The role of glucocerebrosidase in Parkinson disease pathogenesis

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Abbreviations: ALP, autophagy lysosome pathway; ALR, autophagy lysosome reformation; AP, autophagosome; CBE, conduritol β-epoxide; CMA, chaperone mediated autophagy; CNS, central nervous system; DLB, dementia with Lewy bodies; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GCase, glucocerebrosidase; GD, Gaucher disease; GlcCer, glucosylceramide; GlcSph, glucosylsphingosine; iPS, inducible pluripotent stem cells; KD, knock down; KO, knock out; MEFs, mouse embryonic fibroblasts; PD, Parkinson disease; PD+GBA, PD with GBA mutations; TH, tyrosine hydroxylase; UPR, unfolded protein response; WT, wild type.

Keywords: Glucocerebrosidase, Parkinson disease, α-synuclein, unfolded protein response, autophagy.

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Abstract

GBA encodes the lysosomal enzyme glucocerebrosidase (GCase), an enzyme involved in sphingolipid metabolism. Mutations in the *GBA* gene are numerically the most important risk factor for developing Parkinson disease (PD) accounting for at least 5% of all PD cases. Furthermore, loss of GCase activity is found in sporadic PD brains. Lysosomal dysfunction is thought to play a principal role in PD pathogenesis and in particular its effect on the metabolism of α-synuclein. A hallmark of PD is the presence intraneuronal protein inclusions called Lewy bodies, which are composed mainly of α-synuclein. Cellular and animal models of GCase deficiency result in lysosomal dysfunction, and in particular the autophagy lysosome pathway, resulting in the accumulation of α-synuclein. Some forms of mutant GCase unfold in the endoplasmic reticulum activating the unfolded protein response, which might also contribute to PD pathogenesis. It has also been suggested that accumulation of GCase substrates glucosylceramide/glucosylsphingosine may contribute to *GBA*-PD pathogenesis. Mitochondrial dysfunction and neuroinflammation are associated with GCase deficiency and have also been implicated in the aetiology of PD. This review discusses these points and highlights potential treatments that might be effective in treating GCase deficiency in PD.

Glucocerebrosidase

The lysosomal enzyme glucocerebrosidase (GCase; also known as glucosylceramidase; EC. 3.2.1.45) is involved in sphingolipid metabolism catalysing the breakdown of glucosylceramide (GlcCer) to glucose and ceramide. Ceramide is a precursor for complex sphingolipids such as glycosphingolipids (e.g. GM1, GM2, GM3 gangliosides) and sphingomyelin, and can also act as a second messenger [1].

GCase is encoded by the *GBA* gene (1q22) and homozygous *GBA* mutations cause Gaucher disease (GD), the most common lysosomal storage disorder. More than 300 *GBA* mutations have been reported and can be point mutations, insertions, deletions, frame shifts, splice-site alterations or recombinant alleles. The point mutations c.1226A>G (N370S) and c.1448T>C (L444P) are the most commonly associated with GD [2]. The accumulation of GlcCer in macrophages in visceral tissue is the principal feature of GD [3,4], leading to hepatosplenomegaly, anaemia, thrombocytopenia and bone marrow infiltration [5]. There are three types of GD: type 1 has the visceral manifestations above, while types 2 and 3 also exhibit these symptoms but are also neuronopathic, with a median age of death at 9 months (type 2) or childhood to early adult hood (type 3)[5]. The N370S allele in combination with another *GBA* mutant allele (e.g. N370S or *GBA* compound heterozygote) is predictive of type 1 GD. L444P in combination with a complex allele (e.g. a *GBA* allele that has undergone recombination such as Rec*Nci*I) tends to result in type 2, while homozygous L444P alleles or compound heterozygote L444P with null alleles result in type 3 GD [2,5]. However, there is wide heterogeneity in clinical manifestation, even between patients with the same genotype [2,5]

Studies of endogenous GCase in fibroblasts or expression of recombinant proteins suggest that the intrinsic catalytic activity of N370S and L444P mutant GCase is decreased by 80-95% compared to wild-type [6–8]. Loss of GCase activity is not solely due to reduced catalytic activity, but also a reduction in GCase protein levels. Several GCase mutations, including N370S and L444P, unfold in the endoplasmic reticulum (ER) activating the unfolded protein response (UPR). When they are unable to be refolded, the mutant protein is extracted by chaperones and degraded by the proteasome [9–11], a process known as ER-associated degradation (ERAD). It has also been reported that N370S GCase is less able to associate with the physiological GCase activator saposin C and anionic phospholipids [7,12].

GBA and Parkinson disease

Parkinson disease (PD) is the second most common neurodegenerative disorder. The disease is characterised by the loss of dopaminergic neurons in the substantia nigra resulting in the typical symptoms of PD: bradykinesia, resting tremor and rigidity [13]. The pathological hallmark of PD is the presence in surviving neurons of protein inclusions known as Lewy bodies, which are predominantly composed of the protein α-synuclein.

Although type 1 GD patients were not thought to develop neurodegeneration, clinicians started to report that a subset of GD patients exhibited typical parkinsonian features [14,15]. A multicentre genetic analysis by Sidransky et al in 2009 confirmed the association between PD and *GBA* mutations [16]. This study calculated that the odds ratio of carrying a *GBA* mutation in patients with PD (heterozygote or homozygote) was 5.4 versus controls [16]. A smaller study in the British population reported an odds ratio of 3.7 [17]. The two most frequent *GBA* mutations associated with PD are N370S and L444P, accounting for up to 17-31% of all PD patients in the European Ashkenazi Jewish population, and 3% in non-Ashkenazi populations [16–18]. In addition to pathogenic GD-causing mutations, the *GBA* variant E326K predisposes to PD [19–21], with a high frequency (7.5%) in early age onset (≤50 years old) British PD cases [19].

While *GBA* mutations are numerically the most important genetic risk factor for developing PD, it should be noted that the majority of people with GD or heterozygote *GBA* mutations do not develop PD. By the age of 80, it has been estimated that 9.1% of GD patients will develop PD [22]. Estimates for heterozygote *GBA* mutation, range from 7.7% by age 80 in a United States study [22] to 15% in a UK cohort [23] and 29.7% in a French population [24],

although the latter study may be skewed as data was only from a familial PD cohort and therefore may contain other genetic risk factors.

No differences in Lewy body pathology have been reported between PD with GBA mutations (PD+GBA) and sporadic PD [17,25]. However the age of onset is approximately five years earlier in PD+GBA patients [16,17,26]. Furthermore, onset of PD in GD patients is reported to be earlier than heterozygote *GBA* carriers [22]. In addition to gene dosage, *GBA* mutations have been stratified into mild (cause type 1 GD; e.g. N370S) or severe mutations (cause type 2 or 3; e.g. L444P). Analysis has suggested that carriers of severe mutations have an earlier age of onset and a much greater odds ratio of developing PD, when compared to mild mutations [27].

Cognitive impairment is also thought to be more frequent in PD+GBA patients, when compared to sporadic PD [28–30], with carriers of severe mutations reported to be at greater risk of dementia than mild mutations [31]. *GBA* mutations have also been associated with an increased risk of developing dementia with Lewy bodies (DLB) and PD with dementia (odds ratios of 8.3 and 6.5, respectively)[32].

GCase activity in PD

GCase activity was found to be significantly decreased in post-mortem brain tissue from PD brains with heterozygote *GBA* mutations, with the greatest decrease of 58% found in the substantia nigra [33]. Western blotting of the same samples indicated that this was not only due to loss of catalytic activity, but also decreased protein expression. The mutations included N370S and L444P and it is likely that this decrease in protein expression was due to

ERAD. Markers of the UPR were increased in these brains [33], although other factors including calcium dysregulation and α-synuclein accumulation may contribute to this.

Analysis of sporadic PD brains also indicated a significant 33% decrease in GCase activity in the substantia nigra and was concomitant with a decrease in protein levels of the enzyme [33], The activities or expression of other lysosomal enzymes were not affected indicating that the decrease in GCase activity was not simply due to a general loss of lysosomal content or neuronal number. Other studies have also reported a decrease in GCase activity and protein expression in the anterior cingulate cortex of sporadic PD brains, relative to other lysosomal proteins except LAMP2 [34], and decreased GCase activity in the substantia nigra, caudate, putamen and hippocampus [35,36]. It should be noted that in one of these studies 2/26 PD samples had a heterozygote *GBA* mutation [36]. Decreased GCase activity has also been reported to be significantly decreased in the cerebrospinal fluid and dried blood spots of sporadic PD patients [20,37]. Analysis of GCase activity in these two bodily fluids may therefore be a useful biomarker for PD diagnosis.

GCase, lysosomal dysfunction and α-synuclein metabolism

In recent years dysfunction of the autophagy lysosome pathway (ALP) has become a principal suspect in PD pathogenesis. In particular, impairment in macroautophagy and chaperone mediated autophagy (CMA) have been implicated in the accumulation, aggregation and cell to cell transmission of α-synuclein.

Macroautophagy degrades macromolecules such as protein and lipids, but also larger structures, such as aggregated proteins and damaged organelles like mitochondria [38]. Cargo for degradation is engulfed by a phagophore membrane, which then expands to form a

double-membrane vesicle known as an autophagosome (AP). The AP then fuses with a lysosome, resulting in degradation of the sequestered cargo [39,40].

CMA involves the degradation of soluble monomeric proteins containing the pentapeptide motif KFERQ. Unfolded proteins are delivered to the lysosome by the chaperone hsc70, where the protein is translocated directly in to the lysosome by the integral membrane protein LAMP2A for degradation [38,41]. Degradation of α -synuclein can occur via both CMA and macroautophagy, with both processes reported to be impaired in PD [42–44].

Given the lysosomal localisation of GCase and the link between lysosomal dysfunction and PD, research has focused on the effect of decreased GCase activity on α-synuclein metabolism in cell and animal models.

Several papers have reported modest but significant increases in intracellular α-synuclein levels by western blotting in human midbrain neurons differentiated from inducible pluripotent stem cells (iPS) taken from GD patients (with or without PD) or PD patients with heterozygous *GBA* mutations [45–48]. These mutations included the common mutations N370S, L444P, recombinant alleles and null mutants. In Schondorf et al [46] neither the gene dosage, nor type of mutation, appeared to noticeably affect the degree of α-synuclein accumulation. Importantly they also showed that gene correction of *GBA* mutations lowered α-synuclein levels. Immunofluorescence for both α-synuclein and tyrosine hydroxylase (TH) suggested that in dopaminergic neurons with *GBA* mutations, the amount of α-synuclein detected in the soma was increased [47,49]. Cells differentiated from adipose stem cells taken from PD patients with heterozygote *GBA* mutations also exhibited an increase in a-synuclein levels [50]. The accumulation of α-synuclein was coincident with impaired lysosomal proteolysis of both long and short lived proteins and inhibition of macroautophagy flux [45,46,50]. Pulse chase experiments in neurons isolated from mice expressing human α-

synuclein (wild-type (WT) or A53T) indicated that the half-life of α-synuclein was increased in neurons with heterozygote L444P *Gba* compared to cells expressing wild-type *Gba* [51].

One iPS study derived from WT/N370S PD patients, did report inhibition of macroautophagy flux. However, instead of this resulting in accumulation of intracellular α-synuclein, this study found an increase in extracellular α-synuclein [52]. It is established that α-synuclein is released from cells under physiological conditions [53,54], and can be increased following ALP dysfunction [55]. Cell to cell transmission of a-synuclein has been implicated in the spreading of α-synuclein pathology in the brain [56,57] to account for the spread of Lewy bodies proposed by Braak et al [58]. Inhibition of GCase activity in primary mouse cortical neurons with the inhibitor conduritol β-epoxide (CBE) results in impaired macroautophagy flux, and both an increase in intracellular and extracellular α-synuclein [59]. Similarly, SH-SY5Y cells in which *GBA* expression was ablated using zinc finger nuclease technology, caused lysosomal dysfunction, and promoted the cell to cell transmission of α-synuclein aggregates [60].

The accumulation of α-synuclein has also been reported in mouse models, although these tend to be GD models, rather than heterozygote *Gba* models. The *Gba* knock out (KO) mouse models developed by Enquist et al die within weeks of birth [61]. However, evidence of increased insoluble oligomeric a-synuclein in the midbrain and α-synuclein deposits in the brain stem were detected prior to the neurodegeneration and neuroinflammation observed in this model [62]. Note that there was no evidence of α-synuclein accumulation or neurodegeneration in $Gba^{+/-}$ mice aged for 6 or 24 months [63,64]. A *Gba* KO model that also expresses human α-synuclein and does survive to adulthood exhibits increased phosphorylated α-synuclein (S129) in the CA3 region of the hippocampus and elevated aggregated α-synuclein species in brain homogenates [65]. Chronic administration of the

GCase inhibitor CBE to mice also results in α-synuclein deposits in the substantia nigra [66,67], including proteinase K insensitive aggregates [67].

Although *Drosophila melanogaster* do not express α-synuclein, KO of the *Drosophila GBA* homologs (*dGBA1a/b*), does result in accumulation of ubiquitin positive aggregates and increased levels of Ref(2)P, the *Drosophila* homolog of the autophagic protein p62 [68,69].

Mice with pathogenic GD mutations also exhibit α-synuclein pathology. Progressive accumulation of α-synuclein deposits was observed in the hippocampus and frontal cortex of the *Gba* D409V/D409V mouse [63]. These deposits were also ubiquitin-positive and proteinase K insensitive. *Gba* D409H or V394L GD mice that also bear a prosaposin hypomorph exhibit α-synuclein deposits [70]. Size exclusion chromatography and western blotting studies suggested that there was an increase in both soluble monomeric α-synuclein, as well as both soluble and insoluble oligomeric α-synuclein species in these mice [45].

In terms of heterozygote mutant *Gba* mouse models, total α -synuclein levels were the same in mice expressing human A53T α-synuclein regardless of whether it was WT or heterozygous L444P *Gba* [51]. However, there was a trend for increased phosphorylated S129 α-synuclein in the hippocampus [51]. However, when heterozygous L444P mice with endogenous α-synuclein were aged for 24 months a significant increase in α-synuclein was observed in the striatum [64]. Furthermore, when these mice were injected with AAV virus encoding human α-synuclein immediately dorsal to the substantia nigra, the loss of TH positive neurons was increased compared to wild-type mice

The use of viral vectors encoding *GBA* has reinforced the idea that GCase activity in the brain is critical for α-synuclein metabolism. Proteinase K insensitive α-synuclein aggregates have

been shown to be reduced in GD or A53T α-synuclein mouse models following expression of human recombinant GCase via viral vectors [71–73].

Mechanism for perturbed α-synuclein metabolism

While it is apparent that loss of lysosomal GCase affects a-synuclein metabolism, the exact mechanism is unclear and may differ according to genotype (e.g. GD versus heterozygote *GBA* mutations). Various markers of lysosomal dysfunction such as altered lysosomal content, abnormal lysosomal morphology and increased lysosomal pH have all been reported in cell models irrespective of whether the background is heterozygote or homozygote *GBA* mutations, KO, knockdown (KD) or CBE inhibition [45,46,52,59,60]. Inhibition of macroautophagy flux is consistently reported in neuronal models, and in particular the fusion of APs with lysosomes [46,52,59]. To date the effect of GCase deficiency on CMA has not been directly measured (Figure 1).

Since GCase is involved in sphingolipid metabolism, it is tempting to speculate that changes in the lipid composition of cells are affecting autophagy/lysosomal function. Whether this is due to the accumulation of the GCase substrate GlcCer and/or the deacylated version glucosylsphingosine (GlcSph), or changes in other lipids, is open to debate. The mouse models in which α-synuclein aggregates were observed are GD models with accumulation of GlcSph/GlcCer [63,65,67,70]. However, heterozygote *GBA* carriers are not expected to have substrate accumulation. Indeed analysis of GlcCer/GlcSph in homogenates of putamen and cerebellum of PD brains with *GBA* mutations [74], or the primary motor cortex from patients with *GBA* mutations and a variety of Lewy body disorders, including PD [75], did not exhibit any increase in GCase substrate. In sporadic PD brains, where GCase activity is also decreased, GlcCer has also been reported to be unchanged [76], while only one paper has

shown an increased GlcSph levels in the substantia nigra and hippocampus, but not the putamen, frontal cortex or cerebellum [35].

These studies were all on post-mortem tissue and therefore contain a mix of neurons and glia, so it cannot be discounted that particular cell types do accumulate GCase substrates, or that subtle changes in subcellular locations can have an effect on α-synuclein metabolism. Midbrain neurons differentiated from iPS with heterozygote *GBA* mutations have been reported to either increase [46] or not accumulate GlcCer [52]. Further work is necessary to clarify whether GlcCer and GlcSph does increase in heterozygote *GBA* PD patients.

Detergent resistant membranes (also referred to as lipid rafts) are cholesterol and sphingomyelin-rich membrane domains. GlcCer has been reported to accumulate in this fraction [77]. GlcCer can stabilise sphingomyelin/cholesterol-enriched liquid domains, however as the proportion of GlcCer rises membrane order is increased [78]. Indeed membrane fluidity is decreased in CBE-treated fibroblasts or GD type 1 fibroblasts [78]. Should this occur in PD brains, given that membrane dynamics are required for both macroautophagy and CMA (see below) this could greatly affect α-synuclein degradation. *In vitro* studies have also suggested that GlcCer or GlcSph can directly cause monomeric α-synuclein to aggregate [45,65]. Furthermore, treatment of HEK293 cells with these α-synuclein species could act as a seed to propagate the aggregation of GFP-tagged α-synuclein expressed by these cells [65].

Trends or significant changes in a variety of sphingolipids (e.g. sphingomyelin, ceramide, gangliosides) have been reported in PD brains with or without *GBA* mutations [34,74,75,79] and could contribute to impairment of the ALP. Cell models of GCase deficiency, including neurons, have also shown elevated cholesterol levels [59,80,81]. Changes in cholesterol are well known to affect membrane rigidity and both the localisation and activity of proteins at

discrete membrane locations. Increased lysosomal cholesterol can impair macroautophagy flux, perhaps at the step of lysosome fusion with APs [82,83]. The LAMP2A translocation pore is required for α-synuclein degradation by CMA and is active in lysosomal membranes outside of sphingolipid and cholesterol rich microdomains. Increased lysosomal cholesterol content impairs this translocation and thus α-synuclein degradation is decreased [84].

Another mechanism by which GCase deficiency may impair the ALP is via a process termed autophagic lysosome reformation (ALR) [85,86]. Following the termination of macroautophagy, proto-lysosomal tubules have been shown to extrude from the autophagolysome which then mature into functional lysosomes and thus restore the cell's full complement of lysosomes [85,87]. This process is dependent on mTOR [85,86]. *GBA* KO or KD has been shown to result in decreased mTOR activity in both cellular and *Drosophila* models [59,69], with ALR appearing to be inhibited in GCase deficient MEFs, SH-SY5Y cells and neurons [59]. In the latter two cell models, the decrease in ALR was coincident with an inhibition of macroautophagy flux, elevated intracellular levels of α-synuclein and phosphorylated α-synuclein (S129) and increased release of α-synuclein in to media.

Decreased GCase activity in sporadic PD

There are several possible mechanisms that might contribute to the decrease of GCase activity and protein expression in sporadic PD brains. The greatest known risk factor for developing PD is aging, and studies in humans, monkeys and mice have shown that GCase activity declines with age in the midbrain regions such as the substantia nigra, striatum and putamen [35,65,88]. The decrease in GCase activity in the striatum and hippocampus of

monkeys was coincident with an increase in α-synuclein oligomers in the striatum and hippocampus [88].

The bidirectional relationship between GCase and α-synuclein was first reported by Mazzulli *et al* [45]. Following translation of GCase at the ER, GCase is transported via the Golgi to lysosomes via the transporter protein LIMP2, undergoing several glycosylation modifications on the way [89–91]. Analysis of the glycosylated forms of GCase in the cortex of control human brains suggested that lysosomal maturation of GCase was diminished in brains with higher amounts of α-synuclein [45]. A correlation between increasing α-synuclein levels and decreasing GCase activity was then convincingly shown in sporadic PD brains [34].

In rodents, α-synuclein KO mice have increased GCase activity [51], while two reports have shown that expression of human A53T in mice caused a decrease in GCase activity [71,92]. However not all mouse models with increased expression of human α-synuclein exhibit decreased GCase activity [51,93].Cellular models in which human α-synuclein is either over expressed [33,94–96] or human midbrain neurons differentiated from iPS cells taken from PD patients with triplication of the α-synuclein gene [94] have also shown a reduction in GCase protein levels and GCase activity. These studies indicate that increased α-synuclein levels interfere with the transport of GCase through the secretory pathway to the lysosome (Figure 1). α-synuclein has been reported to interrupt ER to golgi transport [97,98]. In the triplication α-synuclein neurons, accumulation of α-synuclein in the cell body disrupted the localisation of rab1a to the ER-Golgi, while overexpression of rab1a restored trafficking of lysosomal enzymes such as GCase to the lysosome [94]. Increased intracellular α-synuclein levels are also known to induce ER stress [99,100] and this might be another mechanism by which GCase transport to the lysosome is affected.

Mitochondrial dysfunction and oxidative stress may also play a role in loss of GCase activity in human dopaminergic neurons. Mitochondrial derived oxidant stress has recently been shown to induce the oxidation of dopamine, which subsequently inhibited GCase activity, resulting in lysosomal dysfunction [101]. GCase activity has also found to be reduced in SH-SY5Y cells with constitutive KD of PINK1 [33]. Mutations in PINK1 are a cause of familial PD, with loss of PINK1 activity known to cause mitochondrial dysfunction and oxidative stress [102–106].

ER stress, mitochondrial dysfunction and neuroinflammation

In addition to the ALP dysfunction described above, loss of GCase activity could contribute to the pathogenesis of PD via ER stress, mitochondrial dysfunction and neuroinflammation.

Activation of the UPR by *GBA* mutations such as L444P and N370S in GD fibroblasts has been well documented [9,11,107,108]. Human midbrain neurons differentiated from iPS derived from PD patients with *GBA* mutations have shown increased expression of chaperones associated with the UPR, including BiP and calnexin, in addition to the activation of the IRE arm of the UPR [52]. Increased release of calcium from the ER has also been observed in iPS-derived neurons [46] or fibroblasts [109] from patients with GBA mutations and PD. *Drosophila* models expressing PD associated *GBA* mutations have also exhibited activation of the UPR [110–112]. *Drosophila* does not have a homolog of α-synuclein, and therefore the loss of dopaminergic neurons [112] and locomotor defects [112,113] were independent of α-synuclein pathology. Notably the locomotor deficits observed in these flies were reversed when ER stress was alleviated.

Mitochondrial dysfunction has long been associated with PD pathogenesis [114] and has been reported in *GBA* cell and animal models [62,80,115–118]. The cause for the dysfunction is

unclear but is likely to be a secondary event, perhaps a result of impaired clearance of damaged mitochondria by macroautophagy (mitophagy), accumulation of α-synuclein and/or dysregulation of calcium.

Neuroinflammation signalling pathways are increasingly being associated with PD pathogenesis [119–121]. Animals in which the glucocerebrosidase gene is KO or mice were treated with CBE show considerable neuroinflammation, and in particular activation of microglia [67,115,122,123]. This is thought to be due to the accumulation of substrate in the neurons, which can then activate microglia [61,124,125]. While it is uncertain to what extent substrate accumulation occurs in GBA-associated PD, it is likely that glia are going to be affected. As described above, neurons with GCase deficiency can increase the release of α-synuclein [52,59,60]. Not only is this extracellular α-synuclein going to be transmitted to neurons, but also astrocytes and microglia where it can be degraded [126,127]. Since loss of GCase impairs the ALP in cells ranging from fibroblasts to neurons [46,52,59,80], it is likely that glia containing heterozygote *GBA* mutations will also be affected, and may contribute to increased spread of pathology. Furthermore, α-synuclein has been shown to activate microglia by binding to toll-like receptors [128–130], so increased release from GCase deficient neurons may also activate glia this way.

Therapy for GCase deficiency in PD

Since *GBA* mutations are numerically the greatest known genetic cause of PD and that loss of GCase activity also occurs in sporadic PD, treatments to restore GCase activity are an attractive target for drug development (Table 1). While enzyme replacement therapy is an effective treatment for type 1 GD patients. Unfortunately the enzyme cannot cross the blood

brain barrier, and is therefore not a treatment for type 2 or 3 neuronopathic GD and will also not be a suitable for PD therapy.

Studies in which virus encoding human recombinant GCase have been injected in to the brain of mouse GD models have shown to be effective in reducing α -synuclein accumulation [63,71,72]. Furthermore, a new AAV virus has been shown to be able to deliver *GBA* to the brain via intravenous injection, reducing α-synuclein inclusions in a human A53T mouse model [73]. However, while neuronal transduction was very good to the cortex, hippocampus, cerebellum and spinal cord, delivery to the substantia nigra was very limited.

Another approach being pursued is the use of blood brain barrier permeant molecules that can either increase GCase activity in lysosomes or modulate the lipid imbalance as a result of GCase deficiency. In the latter case, a potent and orally available inhibitor of GlcCer synthase GZ667161 has been shown to decrease the levels of GlcCer and GlcSph in a GD mouse model, resulting in a decreased number of proteinase K-resistant α-synuclein aggregates [131]. The drug was also effective in reducing α-synuclein aggregation in mice expressing human A53T α-synuclein [131].

Small molecule chaperones for GCase bind mutant GCase in the ER, helping them refold, and thus facilitate trafficking to the lysosome. This type of therapy for PD will in theory have two effects: (a) improve lysosomal function and thus degradation of α-synuclein (b) reduce ER stress. Several drug screens have identified a number of candidates, including already known drugs such as ambroxol and isofagomine [132,133] and novel chaperones [48,134]. These chaperones have been shown to increase the activity, protein expression and lysosomal localisation of mutant GCase such as L444P and N370S in fibroblasts and neurons [48,50,95,112,132,133,135,136]. Importantly in neurons containing either *GBA* mutations or

triplication of the α-synuclein gene, chaperone treatment reduced the aberrant accumulation of α-synuclein observed in these cells [48,50,95].

In *Drosophila* both ambroxol and isofagomine have been shown to effectively reduce the ER stress induced by mutant human *GBA* [110,112,113] and also reverse the locomotor deficit observed in these models [112,113].

Oral administration of ambroxol to heterozygote L444P *Gba* mice increased GCase activity in the brain stem, midbrain, striatum and cortex [137]. Furthermore, ambroxol treatment increased wild-type GCase activity in mouse brain, which in a transgenic mouse expressing human α-synuclein resulted in a decrease of α-synuclein in the striatum and brainstem [137]. In a different transgenic mouse expressing human α-synuclein, isofagomine has also been shown to increase wild-type GCase activity, reduce α-synuclein immunoreactivity in the dopaminergic neurons of the substantia nigra, and improve motor and non-motor function [93]. The observation that these two chaperones increase wild-type GCase activity *in vivo*, and that ambroxol, isofagomine and other small molecule chaperones can increase wild-type GCase activity *in vitro* [48,95,112,135,136,138] suggests that small molecule chaperones could also be used as a treatment for sporadic PD.

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Treatment	Strategy	Drug Name	References
Gene therapy	Replace GCase activity in brain	Viral-mediated delivery of *GBA*	[63,71–73]
Substrate reduction	Reduction of glycosphingolipids in the CNS	GZ667161	[131]
Small molecule chaperones	Refolding mutant GCase in the ER and thus improving trafficking to the lysosome and reducing ER stress	ambroxol isofagomine NCGC00188758 NCGC00241607	[48,50,93,95,110, 112,113,133,135, 137]

Table 1. Potential therapies for treating GCase deficiency in Parkinson disease

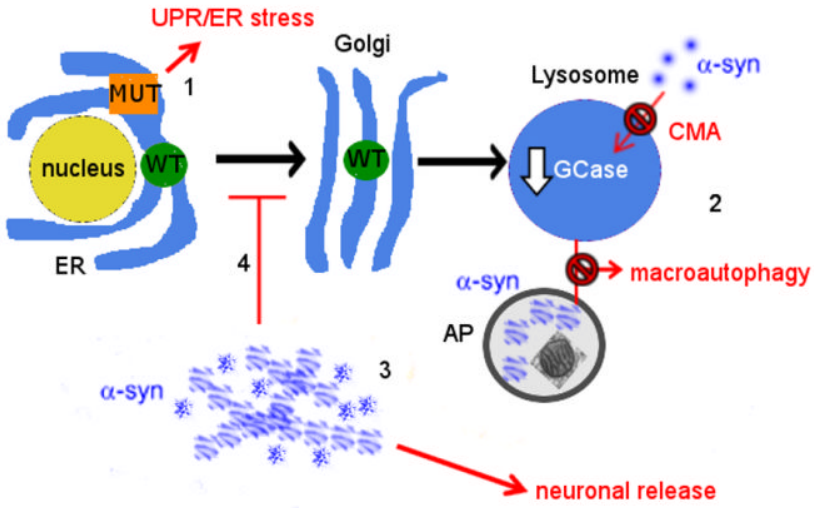

Figure 1. The proposed bidirectional loop between GCase and α-synuclein.

Wild-type GCase (WT, green) is translated in the ER and then transported to the lysosome via the Golgi. Several point mutations of GCase (MUT, orange) unfold in the ER, activating the UPR and ER stress [1]. When lysosomal GCase activity is decreased in neurons (via the UPR or null alleles), the autophagy lysosomal pathway is inhibited leading to the accumulation of α-synuclein [2][3]. Autophagosome (AP) fusion with the lysosome is known to be impaired during macroautophagy which degrades aggregated proteins such as α-synuclein and damaged organelles like mitochondria. The direct effect of GCase deficiency on the degradation of monomeric α-synuclein by chaperone mediated autophagy (CMA) is unclear. In addition to intracellular accumulation of α-synuclein, increased release from neurons has been reported, which might be transmitted to neighbouring neurons [3]. Increased levels of α-synuclein are thought to decrease WT GCase trafficking to the lysosome [4]. This might be a mechanism by which GCase activity is decreased in sporadic PD.