

## Enhancing the Activity of Glucocerebrosidase as a Treatment for Parkinson Disease

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### Abstract (217 words)

Mutations in the glucocerebrosidase (*GBA1*) gene are the most common genetic risk factor for Parkinson disease (PD). Homozygous or compound heterozygous *GBA1* mutations cause the lysosomal storage disorder Gaucher disease (GD), characterized by deficient activity of the glucocerebrosidase enzyme (GCCase). Both individuals with GD type 1 and heterozygous carriers of pathogenic variants of *GBA1* have an increased risk of developing PD, by approximately 10-20-fold compared to non-carriers. GCCase activity is also reduced in PD patients without *GBA1* mutations, suggesting that the GCCase lysosomal pathway might be involved in PD pathogenesis. Available evidence indicates that GCCase can affect  $\alpha$ -synuclein pathology in different ways. Misfolded GCCase proteins are retained in the endoplasmic reticulum, altering the lysosomal trafficking of the enzyme and disrupting protein trafficking. Also, deficient GCCase leads to accumulation of substrates that in turn may bind  $\alpha$ -synuclein and promote pathological formation of aggregates. Furthermore,  $\alpha$ -synuclein itself can lower the enzymatic activity of GCCase, indicating that a bidirectional interaction exists between GCCase and  $\alpha$ -synuclein. Targeted therapies aimed at enhancing GCCase activity, augmenting the trafficking of misfolded GCCase proteins by small molecule chaperones, or reducing substrate accumulation, have been tested in preclinical and clinical trials. This article reviews the molecular mechanisms linking GCCase to  $\alpha$ -synuclein and discusses the therapeutic drugs that by targeting the GCCase pathway can influence PD progression.

### Key points

- Mutations in the *GBA1* gene, encoding for the lysosomal enzyme glucocerebrosidase, are the most common genetic risk factor for Parkinson disease.
- A complex interaction exists between glucocerebrosidase and  $\alpha$ -synuclein.
- Gene therapy, small molecule chaperones and modulators targeting the glucocerebrosidase pathway, are promising therapies for Parkinson disease.

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## **1. Introduction**

“There appears to be sufficient reason for hoping that some remedial process may ere long be discovered, by which, at least, the progress of the disease may be stopped”. After more than 200 years since James Parkinson wrote this prediction, we are now living in a time when the chance to slow the progression of Parkinson disease (PD) is probably more than just a hope. Novel treatments manipulating the pathway involving Glucocerebrosidase enzyme (GCase), encoded by the *GBA1* gene, offer potential to slow or even prevent PD in both individuals with and without *GBA1* mutations.

In this article, we provide an overview of the epidemiological burden of *GBA1* mutations in the PD population, review the mechanisms by which GCase dysfunction is thought to impact PD onset and progression, and conclude by examining the available treatments for PD that target the GCase pathway.

## **2. *GBA1* Mutations and Gaucher’s disease**

GCase (also known as glucosylceramidase; EC 3.2.1.45) is a lysosomal enzyme which hydrolyses glucosylceramide (GlcCer) to ceramide and glucose. GCase is encoded by the *GBA1* gene located on chromosome 1q22. Homozygous or compound heterozygous mutations in the *GBA1* gene cause a marked decrease in GCase activity and accumulation of its substrates in the lysosomes, resulting in the autosomal recessive disorder Gaucher disease (GD) [1]. The incidence of GD is around 1/40,000 to 1/50,000 births in the general population, and up to 1/800 births in the Ashkenazi Jewish population [1, 2]. GD is a systemic disorder that can present with a variety of visceral manifestations (hepatosplenomegaly, anaemia, thrombocytopenia) and neurological involvement. The degree of neurological involvement permits further categorization into three clinical subtypes: GD type I (OMIM 230800), the most common form, characterized by no neurological involvement; and GD type II (OMIM 230900) and type III (OMIM 231000), the neuronopathic variants [1]. More than 300 *GBA1* pathogenic variants have been associated with GD, including point mutations, insertions, deletions, splice-site mutations, and recombinant alleles [3], with the two most common being N370S and L444P [4]. The “mild” N370S mutation, which results in *in-vitro* residual enzyme activity of 32-38%, is associated only with GD type I. The “severe” L444P mutation, with *in-vitro* residual activity of 13-24%, is more often associated with GD types II and III [5, 6].

## **3. *GBA1* Mutations and Parkinson disease**

In the 1990s, some patients with GD [7] and asymptomatic *GBA1* carriers [8] were noted to have parkinsonian features. Neuropathologic findings subsequently confirmed classic PD pathology with aggregation of misfolded  $\alpha$ -synuclein into Lewy bodies (LBs) and neurites [9] in parkinsonian patients with GD [10, 11]. Heterozygous mutations in *GBA1* gene (OMIM 606463) are now considered the most significant genetic risk factor for PD, being present in 5% to 15% of Caucasian PD patients, 25% of Ashkenazi Jewish PD patients, and less than 1% of the general population [12]. More than 50 genetic studies have screened the *GBA1* gene in PD populations [13]: among the 130 variants of *GBA1* which have been reported in association with PD, 11 of them have been demonstrated to increase significantly the risk of PD worldwide, with odds ratios (ORs) ranging from 1.78 to 10.49 [12]. The common N370S and L444P mutations increase the risk of PD by approximately 4- and 8-to-12-fold, respectively [12]. The penetrance of *GBA1* variants is incomplete, variable across studies (approximately estimated between 10% and 30% in carriers), and age-specific [14, 15], with severe variants leading to a 3- to 4-fold higher risk and earlier disease onset compared to mild variants [16]. *GBA1* mutations have also been associated with increased risk of Dementia with Lewy Bodies (DLB), another form of

synucleinopathy, with OR of 8.28 [17]. This association was recently confirmed by a genome-wide associated study [18], further supporting the relationship between *GBA1* pathway and synucleinopathies.

Notwithstanding the above, some caveats concerning the relationship between the *GBA1* pathway and PD need to be highlighted. First, PD has a similar incidence in homozygous and heterozygous carriers, though homozygous have a slightly earlier disease onset [19]. Second, common *GBA1* variants, such as E326K, predispose to PD by increasing the risk approximately 1.5-fold, but do not cause GD [12]. Third, GCase activity is reduced in brain tissue, cerebrospinal fluid and dried blood spots of PD patients carrying *GBA1* mutations as well as non-carriers [20-22]. Overall, this suggests that the connection between GCase and PD goes beyond the pathogenicity of mutations in the *GBA1* gene, and that additional mechanisms causing accumulation of  $\alpha$ -synuclein aggregates, such as age-related proteostasis dysfunction, might be involved in PD pathogenesis [23]. Nonetheless, unravelling the molecular mechanisms linking GCase with  $\alpha$ -synuclein is necessary to understand the pathogenesis of both sporadic and genetic forms of PD, and thus to develop specific targeted treatments.

#### **4. The Link Between *GBA1* Mutations, GCase and $\alpha$ -synuclein**

Three possible mechanisms linking *GBA1* mutations, GCase dysfunction and  $\alpha$ -synuclein pathology have been proposed.

##### **4.1. The GCase Gain-Of-Function and Endoplasmic Reticulum Stress Mechanism**

The first mechanism accounts for a gain-of-function role for *GBA1* mutations. The majority of *GBA1* mutations are missense mutations affecting the correct folding and maturation of the protein [4]. As such, misfolded GCase accumulates in the endoplasmic reticulum (ER) triggering an upregulation of ER stress response [24-26]. GCase is then translocated to the cytosol to be eliminated by the ubiquitin proteasome system, the so-called ER-associated degradation (ERAD); this process impedes mutant GCase from reaching the lysosome and impairs lysosomal GCase activity and function [27]. The presence of misfolded GCase, in turn, triggers the unfolded protein response (UPR) and interferes with the degradation of other proteins [28]. That said, how can misfolded GCase influence  $\alpha$ -synuclein levels? Several lines of evidence support a direct interaction between GCase and  $\alpha$ -synuclein [4]. The expression of mutant *GBA1* proteins promoted  $\alpha$ -synuclein accumulation in neuronal cells [29], as well as *in vivo* models of GD mice [29-31]. Similarly, intrastriatal injections of N370S mutant *GBA1* induced local increase of  $\alpha$ -synuclein secretion in mice overexpressing  $\alpha$ -synuclein [32]. The half-life of  $\alpha$ -synuclein in cells expressing mutant GCase was found to be significantly elevated in *Drosophila* [33] and mouse models [34], leading to its accumulation and aggregation. In humans, GCase has been found in 32-90% of LBs of brain tissue samples from PD patients with *GBA1* mutations, and has been shown to co-localize with  $\alpha$ -synuclein [35]. It has also been reported that normal GCase can directly interact with the  $\alpha$ -synuclein C-terminal residues in a pH-dependent manner, that is within the lysosome [36]. Taken together, these findings support the strategy of regulating GCase trafficking as a potential treatment for PD.

##### **4.2. The GCase Loss-Of-Function/Haploinsufficiency and Substrate Accumulation Mechanism**

The second mechanism linking the *GBA1* pathway to PD proposes that the enzymatic deficiency resulting from loss-of-function or haploinsufficiency of GCase, leads to accumulation of substrates, thus affecting lipid homeostasis and  $\alpha$ -synuclein trafficking and clearance [4]. It has been demonstrated

in both *in vitro* and *in vivo* models that the GCCase inhibitor conduritol- $\beta$ -epoxide (C $\beta$ E) increases  $\alpha$ -synuclein levels [32, 37-39]. Even partial GCCase inhibition caused by low doses of C $\beta$ E could promote marked microglia activation and enhance toxin-induced neurodegeneration in mice [40]. However, in primary neuron cultures, C $\beta$ E alone was not able to cause *de novo*  $\alpha$ -synuclein aggregation, but only to induce a dose-dependent increase when the pathological burden of  $\alpha$ -synuclein was initiated by preformed fibrils [41]. In addition to aggregation process, GCCase depletion was also reported to be involved in cell-to-cell transmission of  $\alpha$ -synuclein aggregates [42].

Controversial data exist around the concept that GCCase substrate GlcCer, or other sphingolipids accumulating in GD such as glucosylsphingosine (GlcSph), accumulate in the lysosome and trigger toxic  $\alpha$ -synuclein conformational change in *GBA1*-PD and GD-PD. Experiments conducted using induced pluripotent stem cells (iPSC) derived from GD patients and PD patients harbouring alpha-synuclein gene (*SNCA*, OMIM 163890) triplication, showed that elevated GlcCer directly promoted the conversion of physiological  $\alpha$ -synuclein into insoluble forms and that the reduction of GlcCer was able to reverse the toxic conversion of  $\alpha$ -synuclein [43]. Furthermore, in human postmortem brain samples from GD patients, GlcCer levels controlled the formation of  $\alpha$ -synuclein oligomeric intermediates and induced polymerization of fibrils [44]. GlcSph has been shown to promote the formation of oligomeric  $\alpha$ -synuclein in young GD/PD mice brains [45]. In double mutant mice obtained by crossing *SNCA* transgenic mice with *GBA1* heterozygous knockout mice, phosphorylated  $\alpha$ -synuclein was highly expressed in regions vulnerable to early-stage PD pathology, such as the olfactory bulb, and increased levels of GlcSph were reported [46]. In line with these data, significantly reduced GCCase activity and increased GlcSph levels were detected in the substantia nigra and hippocampus of idiopathic PD patients during their sixth decade of life [47]. However, no accumulation of GlcCer and GlcSph was observed in the putamen and cerebellum samples from *GBA1*-PD patients [48]. Strategies to reduce substrate accumulation are currently being assessed in PD.

Ceramide homeostasis may also be implicated in PD pathogenesis. Data from postmortem brain studies of PD patients show that total ceramide levels including C18-ceramide are reduced in the anterior cingulate cortex, a region with significant PD pathology [49]. Decreased C18-ceramide species levels in GCCase-deficient cells were shown to impair bafilomycin-A1-induced  $\alpha$ -synuclein secretion resulting in increased intracellular  $\alpha$ -synuclein accumulation, thus suggesting impaired secretory autophagy [50]. This secretory defect was rescued by exogenous C18-ceramide or chemical inhibition of lysosomal enzyme acid ceramidase (with carmofur) which resulted in decreased  $\alpha$ -synuclein in *GBA1*-PD patient-derived dopaminergic neurons, increased ceramide levels and decreased GlcSph levels [50]. It is known that ceramide acts as a lipid second messenger to bind and activate cathepsin D (CTSD) [51], a lysosomal aspartic endo-protease which is involved in  $\alpha$ -synuclein degradation [52]. In *GBA1* mutant (WT/N370S) PD neurons, decreased levels of CTSD protein and its enzymatic activity were reported, together with monomeric  $\alpha$ -synuclein accumulation, implying that reduced CTSD occurred as a consequence of reduced mutant GCCase activity and reduced ceramide levels [53]. Interestingly, GCCase replacement therapy failed to lower  $\alpha$ -synuclein levels in the presence of CTSD inhibitor [53], suggesting that the ceramide-CTSD pathway can impact  $\alpha$ -synuclein levels and therefore might be considered as a target to reduce  $\alpha$ -synuclein pathology.

### **4.3. The Positive Feedback Loop of GCCase and $\alpha$ -synuclein**

The pathological cascade of GCCase and  $\alpha$ -synuclein alterations can rely on a third mechanism involving a bidirectional and reciprocal effect between GCCase and  $\alpha$ -synuclein: increased  $\alpha$ -synuclein levels result in aberrant maturation and ER/Golgi apparatus trafficking of GCCase, while GCCase deficiency can promote  $\alpha$ -synuclein accumulation [44]. Two main studies support this hypothesis. In the first, overexpression of  $\alpha$ -synuclein in human primary cortical neurons altered the post-ER/ER GCCase forms ratio by causing reduction of the former and accumulation of the latter, and decreased the GCCase

lysosomal activity [44]. On the other hand, GCase deficiency was shown to promote the formation of soluble oligomeric and insoluble  $\alpha$ -synuclein *in vivo* [44]. In the second study, human neuroblastoma SH-SY5Y cell lines over-expressing high levels of exogenous  $\alpha$ -synuclein (high  $\alpha$ -synuclein) were compared to cells expressing 10-fold lower levels of exogenous  $\alpha$ -synuclein: in high  $\alpha$ -synuclein cells, GCase activity and protein level were decreased by 70% and 87%, respectively [20]. After endo-H digestion, high  $\alpha$ -synuclein cells showed an additional GCase band of lower molecular weight, indicating that a proportion of GCase was trapped prior to reaching the mid-Golgi apparatus [20]. Taken as a whole, these results suggest that therapeutic strategies aimed at restoring or improving GCase intracellular trafficking might be beneficial in the context of PD.

#### **4.4. Autophagy and GCase: Another Possible Link Towards Synucleinopathy**

Accumulating evidence suggests the prominent role of autophagy-lysosomal pathways (ALP) in PD pathogenesis [54]. Defects in the ALP system have been reported in human iPSC-derived dopamine neuron cultures from GD and PD patients carrying *GBA1* mutations, leading to increased release of extracellular  $\alpha$ -synuclein in these neurons [24, 55]. Similarly, adeno-associated virus (AAV)-mediated overexpression of N370S mutant *GBA1* in the mouse striatum resulted in increased local  $\alpha$ -synuclein secretion, and increased expression of ALP biomarkers [32]. In another report, GCase deficient cells showed autophagy lysosomal reformation dysfunction, which was accompanied by increased levels of total and phosphorylated monomeric  $\alpha$ -synuclein, oligomer deposition, and enhanced  $\alpha$ -synuclein release, indicating that defective lysosomal function may contribute to impaired ALP and  $\alpha$ -synuclein pathology [56]. Furthermore, rapamycin demonstrated beneficial effects in PD neuronal cell models, supporting that enhancing the ALP pathway may represent a useful therapeutic strategy in PD [57].

### **5. From Mechanisms to Treatments: How We Can Target the GCase Pathway**

Due to the increased understanding of the pathogenetic mechanisms linking GCase dysfunction and  $\alpha$ -synuclein neuropathology, the manipulation of the GCase pathway has become an appealing therapeutic target in PD. Following the three main hypotheses explained above, most preclinical and clinical research has focused on A) enhancing GCase activity by replacing the mutant form of GCase, B) stabilizing the GCase mutant forms through molecular chaperones, and C) reducing the substrate accumulation by targeting the glycosphingolipid synthesis. Regardless which strategy is applied, the main purpose is to lower  $\alpha$ -synuclein levels and hence hopefully slow or halt the disease progression.

#### **5.1. Enhancing GCase Activity: Enzyme Replacement Therapy, Gene Therapy and other Modulators**

While enzyme replacement therapy has remained one of the standard treatments for GD since 1991 [58], recombinant GCase imiglucerase does not cross the blood–brain barrier (BBB) and therefore has no effect on the CNS [59].

Alternative strategies to restore physiological GCase activity in the brain include *GBA1* overexpression by AAV. Viral delivery of wild-type *GBA1* showed a trend to reduce  $\alpha$ -synuclein abnormalities and memory deficits in a GD mouse model [60]. Similar beneficial effects were replicated in mouse models that did not carry any *GBA1* mutations, indicating that enhancing GCase activity by gene therapy could prevent synucleinopathy regardless of *GBA1* mutational status [30, 61-63]. Moreover, ectopic expression of wild-type *GBA1* was reported to reverse the effects of *GBA1* gene deletion on the propagation and cell-to-cell transmission of  $\alpha$ -synuclein aggregates [42], suggesting its ability to impact  $\alpha$ -synuclein pathology at different steps. A multicenter, randomized, double-blind, sham procedure-

controlled, phase 1/2a clinical trial has recently started recruiting patients with moderate to severe PD with at least 1 pathogenic *GBA1* mutation, to evaluate the safety of intra-cisternal PR001A administration (single-dose gene therapy using an AAV9 viral vector), with two escalating dose cohorts (PROPEL study, NCT04127578). A phase 1/2 study testing PR001A in neuronopathic GD is also expected to begin soon.

Regulators of gene expression may also be appealing therapeutic strategies. In this context, a very important role is played by microRNAs (miRNAs), a class of small non-coding RNAs, which can mediate gene silencing post-transcriptionally by mRNA degradation and/or repression of translation [64]. In human PD brains, miR-127-5p, which has been shown to reduce GCCase activity, and miR-16-5p, which has been shown to enhance GCCase protein levels [65], were found to be downregulated and upregulated, respectively [66]. An additional regulatory circuit possibly associated with PD, could involve the *GBA1* pseudogene through its ability to act as a competing-endogenous RNA (ceRNA) titrating miR-22-3p (“miRNA sponging”) and thus up-regulating the levels of *GBA1* mRNA [67].

Next to recombinant GCCase or gene therapy, the activity of wild-type GCCase can be enhanced using small molecular modulators of GCCase. The rationale of using such molecules is that individuals carrying *GBA1* mutations such as 84GG, do not express the mutant protein [68], and wild-type GCCase enzyme activity was reported to be reduced in iPSC-derived dopaminergic neurons from patients with genetic or idiopathic PD who did not harbour *GBA1* mutations [69, 70]. For instance, the GCCase modulator S-181 was shown to ameliorate lysosomal dysfunction and  $\alpha$ -synuclein accumulation in both *GBA1* and non-*GBA1* PD iPSC-derived dopaminergic neurons [68]. In a *GBA1* heterozygous mouse model, S-181 was able to penetrate the CNS, activate the wild-type GCCase and reduce  $\alpha$ -synuclein [68]. Similarly, small molecular chaperones such as ambroxol were reported to increase *GBA1* mRNA and protein levels and enzymatic activity in several cell and animal models (see below) [26, 71-73].

In conclusion, the development of targeted therapies that are based on the genetic background of PD is likely to have an important impact on the disease course, and future studies are needed to test the efficacy of *GBA1*-overexpressing therapies and/or develop the most effective wild-type GCCase modulators for testing in human clinical trials.

## 5.2. Substrate Reduction Therapy

Through the inhibition of glycosphingolipid synthesis and the restoration of a more physiological GCCase substrate ratio, substrate reduction therapies (eliglustat and miglustat) represent a valid alternative to control visceral symptoms in patients with GD type I. Miglustat has been shown to reduce glycosphingolipid accumulation and  $\alpha$ -synuclein preformed fibril toxicity in human dopaminergic neuronal cells [74]. However, the effectiveness of miglustat or eliglustat is limited in neuronopathic GD and PD due to their inability to pass the BBB [59]. Venglustat (GZ/SAR402671) is an oral GlcCer synthase inhibitor, which can cross the BBB. A multicenter, randomized, double-blind, placebo-controlled phase 2 study is currently ongoing to assess the safety, drugs dynamics and efficacy of venglustat in *GBA1* carriers with early-stage PD (MOVES-PD study, NCT02906020). Another CNS-penetrant GlcCer synthase inhibitor, GZ667161, has been reported to improve  $\alpha$ -synuclein processing and behavioural outcomes in both  $\alpha$ -synuclein overexpressing mice and mice carrying homozygous *GBA1* mutations [75], further indicating a potential for clinical applications of GlcCer synthase inhibitors in PD patients.

## 5.3. Stabilizing GCCase Mutant: Small Molecular Chaperones

Based on the observation that misfolded GCase is sequestered within the ER, small chemical chaperones can act as cofactors that stabilize and refold misfolded protein, in turn upregulating post-ER trafficking of mutant GCase to the lysosome [76]. As they are small enough to cross the BBB, they represent a promising therapeutic option in PD [77]. Molecular chaperones can be categorized into two groups. The first one includes inhibitory chaperones, that can directly bind to the active site of the GCase protein, antagonizing the binding of the enzyme substrates and hence reducing the enzymatic activity [76]. To date, the majority of GCase chaperones discovered are inhibitory. However, if the affinity of the chaperone for the substrate is too high, the active site will remain bound to the chaperone: as a result, the chaperone will inhibit rather than increase GCase activity within the lysosome [76]. Within this category, the compound AT2101 (afegostat-tartrate, or isofagomine) has shown promising results in *in vitro* and *in vivo* studies [78-80], however clinical trials testing this compound in patients with GD were unsuccessful, possibly due to the high-binding affinity to the active site up to pH 4 [76]. The second group of compounds includes non-inhibitory chaperones, that can bind the enzyme out of the active site, but yet are still able to modulate the folding sufficiently to restore or partially restore posttranslational folding [81]. Recently, a number of non-inhibitory chaperones of GCase have been identified through high-throughput screening systems using the spleen-lysate and patient's fibroblast [82, 83]. It was shown that the lead compound NCGC00188758 of a class of noninhibitory chaperone molecules potently activated and promoted the translocation of mutant GCase into lysosomes [84]. Some of these GCase modulators, such as NCGC00188758 [85] or NCGC607 [86], were applied to stable cell lines and multiple iPSC neuronal lines derived from PD patients harbouring distinct mutations in *SNCA* or *GBA1*, as well as idiopathic PD. The results showed that both NCGC607 and NCGC00188758 could enhance GCase lysosomal activity, reduce  $\alpha$ -synuclein accumulation and reverse neurotoxicity [85, 86].

Among the class of small chaperones, ambroxol is one of the most promising candidates as a disease-modifying treatment in PD. Used as an expectorant for over 30 years within the dose range of 75–100 mg/day for adults, ambroxol is characterized by good lipophilicity and low polar surface area, thus predicting good CNS penetration [87]. It also has an excellent safety record with its principal adverse effects being gastrointestinal disturbance and a small risk of anaphylaxis [76]. In 2009, a screen of a FDA-approved drug library identified ambroxol as a pH-dependent mixed inhibitor of GCase [88], and more than 10 years after, it is currently in clinical trials in PD patients [87, 89]. Ambroxol can bind both active and non-active site residues of GCase protein [88], thus enhancing the folding of mutant GCase in the ER and facilitating the shuttling to the lysosome [90]. Within the lysosome, it is then eluted, allowing normal catalysis to resume and restoring lysosomal function [89]. A number of *in vitro* and *in vivo* studies have reported that ambroxol can increase GCase activity and also reduce  $\alpha$ -synuclein levels [26, 71-73, 90, 91]. The modulation of  $\alpha$ -synuclein levels can rely on different mechanisms, such as by 1) upregulating GCase expression through the transcription factor EB pathway and thus stimulating lysosomal exocytosis [71, 92], and/or 2) correcting the GCase posttranslational folding and mitigating the UPR [90]. A single-centre open-label noncontrolled clinical trial of oral ambroxol therapy (escalating dose to 1.26 g per day) was recently conducted in 17 patients with moderate PD with and without *GBA1* mutations [89]. The study confirmed that ambroxol is able to penetrate CSF and is well tolerated in patients with PD (even at a 10-times higher dose and longer duration of administration than specified in its license). A significant increase in CSF  $\alpha$ -synuclein concentration and GCase protein was observed, together with a significant improvement in motor symptoms severity [89]. Another phase II, single-center, double-blind, randomized placebo-controlled trial (NCT02914366) involving 75 individuals with mild to moderate PD-Dementia, randomly assigned to high-dose (1050 mg/day), low-dose (525 mg/day), or placebo treatment arms, is currently on-going. Primary outcome measures will be any changes in the cognitive scales [87].

Next to intrinsic chaperones, extrinsic chaperones are proteins that serve to stabilize and refold proteins during periods of extreme stress that disrupt proteostasis, such as heat shock [77]. Within this group, members of the heat shock protein 70 (HSP70) family have been shown to be essential for proper

lysosomal and GCase function [93]. Arimoclomol, a small molecule amplifier of HSP70, was shown to enhance the folding, maturation, activity, and cellular localization of mutated GCase in primary fibroblasts and neuronal-like cells from GD patients [93]. This compound is currently evaluated in a clinical trial for GD type I and III (NCT03746587), but it has not yet been studied in PD.

#### 5.4. Alternative Approaches to Manipulate the GCase Pathway

It is well-known that GCase is transported from the ER by the GCase transporter lysosomal integral membrane protein-2 (LIMP-2), which is encoded by the scavenger receptor class B member 2 (*SCARB2*) gene (OMIM 602257) [94]. Genetic variations in the *SCARB2* gene have been associated with an increased risk of developing PD [95], so manipulation of LIMP-2 expression might represent a valid strategy for PD treatment. A direct relationship between LIMP-2 expression and  $\alpha$ -synuclein accumulation has been demonstrated in LIMP-2<sup>-/-</sup> mouse models where LIMP-2 depletion resulted in accumulation of soluble and insoluble oligomeric  $\alpha$ -synuclein species in brain, likely due to lysosomal deficiency of GCase and subsequent accumulation of substrates [96]. In murine neuroblastoma cell lines, heterologous expression of wild-type LIMP-2 led to a significant reduction in  $\alpha$ -synuclein protein [96]. Structural models of the interaction site between LIMP-2 and GCase have been proposed, paving the way to the design of small molecules targeting the LIMP-2/GCase complex [97].

Recently, the leucine rich repeat kinase 2 (*LRRK2*) gene (OMIM 609007) has been proposed as a negative regulator of lysosomal GCase activity [98]. In this report, *LRRK2* mutations resulted in reduced GCase activity in human dopaminergic neurons, and the inhibition of *LRRK2* kinase activity restored GCase activity in neurons carrying either *LRRK2* or *GBA1* mutations, and rescued neurons from PD phenotype [98]. Therefore, due to its influence on the GCase pathway, *LRRK2* inhibition may be beneficial for PD patients, and the relationship between the two proteins deserves to be further investigated.

Finally, the GCase pathway can be manipulated by the modulation of misfolded GCase degradation through small molecule compounds such as histone deacetylase inhibitors (HDACis). HDACis cause hyperacetylation of chaperones such as Hsp90, limiting their affinity to misfolded GCase protein and preventing the subsequent UPR-related degradation, thus increasing the quantity of GCase and overall catalytic activity of GCase in fibroblasts from GD patients [99, 100].

Taken together, these data suggest that future studies might evaluate the effects of LIMP-2, *LRRK2* inhibition and HDACis in slowing the pathological progression of  $\alpha$ -synuclein in PD models.

## 6. Conclusions

The association between mutations of the *GBA1* gene and PD is one of the most interesting and promising discoveries related to PD pathogenesis. The available evidence from *in vitro* and *in vivo* studies indeed indicates that GCase activity and  $\alpha$ -synuclein levels have a reciprocal relationship. Although which of the molecular mechanisms involved in the GCase pathway prevails over the others, or how they coexist, in determining  $\alpha$ -synuclein pathology remains unclear, ongoing translation studies have been focused on designing molecules that can target the GCase pathway and reduce  $\alpha$ -synuclein accumulation. Clinical trials evaluating some of these compounds are already underway in PD patients, with the purpose of demonstrating their disease-modifying potential, thus making James Parkinson's hope a forthcoming reality.

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