammatory measures, although these changes were notably not associated with changes in lipid accumulation (Sun et al., 2011). In the Thy1-aSyn mouse synucleinopathy model, IFG administration increased GCase trafficking and lysosomal activity, and ameliorated motor deficits (Richter et al., 2014). While IFG treatment in this study reduced the percentage of DA neurons exhibiting elevated levels of a-syn-postive immunofluorescence, overall brain a-syn levels remained unchanged. The expectorant ambroxol is another inhibitory pharmacological chaperone shown to increase GCase lysosomal localization and activity in GD Type I and Type II fibroblasts (Bendikov-Bar et al., 2013). These findings were confirmed in GD fibroblasts and extended to show similar effects in fibroblasts heterozygous for GCase mutation derived from carriers with and without parkinsonism (McNeill et al., 2014). Two clinical pilot studies testing ambroxol treatment in GD patients have been published. In a study of 12 Type I patients, ambroxol led to variable individual improvement of peripheral symptoms without significantly affecting global outcome measures at 6 months (Zimran et al., 2013). In four patients selected for neurological GD symptoms and a positive *in vitro* response to ambroxol observed in fibroblasts, individualized ambroxol dosing regimens improved clinical measures of myoclonus and neuro-opthalmologic function with variable effects on seizure activity, overall improving patients' quality of life (Narita et al., 2016). While preliminary, such results suggest the therapeutic potential of inhibitory chaperone treatment for synucleinopathies, providing that these agents can efficiently dissociate from the GCase active site at acidic pH.

Given the limitations of inhibitory chaperone therapy, recent efforts have been focused on the development of non-iminosugar small molecule chaperones that promote GCase maturation through other mechanisms. For example, a high throughput screening assay testing the effect of small molecule candidates on GCase activity in patient spleen revealed a number of non-inhibitory compounds in the pyrazolopyrimidine family with direct GCase activation and chaperoning activity (Goldin et al., 2012; Patnaik et al., 2012). Lead molecule NCGC758 was subsequently confirmed to increase GCase activation and translocation while reducing lipids in macrophages and monocytes derived from GD iPSCs (Aflaki et al., 2014). Similar effects in macrophages were observed following treatment with another lead candidate, NCGC607, and these findings were translated to iPS-derived DA neurons from 6 GD Type I and Type II patients with and without parkinsonism, demonstrating increased GCase activity and translocation with reductions in both lipid substrates and a-syn accumulation (Aflaki et al., 2016b). A related study tested the effects of NCG758 in iPS DA neurons derived from patients with Type I GD, familial PD mutations, or iPD, and observed specific GCase activation with reduced a-syn accumulation and rescue of other downstream phenotypes across synucleinopathies (Mazzulli et al., 2016b). A chaperoning strategy focuses on modifying the activity of endogenous ER chaperones. For example, a small-molecule activator of the heat-shock response, celastrol, increased GCase activity in GD fibroblasts (Mu et al., 2008) by inhibiting Hsp90 mediated degradation of mutant GCase and increasing transcription of a number of stabilizing heat shock elements, such as BAG3 (Yang et al., 2014). Non-inhibitory small molecule chaperone therapy thus represents a promising approach for treating GCase dysfunction. As a main thesis of this review is the interrelationship between GD and PD pathogenesis, the variety of models used in these studies notably illustrates the potential of GCase chaperoning not only in cases of GBA1 mutation, but also as a treatment for iPD and synucleinopathy more generally (Barkhuizen et al., 2016; Sybertz and Krainc, 2014).

In addition to the development of GCase lysosomal activators, another strategy to reduce GluCer is by inhibiting the Golgi-resident enzyme glucosylceramide synthase (GCS). GCS inhibitors have been under development for the treatment of Gaucher disease, Fabry disease, and other lysosomal diseases characterized by accumulation of glycosphingolipids. Using a GCS inhibitor that is FDA approved for the treatment of type I GD (eliglustat), we recently showed that reducing GluCer in patient-derived iPSn can diminish a-syn pathology, and restore physiological conformations of a-syn (Zunke et al., 2017). Another study used an iminosugar that inhibits GCS, miglustat, to show that GluCer reduction could inhibit a-syn pathology that is artificially induced by the addition of synthetic pre-formed seed fibrils (Kim et al., 2018). While eliglustat and miglustat are effective in neuronal cell culture models, their ability to treat neuronopathic forms of GD or PD are limited due to the lack of penetrance of the blood-brain barrier. A recently developed GCS inhibitor that can pass the blood-brain barrier has been tested in animal models of GD and PD, and can reduce a-syn, tau, and improve cognitive function (Sardi et al., 2017). Therapeutic efficacy of GCS inhibitors will be assessed through a recently initiated clinical trial for PD patients that carry a *GBA1* mutation (NCT02906020 on clinicaltrials.gov).

Concluding Remarks

Significant progress has been made over the past several years regarding the mechanisms that cause neuronal dysfunction in GD and GBA1-PD. Much of the evidence indicates that loss-of-enzymatic activity of GCase leads to lysosomal substrate accumulation, disruption of autophagic-lysosomal function, and induction of p62 and a-syn pathology that could contribute to downstream disruption of essential cellular pathways (see the figure). Several studies have documented perturbations in the ER, including distorted ER morphology and elevated ER-derived cytosolic calcium, implicating a role for ER dysfunction as a contributor to cell death (see the figure). Emerging data over the last several years has shown that a-syn accumulation disrupts lysosomal function through direct or indirect (ie protein trafficking disruptions) mechanisms. A combination of damaging genetic variants, including *GBA1* mutations, along with other factors that contribute to a-syn accumulation may dictate the onset and severity of parkinsonism in *GBA1* carriers. Together, this indicates that *GBA1* mutations disrupt nearly all pathways that are essential for maintaining protein homeostasis, including protein folding in the ER, trafficking at the level of autophagic-lysosome or ER-Golgi, and cellular degradation through lysosomes. Compounds that specifically enhance each of these pathways in a controllable manner, including GCase chaperones, lysosomal activators, or general autophagic enhancers may have potential as promising therapies for GD and PD. Further development of GluCer reducing agents including brain-penetrant GCS inhibitors may hold promise for the treatment of neuronopathic GD and GBA1-PD. The use of patient-derived iPSn models to test therapeutic efficacy of these agents *in vitro* may help to guide future clinical trials in optimizing patient selection, by measuring how patient neurons respond to these treatments using established pathogenic phenotypes including a-syn accumulation and neurotoxicity.

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References

Aflaki E, Borger DK, Moaven N, Stubblefield BK, Rogers SA, Patnaik S, Schoenen FJ, Westbroek W, Zheng W, Sullivan P, et al. 2016a; A New Glucocerebrosidase Chaperone Reduces alpha-Synuclein and Glycolipid Levels in iPSC-Derived Dopaminergic Neurons from Patients with Gaucher Disease and Parkinsonism. J Neurosci. 36:7441–7452. [PubMed: 27413154]

Aflaki E, Moaven N, Borger DK, Lopez G, Westbroek W, Chae JJ, Marugan J, Patnaik S, Maniwang E, Gonzalez AN, et al. 2016b; Lysosomal storage and impaired autophagy lead to inflammasome activation in Gaucher macrophages. Aging Cell. 15:77–88. [PubMed: 26486234]

Aflaki E, Stubblefield BK, Maniwang E, Lopez G, Moaven N, Goldin E, Marugan J, Patnaik S, Dutra A, Southall N, et al. 2014; Macrophage Models of Gaucher Disease for Evaluating Disease Pathogenesis and Candidate Drugs. Science translational medicine. 6:240ra273–240ra273.

Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, Mazzoni P, Pauciulo MW, Nichols WC, Gan-Or Z, et al. 2015; Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. Brain. 138:2648–2658. [PubMed: 26117366]

Alvarez-Erviti L, Rodriguez-Oroz MC, Cooper JM, Caballero C, Ferrer I, Obeso JA, Schapira AHV. 2010; Chaperone-Mediated Autophagy Markers in Parkinson Disease Brains. Archives of Neurology. 67:16–25. [PubMed: 20065124]

Bae EJ, Yang NY, Song M, Lee CS, Lee JS, Jung BC, Lee HJ, Kim S, Masliah E, Sardi SP, et al. 2014; Glucocerebrosidase depletion enhances cell-to-cell transmission of alpha-synuclein. Nat Commun. 5:4755. [PubMed: 25156829]

Barkhuizen M, Anderson DG, Grobler AF. 2016; Advances in GBA-associated Parkinson's disease – Pathology, presentation and therapies. Neurochemistry International. 93:6–25. [PubMed: 26743617]

Basu A, Glew RH, Wherrett JR, Huterer S. 1986; Comparison of the ability of phospholipids from rat liver lysosomes to reconstitute glucocerebrosidase. Archives of biochemistry and biophysics. 245:464–469. [PubMed: 3954363]

Bendikov-Bar I, Horowitz M. 2012; Gaucher disease paradigm: from ERAD to comorbidity. Human mutation. 33:1398–1407. [PubMed: 22623374]

Bendikov-Bar I, Maor G, Filocamo M, Horowitz M. 2013; Ambroxol as a pharmacological chaperone for mutant glucocerebrosidase. Blood cells, molecules & diseases. 50:141–145.

Bennett LL, Mohan D. 2013; Gaucher Disease and Its Treatment Options. Annals of Pharmacotherapy. 47:1182–1193. [PubMed: 24259734]

Berg-Fussman A, Grace ME, Ioannou Y, Grabowski GA. 1993; Human acid beta-glucosidase. N-glycosylation site occupancy and the effect of glycosylation on enzymatic activity. J Biol Chem. 268:14861–14866. [PubMed: 8325864]

Beutler E. 1992; Gaucher disease: new molecular approaches to diagnosis and treatment. Science (New York, NY). 256:794–799.

Blanz J, Saftig P. 2016; Parkinson's disease: acid-glucocerebrosidase activity and alpha-synuclein clearance. J Neurochem. 139(Suppl 1):198–215. [PubMed: 26860955]

Blanz J, Zunke F, Markmann S, Damme M, Braulke T, Saftig P, Schwake M. 2015; Mannose 6-phosphate-independent Lysosomal Sorting of LIMP-2. Traffic (Copenhagen, Denmark). 16:1127–1136.

Cao K, Graziotto JJ, Blair CD, Mazzulli JR, Erdos MR, Krainc D, Collins FS. 2011; Rapamycin reverses cellular phenotypes and enhances mutant protein clearance in Hutchinson-Gilford progeria syndrome cells. Sci Transl Med. 3:89ra58.

Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, Kerchner GA, Ayalon G, Bingol B, Sheng M, et al. 2017; A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nature genetics. 49:1511–1516. [PubMed: 28892059]

Chen L, Xie Z, Turkson S, Zhuang X. 2015; A53T Human -Synuclein Overexpression in Transgenic Mice Induces Pervasive Mitochondria Macroautophagy Defects Preceding Dopamine Neuron Degeneration. Journal of Neuroscience. 35:890–905. [PubMed: 25609609]

Choi SH, Kim YH, Hebisch M, Sliwinski C, Lee S, D'Avanzo C, Chen H, Hooli B, Asselin C, Muffat J, et al. 2014; A three-dimensional human neural cell culture model of Alzheimer's disease. Nature. 515:274–278. [PubMed: 25307057]

Choubey V, Safiulina D, Vaarmann A, Cagalinec M, Wareski P, Kuum M, Zharkovsky A, Kaasik A. 2011; Mutant A53T α-Synuclein Induces Neuronal Death by Increasing Mitochondrial Autophagy. Journal of Biological Chemistry. 286:10814–10824. [PubMed: 21252228]

Chung, Sun Y; Kishinevsky, S; Mazzulli, Joseph R; Graziotto, J; Mrejeru, A; Mosharov, Eugene V; Puspita, L; Valiulahi, P; Sulzer, D; Milner, Teresa A; , et al. 2016; Parkin and PINK1 Patient iPSC-Derived Midbrain Dopamine Neurons Exhibit Mitochondrial Dysfunction and α-Synuclein Accumulation. Stem Cell Reports. 7:664–677. [PubMed: 27641647]

Clark LN, Ross BM, Wang Y, Mejia-Santana H, Harris J, Louis ED, Cote LJ, Andrews H, Fahn S, Waters C, et al. 2007; Mutations in the glucocerebrosidase gene are associated with early-onset Parkinson disease. Neurology. 69:1270–1277. [PubMed: 17875915]

Cleeter MWJ, Chau KY, Gluck C, Mehta A, Hughes DA, Duchen M, Wood NW, Hardy J, Mark Cooper J, Schapira AH. 2013; Glucocerebrosidase inhibition causes mitochondrial dysfunction and free radical damage. Neurochemistry international. 62:1–7. [PubMed: 23099359]

Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathearn KE, Liu F, et al. 2006; Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science (New York, NY). 313:324–328.

Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T, et al. 2012; Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. Sci Transl Med. 4:141ra190.

Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. 2004; Impaired Degradation of Mutant -Synuclein by Chaperone-Mediated Autophagy. Science. 305:1292–1295. [PubMed: 15333840]

Dandana A, Ben Khelifa S, Chahed H, Miled A, Ferchichi S. 2016; Gaucher Disease: Clinical, Biological and Therapeutic Aspects. Pathobiology: journal of immunopathology, molecular and cellular biology. 83:13–23.

Davis MY, Trinh K, Thomas RE, Yu S, Germanos AA, Whitley BN, Sardi SP, Montine TJ, Pallanck LJ. 2016; Glucocerebrosidase Deficiency in Drosophila Results in α-Synuclein-Independent Protein Aggregation and Neurodegeneration. PLoS Genetics. 12:e1005944. [PubMed: 27019408]

Farfel-Becker T, Vitner EB, Kelly SL, Bame JR, Duan J, Shinder V, Merrill AH, Dobrenis K, Futerman AH. 2014; Neuronal accumulation of glucosylceramide in a mouse model of neuronopathic Gaucher disease leads to neurodegeneration. Human Molecular Genetics. 23:843–854. [PubMed: 24064337]

Fernandes HJ, Hartfield EM, Christian HC, Emmanoulidou E, Zheng Y, Booth H, Bogetofte H, Lang C, Ryan BJ, Sardi SP, et al. 2016; ER Stress and Autophagic Perturbations Lead to Elevated Extracellular alpha-Synuclein in GBA-N370S Parkinson's iPSC-Derived Dopamine Neurons. Stem Cell Reports. 6:342–356. [PubMed: 26905200]

Fernández-Santiago R, Carballo-Carbajal I, Castellano G, Torrent R, Richaud Y, Sánchez-Danés A, Vilarrasa-Blasi R, Sánchez-Pla A, Mosquera JL, Soriano J, et al. 2015; Aberrant epigenome in iPSC-derived dopaminergic neurons from Parkinson's disease patients. EMBO Molecular Medicine. 7:1529–1546. [PubMed: 26516212]

Forman MS, Trojanowski JQ, Lee VM. 2004; Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med. 10:1055–1063. [PubMed: 15459709]

Friedman LG, Lachenmayer ML, Wang J, He L, Poulose SM, Komatsu M, Holstein GR, Yue Z. 2012; Disrupted Autophagy Leads to Dopaminergic Axon and Dendrite Degeneration and Promotes Presynaptic Accumulation of -Synuclein and LRRK2 in the Brain. Journal of Neuroscience. 32:7585–7593. [PubMed: 22649237]

Gegg ME, Burke D, Heales SJR, Cooper JM, Hardy J, Wood NW, Schapira AHV. 2012; Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. Annals of Neurology. 72:455–463. [PubMed: 23034917]

Goker-Alpan O, Giasson BI, Eblan MJ, Nguyen J, Hurtig HI, Lee VMY, Trojanowski JQ, Sidransky E. 2006; Glucocerebrosidase mutations are an important risk factor for Lewy body disorders. Neurology. 67:908–910. [PubMed: 16790605]

Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. 2004; Parkinsonism among Gaucher disease carriers. Journal of medical genetics. 41:937–940. [PubMed: 15591280]

Goker-Alpan O, Stubblefield BK, Giasson BI, Sidransky E. 2010; Glucocerebrosidase is present in alpha-synuclein inclusions in Lewy body disorders. Acta neuropathologica. 120:641–649. [PubMed: 20838799]

Goldin E, Zheng W, Motabar O, Southall N, Choi JH, Marugan J, Austin CP, Sidransky E. 2012; High Throughput Screening for Small Molecule Therapy for Gaucher Disease Using Patient Tissue as the Source of Mutant Glucocerebrosidase. PLoS ONE. 7:e29861. [PubMed: 22272254]

Grabowski GA. 2008; Phenotype, diagnosis, and treatment of Gaucher's disease. Lancet. 372:1263–1271. [PubMed: 19094956]

Grace ME, Grabowski GA. 1990; Human acid beta-glucosidase: glycosylation is required for catalytic activity. Biochemical and biophysical research communications. 168:771–777. [PubMed: 2110456]

Halperin A, Elstein D, Zimran A. 2006; Increased incidence of Parkinson disease among relatives of patients with Gaucher disease. Blood cells, molecules & diseases. 36:426–428.

Hernandez DG, Reed X, Singleton AB. 2016; Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. J Neurochem. 139(Suppl 1):59–74. [PubMed: 27090875]

Jian J, Zhao S, Tian QY, Liu H, Zhao Y, Chen WC, Grunig G, Torres PA, Wang BC, Zeng B, et al. 2016Association Between Progranulin and Gaucher Disease. EBioMedicine. :127–137.

Jo J, Xiao Y, Sun AX, Cukuroglu E, Tran HD, Goke J, Tan ZY, Saw TY, Tan CP, Lokman H, et al. 2016; Midbrain-like Organoids from Human Pluripotent Stem Cells Contain Functional Dopaminergic and Neuromelanin-Producing Neurons. Cell Stem Cell. 19:248–257. [PubMed: 27476966]

Joksimovic M, Yun BA, Kittappa R, Anderegg AM, Chang WW, Taketo MM, McKay RD, Awatramani RB. 2009; Wnt antagonism of Shh facilitates midbrain floor plate neurogenesis. Nat Neurosci. 12:125–131. [PubMed: 19122665]

Jovic M, Kean MJ, Szentpetery Z, Polevoy G, Gingras AC, Brill JA, Balla T. 2012; Two phosphatidylinositol 4-kinases control lysosomal delivery of the Gaucher disease enzyme, beta-glucocerebrosidase. Mol Biol Cell. 23:1533–1545. [PubMed: 22337770]

Jung O, Patnaik S, Marugan J, Sidransky E, Westbroek W. 2016; Progress and potential of non-inhibitory small molecule chaperones for the treatment of Gaucher disease and its potential implications for Parkinson disease. Expert review of proteomics. 13:471–479. [PubMed: 27098312]

Khanna R, Benjamin ER, Pellegrino L, Schilling A, Rigat BA, Soska R, Nafar H, Ranes BE, Feng J, Lun Y, et al. 2010; The Pharmacological Chaperone Isofagomine Increases Activity of the Gaucher Disease L444P Mutant Form of β-Glucosidase. The FEBS journal. 277:1618–1638. [PubMed: 20148966]

Kim S, Yun SP, Lee S, Umanah GE, Bandaru VVR, Yin X, Rhee P, Karuppagounder SS, Kwon SH, Lee H, et al. 2018GBA1 deficiency negatively affects physiological alpha-synuclein tetramers and related multimers. Proc Natl Acad Sci U S A.

Kinghorn KJ, Grönke S, Castillo-Quan JI, Woodling NS, Li L, Sirka E, Gegg M, Mills K, Hardy J, Bjedov I, et al. 2016; A Drosophila Model of Neuronopathic Gaucher Disease Demonstrates Lysosomal-Autophagic Defects and Altered mTOR Signalling and Is Functionally Rescued by Rapamycin. The Journal of neuroscience: the official journal of the Society for Neuroscience. 36:11654–11670. [PubMed: 27852774]

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, et al. 2006; Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 441:880–884. [PubMed: 16625205]

Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, et al. 2007; Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell. 131:1149–1163. [PubMed: 18083104]

Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, et al. 2011; Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature. 480:547–551. [PubMed: 22056989]

Li W, Lesuisse C, Xu Y, Troncoso JC, Price DL, Lee MK. 2004; Stabilization of alpha-synuclein protein with aging and familial parkinson's disease-linked A53T mutation. The Journal of neuroscience: the official journal of the Society for Neuroscience. 24:7400–7409. [PubMed: 15317865]

Li Y, Li P, Liang H, Zhao Z, Hashimoto M, Wei J. 2015; Gaucher-Associated Parkinsonism. Cellular and molecular neurobiology. 35:755–761. [PubMed: 25820783]

Liou B, Peng Y, Li R, Inskeep V, Zhang W, Quinn B, Dasgupta N, Blackwood R, Setchell KD, Fleming S, et al. 2016; Modulating ryanodine receptors with dantrolene attenuates neuronopathic phenotype in Gaucher disease mice. Hum Mol Genet. 25:5126–5141. [PubMed: 27655403]

Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, et al. 2011; Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature. 471:68–73. [PubMed: 21289626]

Magalhaes J, Gegg ME, Migdalska-Richards A, Doherty MK, Whitfield PD, Schapira AHV. 2016; Autophagic lysosome reformation dysfunction in glucocerebrosidase deficient cells: relevance to Parkinson disease. Human Molecular Genetics. 25:3432–3445. [PubMed: 27378698]

Manning-Boğ AB, Schüle B, Langston JW. 2009; Alpha-synuclein-glucocerebrosidase interactions in pharmacological Gaucher models: A biological link between Gaucher disease and parkinsonism. NeuroToxicology. 30:1127–1132. [PubMed: 19576930]

Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, Hodara R, Fredenburg R, Wu DC, Follenzi A, et al. 2008; Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest. 118:777–788. [PubMed: 18172548]

Mazzulli, Joseph R; Xu, YH; Sun, Y; Knight, Adam L; McLean, Pamela J; Caldwell, Guy A; Sidransky, E; Grabowski, Gregory A; Krainc, D. 2011; Gaucher Disease Glucocerebrosidase and α-Synuclein Form a Bidirectional Pathogenic Loop in Synucleinopathies. Cell. 146:37–52. [PubMed: 21700325]

Mazzulli JR, Zunke F, Isacson O, Studer L, Krainc D. 2016a; alpha-Synuclein-induced lysosomal dysfunction occurs through disruptions in protein trafficking in human midbrain synucleinopathy models. Proc Natl Acad Sci U S A. 113:1931–1936. [PubMed: 26839413]

Mazzulli JR, Zunke F, Tsunemi T, Toker NJ, Jeon S, Burbulla LF, Patnaik S, Sidransky E, Marugan JJ, Sue CM, et al. 2016b; Activation of beta-Glucocerebrosidase Reduces Pathological alpha-Synuclein and Restores Lysosomal Function in Parkinson's Patient Midbrain Neurons. J Neurosci. 36:7693–7706. [PubMed: 27445146]

McNeill A, Magalhaes J, Shen C, Chau KY, Hughes D, Mehta A, Foltynie T, Cooper JM, Abramov AY, Gegg M, et al. 2014; Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. Brain. 137:1481–1495. [PubMed: 24574503]

Mekhoubad S, Bock C, de Boer AS, Kiskinis E, Meissner A, Eggan K. 2012; Erosion of dosage compensation impacts human iPSC disease modeling. Cell Stem Cell. 10:595–609. [PubMed: 22560080]

Menzies FM, Fleming A, Caricasole A, Bento CF, Andrews SP, Ashkenazi A, Fullgrabe J, Jackson A, Jimenez Sanchez M, Karabiyik C, et al. 2017; Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities. Neuron. 93:1015–1034. [PubMed: 28279350]

Migdalska-Richards A, Schapira AHV. 2016; The relationship between glucocerebrosidase mutations and Parkinson disease. Journal of neurochemistry. 139(Suppl 1):77–90. [PubMed: 26860875]

Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal P, Vera E, Shim J-w, Kriks S, et al. 2013; Human iPSC-based Modeling of Late-Onset Disease via Progerin-induced Aging. Cell stem cell. 13:691–705. [PubMed: 24315443]

Mu TW, Ong DS, Wang YJ, Balch WE, Yates JR 3rd, Segatori L, Kelly JW. 2008; Chemical and biological approaches synergize to ameliorate protein-folding diseases. Cell. 134:769–781. [PubMed: 18775310]

Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, Cooper A, Garner B, Halliday GM. 2014; Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson's disease. Brain. 137:834–848. [PubMed: 24477431]

Murphy KE, Halliday GM. 2014; Glucocerebrosidase deficits in sporadic Parkinson disease. Autophagy. 10:1350–1351. [PubMed: 24915553]

Murray GJ, Jin FS. 1995; Immunoelectron microscopic localization of mannose-terminal glucocerebrosidase in lysosomes of rat liver Kupffer cells. The journal of histochemistry and cytochemistry: official journal of the Histochemistry Society. 43:149–158. [PubMed: 7822772]

Narita A, Shirai K, Itamura S, Matsuda A, Ishihara A, Matsushita K, Fukuda C, Kubota N, Takayama R, Shigematsu H, et al. 2016; Ambroxol chaperone therapy for neuronopathic Gaucher disease: A pilot study. Annals of Clinical and Translational Neurology. 3:200–215. [PubMed: 27042680]

Neudorfer O, Giladi N, Elstein D, Abrahamov A, Turezkite T, Aghai E, Reches A, Bembi B, Zimran A. 1996; Occurrence of Parkinson's syndrome in type 1 Gaucher disease. QJM. 89:691–694. [PubMed: 8917744]

Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, Cortes E, Honig LS, Dauer W, Consiglio A, et al. 2013; Interplay of LRRK2 with chaperone-mediated autophagy. Nature Neuroscience. 16:394–406. [PubMed: 23455607]

Osellame, Laura D; Rahim, Ahad A; Hargreaves, Iain P; Gegg, Matthew E; Richard-Londt, A; Brandner, S; Waddington, Simon N; Schapira, Anthony HV; Duchen, Michael R. 2013;

Mitochondria and Quality Control Defects in a Mouse Model of Gaucher Disease—Links to Parkinson's Disease. Cell Metabolism. 17:941–953. [PubMed: 23707074]

Pan PY, Yue Z. 2014Genetic causes of Parkinson's disease and their links to autophagy regulation.

Pandey MK, Burrow TA, Rani R, Martin LJ, Witte D, Setchell KD, McKay MA, Magnusen AF, Zhang W, Liou B, et al. 2017; Complement drives glucosylceramide accumulation and tissue inflammation in Gaucher disease. Nature. 543:108–112. [PubMed: 28225753]

Park JS, Mehta P, Cooper AA, Veivers D, Heimbach A, Stiller B, Kubisch C, Fung VS, Krainc D, Mackay-Sim A, et al. 2011; Pathogenic effects of novel mutations in the P-type ATPase ATP13A2 (PARK9) causing Kufor-Rakeb syndrome, a form of early-onset parkinsonism. Human mutation. 32:956–964. [PubMed: 21542062]

Pastores GM, Weinreb NJ, Aerts H, Andria G, Cox TM, Giralt M, Grabowski GA, Mistry PK, Tylki-Szymańska A. 2004; Therapeutic goals in the treatment of Gaucher disease. Seminars in hematology. 41:4–14.

Patnaik S, Zheng W, Choi JH, Motabar O, Southall N, Westbroek W, Lea WA, Velayati A, Goldin E, Sidransky E, et al. 2012; Discovery, SAR, and Biological evaluation of Non-Inhibitory Small Molecule Chaperones of Glucocerebrosidase. Journal of Medicinal Chemistry. 55:5734–5748. [PubMed: 22646221]

Pickrell AM, Youle RJ. 2015; The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron. 85:257–273. [PubMed: 25611507]

Plowey ED, Cherra SJ, Liu YJ, Chu CT. 2008; Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. Journal of neurochemistry. 105:1048–1056. [PubMed: 18182054]

Qi X, Grabowski GA. 1998; Acid beta-glucosidase: intrinsic fluorescence and conformational changes induced by phospholipids and saposin C. Biochemistry. 37:11544–11554. [PubMed: 9708990]

Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, et al. 2006; Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nature Genetics. 38:1184. [PubMed: 16964263]

Reczek D, Schwake M, Schroder J, Hughes H, Blanz J, Jin X, Brondyk W, Van Patten S, Edmunds T, Saftig P. 2007; LIMP-2 is a receptor for lysosomal mannose-6-phosphate-independent targeting of beta-glucocerebrosidase. Cell. 131:770–783. [PubMed: 18022370]

Richter F, Fleming SM, Watson M, Lemesre V, Pellegrino L, Ranes B, Zhu C, Mortazavi F, Mulligan CK, Sioshansi PC, et al. 2014; A GCase Chaperone Improves Motor Function in a Mouse Model of Synucleinopathy. Neurotherapeutics. 11:840–856. [PubMed: 25037721]

Robak LA, Jansen IE, van Rooij J, Uitterlinden AG, Kraaij R, Jankovic J, Heutink P, Shulman JM, members IC, et al. International Parkinson's Disease Genomics C. 2017; Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain. 140:3191–3203. [PubMed: 29140481]

Rocha EM, Smith GA, Park E, Cao H, Graham AR, Brown E, McLean JR, Hayes MA, Beagan J, Izen SC, et al. 2015; Sustained Systemic Glucocerebrosidase Inhibition Induces Brain α-Synuclein Aggregation, Microglia and Complement C1q Activation in Mice. Antioxidants & redox signaling. 23:550–564. [PubMed: 26094487]

Ron, IaHM. 2005ER retention and degradation as the molecular basis underlying Gaucher disease heterogeneity.

Roosen DA, Cookson MR. 2016; LRRK2 at the interface of autophagosomes, endosomes and lysosomes. Mol Neurodegener. 11:73. [PubMed: 27927216]

Rothaug M, Zunke F, Mazzulli JR, Schweizer M, Altmeppen H, Lullmann-Rauch R, Kallemeijn WW, Gaspar P, Aerts JM, Glatzel M, et al. 2014; LIMP-2 expression is critical for beta-glucocerebrosidase activity and alpha-synuclein clearance. Proc Natl Acad Sci U S A. 111:15573–15578. [PubMed: 25316793]

Ryan SD, Dolatabadi N, Chan SF, Zhang X, Akhtar MW, Parker J, Soldner F, Sunico CR, Nagar S, Talantova M, et al. 2013; Isogenic human iPSC Parkinson's model shows nitrosative stress-induced dysfunction in MEF2-PGC1alpha transcription. Cell. 155:1351–1364. [PubMed: 24290359]

Sanchez-Danes A, Richaud-Patin Y, Carballo-Carbajal I, Jimenez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giralt A, et al. 2012; Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. EMBO Mol Med. 4:380–395. [PubMed: 22407749]

Sardi SP, Clarke J, Viel C, Chan M, Tamsett TJ, Treleaven CM, Bu J, Sweet L, Passini MA, Dodge JC, et al. 2013; Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. Proceedings of the National Academy of Sciences. 110:3537–3542.

Sardi SP, Viel C, Clarke J, Treleaven CM, Richards AM, Park H, Olszewski MA, Dodge JC, Marshall J, Makino E, et al. 2017; Glucosylceramide synthase inhibition alleviates aberrations in synucleinopathy models. Proc Natl Acad Sci U S A. 114:2699–2704. [PubMed: 28223512]

Savitt IA, Yideng L, Sabitha S, Valina LD, Ted MD, Joseph M. 2012Development and Characterization of a New Parkinson's Disease Model Resulting from Impaired Autophagy.

Sawkar AR, Cheng WC, Beutler E, Wong CH, Balch WE, Kelly JW. 2002; Chemical chaperones increase the cellular activity of N370S β-glucosidase: A therapeutic strategy for Gaucher disease. Proceedings of the National Academy of Sciences of the United States of America. 99:15428–15433. [PubMed: 12434014]

Sawkar AR, Schmitz M, Zimmer KP, Reczek D, Edmunds T, Balch WE, Kelly JW. 2006; Chemical Chaperones and Permissive Temperatures Alter the Cellular Localization of Gaucher Disease Associated Glucocerebrosidase Variants. ACS Chemical Biology. 1:235–251. [PubMed: 17163678]

Schöndorf DC, Aureli M, McAllister FE, Hindley CJ, Mayer F, Schmid B, Sardi SP, Valsecchi M, Hoffmann S, Schwarz LK, et al. 2014; iPSC-derived neurons from GBA1-associated Parkinson's disease patients show autophagic defects and impaired calcium homeostasis. Nature Communications. 5:4028–4028.

Shemesh E, Deroma L, Bembi B, Deegan P, Hollak C, Weinreb NJ, Cox TM. 2015Enzyme replacement and substrate reduction therapy for Gaucher disease. The Cochrane database of systematic reviews. :CD010324–CD010324. [PubMed: 25812601]

Shi Y, Inoue H, Wu JC, Yamanaka S. 2017; Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov. 16:115–130. [PubMed: 27980341]

Sidransky E. 2012; Gaucher disease: insights from a rare Mendelian disorder. Discovery medicine. 14:273–281. [PubMed: 23114583]

Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A, et al. 2009; Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease. New England Journal of Medicine. 361:1651–1661. [PubMed: 19846850]

Soldner F, Stelzer Y, Shivalila CS, Abraham BJ, Latourelle JC, Barrasa MI, Goldmann J, Myers RH, Young RA, Jaenisch R. 2016; Parkinson-associated risk variant in distal enhancer of alpha-synuclein modulates target gene expression. Nature. 533:95–99. [PubMed: 27096366]

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. 1997; Alpha-synuclein in Lewy bodies. Nature. 388:839–840. [PubMed: 9278044]

Steet RA, Chung S, Wustman B, Powe A, Do H, Kornfeld SA. 2006; The iminosugar isofagomine increases the activity of N370S mutant acid β-glucosidase in Gaucher fibroblasts by several mechanisms. Proceedings of the National Academy of Sciences of the United States of America. 103:13813–13818. [PubMed: 16945909]

Steger M, Tonelli F, Ito G, Davies P, Trost M, Vetter M, Wachter S, Lorentzen E, Duddy G, Wilson S, et al. 2016; Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. Elife. 5

Su YC, Qi X. 2013; Inhibition of excessive mitochondrial fission reduced aberrant autophagy and neuronal damage caused by LRRK2 G2019S mutation. Human Molecular Genetics. 22:4545–4561. [PubMed: 23813973]

Sun Y, Liou B, Ran H, Skelton MR, Williams MT, Vorhees CV, Kitatani K, Hannun YA, Witte DP, Xu YH, et al. 2010; Neuronopathic Gaucher disease in the mouse: viable combined selective saposin C deficiency and mutant glucocerebrosidase (V394L) mice with glucosylsphingosine and

glucosylceramide accumulation and progressive neurological deficits. Hum Mol Genet. 19:1088–1097. [PubMed: 20047948]

Sun Y, Ran H, Liou B, Quinn B, Zamzow M, Zhang W, Bielawski J, Kitatani K, Setchell KDR, Hannun YA, et al. 2011; Isofagomine In Vivo Effects in a Neuronopathic Gaucher Disease Mouse. PLoS ONE. 6:e19037. [PubMed: 21533102]

Sybertz E, Krainc D. 2014; Development of targeted therapies for Parkinson's disease and related synucleinopathies. Journal of lipid research. 55:1996–2003. [PubMed: 24668939]

Tamargo RJ, Velayati A, Goldin E, Sidransky E. 2012; The role of saposin C in Gaucher disease. Mol Genet Metab. 106:257–263. [PubMed: 22652185]

Tan, Yun L; Genereux, Joseph C; Pankow, S; Aerts, Johannes MFG; Yates, John R; Kelly, Jeffery W. 2014; ERdj3 Is an Endoplasmic Reticulum Degradation Factor for Mutant Glucocerebrosidase Variants Linked to Gaucher's Disease. Chemistry & Biology. 21:967–976. [PubMed: 25126989]

Tang FL, Liu W, Hu JX, Erion JR, Ye J, Mei L, Xiong WC. 2015a; VPS35 deficiency or mutation causes dopaminergic neuronal loss by impairing mitochondrial fusion and function. Cell reports. 12:1631–1643. [PubMed: 26321632]

Tang FL, Erion JR, Tian Y, Liu W, Yin DM, Ye J, Tang B, Mei L, Xiong WC. 2015b; VPS35 in Dopamine Neurons Is Required for Endosome-to-Golgi Retrieval of Lamp2a, a Receptor of Chaperone-Mediated Autophagy That Is Critical for -Synuclein Degradation and Prevention of Pathogenesis of Parkinson's Disease. Journal of Neuroscience. 35:10613–10628. [PubMed: 26203154]

Tayebi N, Callahan M, Madike V, Stubblefield BK, Orvisky E, Krasnewich D, Fillano JJ, Sidransky E. 2001; Gaucher disease and parkinsonism: a phenotypic and genotypic characterization. Mol Genet Metab. 73:313–321. [PubMed: 11509013]

Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D. 2012; Deficiency of ATP13A2 leads to lysosomal dysfunction, α-synuclein accumulation and neurotoxicity. The Journal of neuroscience: the official journal of the Society for Neuroscience. 32:4240–4246. [PubMed: 22442086]

Wang W, Wang X, Fujioka H, Hoppel C, Whone AL, Caldwell MA, Cullen PJ, Liu J, Zhu X. 2016; Parkinson's disease-associated mutant VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes. Nature medicine. 22:54–63.

Westbroek W, Gustafson AM, Sidransky E. 2011; Exploring the link between glucocerebrosidase mutations and parkinsonism. Trends in Molecular Medicine. 17:485–493. [PubMed: 21723784]

Willemsen R, van Dongen JM, Aerts JM, Schram AW, Tager JM, Goudsmit R, Reuser AJ. 1988; An immunoelectron microscopic study of glucocerebrosidase in type 1 Gaucher's disease spleen. Ultrastructural pathology. 12:471–478. [PubMed: 3194992]

Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S, et al. 2010; α-Synuclein impairs macroautophagy: implications for Parkinson's disease. The Journal of cell biology. 190:1023–1037. [PubMed: 20855506]

Wong K, Sidransky E, Verma A, Mixon T, Sandberg GD, Wakefield LK, Morrison A, Lwin A, Colegial C, Allman JM, et al. 2004; Neuropathology provides clues to the pathophysiology of Gaucher disease. Mol Genet Metab. 82:192–207. [PubMed: 15234332]

Woodard CM, Campos BA, Kuo SH, Nirenberg MJ, Nestor MW, Zimmer M, Mosharov E, Sulzer D, Zhou H, Paull D, et al. 2014; iPS Cell-Derived Dopamine Neurons Reveal Differences between Monozygotic Twins Discordant for Parkinson's Disease. Cell Rep. 9:1173–1182. [PubMed: 25456120]

Wu H, Xu J, Pang ZP, Ge W, Kim KJ, Blanchi B, Chen C, Sudhof TC, Sun YE. 2007; Integrative genomic and functional analyses reveal neuronal subtype differentiation bias in human embryonic stem cell lines. Proc Natl Acad Sci U S A. 104:13821–13826. [PubMed: 17693548]

Xilouri M, Brekk OR, Stefanis L. 2016; Autophagy and Alpha-Synuclein: Relevance to Parkinson's Disease and Related Synucleopathies. Movement Disorders. 31:178–192. [PubMed: 26813776]

Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L. 2009; Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One. 4:e5515. [PubMed: 19436756]

Xu YH, Xu K, Sun Y, Liou B, Quinn B, Li RH, Xue L, Zhang W, Setchell KD, Witte D, et al. 2014; Multiple pathogenic proteins implicated in neuronopathic Gaucher disease mice. Hum Mol Genet. 23:3943–3957. [PubMed: 24599400]

Yamamoto A, Yue Z. 2014; Autophagy and its normal and pathogenic states in the brain. Annu Rev Neurosci. 37:55–78. [PubMed: 24821313]

Yang C, Rahimpour S, Lu J, Pacak K, Ikejiri B, Brady Ro, Zhuang Z. 2013Histone deacetylase inhibitors increase glucocerebrosidase activity in Gaucher disease by modulation of molecular chaperones.

Yang C, Swallows CL, Zhang C, Lu J, Xiao H, Brady RO, Zhuang Z. 2014; Celastrol increases glucocerebrosidase activity in Gaucher disease by modulating molecular chaperones. Proceedings of the National Academy of Sciences of the United States of America. 111:249–254. [PubMed: 24351928]

Yang C, Wang H, Zhu D, Hong CS, Dmitriev P, Zhang C, Li Y, Ikejiri B, Brady RO, Zhuang Z. 2015Mutant glucocerebrosidase in Gaucher disease recruits Hsp27 to the Hsp90 chaperone complex for proteasomal degradation.

Zhang H, Duan C, Yang H. 2015; Defective Autophagy in Parkinson's Disease: Lessons from Genetics. Molecular Neurobiology. 51:89–104. [PubMed: 24990317]

Zimran A, Altarescu G, Elstein D. 2013; Pilot study using ambroxol as a pharmacological chaperone in type 1 Gaucher disease. Blood Cells, Molecules, and Diseases. 50:134–137.

Zunke F, Andresen L, Wesseler S, Groth J, Arnold P, Rothaug M, Mazzulli JR, Krainc D, Blanz J, Saftig P, et al. 2016; Characterization of the complex formed by beta-glucocerebrosidase and the lysosomal integral membrane protein type-2. Proc Natl Acad Sci U S A. 113:3791–3796. [PubMed: 27001828]

Zunke F, Moise AC, Belur NR, Gelyana E, Stojkovska I, Dzaferbegovic H, Toker NJ, Jeon S, Fredriksen K, Mazzulli JR. 2017Reversible Conformational Conversion of alpha-Synuclein into Toxic Assemblies by Glucosylceramide. Neuron.

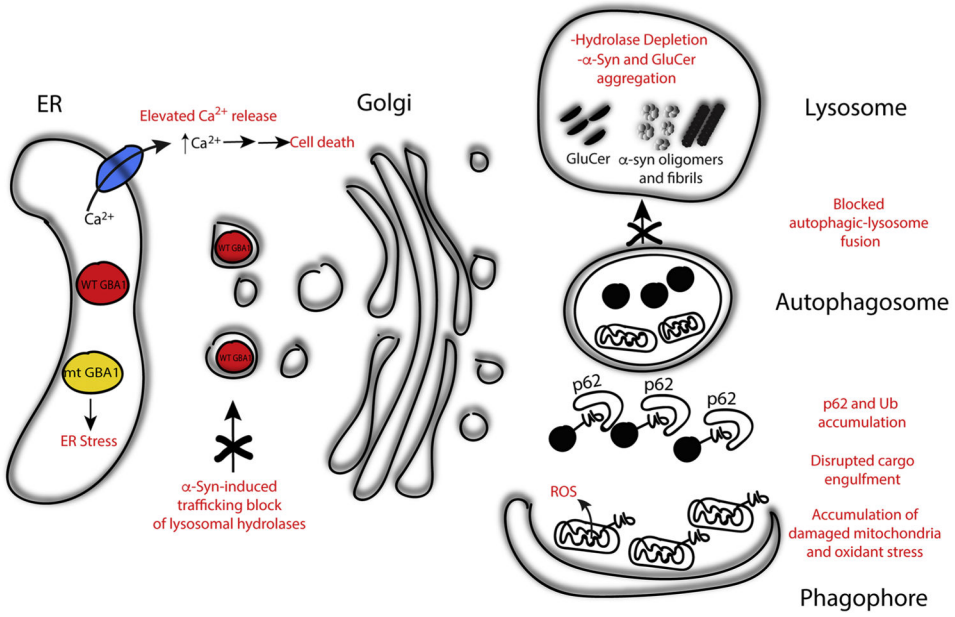

Figure. Mechanisms of GBA1-mutant induced disruption in GD and PD

Mutated *GBA1* (mt) may induce cellular dysfunction through perturbing multiple cellular pathways including ER stress, cytosolic calcium release from the ER, or disrupting lysosomal function. Expression of the mt *GBA1* itself or the accumulation of its substrate, GluCer, may cause disruptions in the autophagic-lysosomal system at many steps of the clearance process including substrate engulfment (as indicated by accumulation of ubiquitinated (Ub) and p62 inclusions), disrupted mitophagy leading to reactive oxygen species (ROS), or blocked autophagic lysosomal function. A-syn, once accumulated in pathogenic conformations of oligomers or fibrils, can further amplify dysfunctional cellular clearance through impeding the ER-Golgi trafficking of hydrolases such as wild-type (WT) *GBA1* into lysosomes, or interfering directly with the autophagic delivery system. *GBA1* mutations likely combine with other cellular perturbations including oxidation or a-syn aggregation, leading to neurotoxicity. Red text indicates possible pathogenic functions induced by *GBA1* mutations. ER, endoplasmic reticulum; GluCer, glucosylceramide.