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# Targeting the *GBA1* pathway to slow Parkinson disease: Insights into clinical aspects, pathogenic mechanisms and new therapeutic avenues

Elisa Menozzi [a,b], Marco Toffoli [a,b], Anthony H.V. Schapira [a,b,*]

[a] Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London, UK
[b] Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, United States of America

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ABSTRACT

The *GBA1* gene encodes the lysosomal enzyme glucocerebrosidase (GCase), which is involved in sphingolipid metabolism. Biallelic variants in *GBA1* cause Gaucher disease (GD), a lysosomal storage disorder characterised by loss of GCase activity and aberrant intracellular accumulation of GCase substrates. Carriers of *GBA1* variants have an increased risk of developing Parkinson disease (PD), with odds ratio ranging from 2.2 to 30 according to variant severity. *GBA1* variants which do not cause GD in homozygosis can also increase PD risk. Patients with PD carrying *GBA1* variants show a more rapidly progressive phenotype compared to non-carriers, emphasising the need for disease modifying treatments targeting the *GBA1* pathway.

Several mechanisms secondary to GCase dysfunction are potentially responsible for the pathological changes leading to PD. Misfolded GCase proteins induce endoplasmic reticulum stress and subsequent unfolded protein response and impair the autophagy-lysosomal pathway. This results in α-synuclein accumulation and spread, and promotes neurodegenerative changes. Preclinical evidence also shows that products of GCase activity can promote accumulation of α-synuclein, however there is no convincing evidence of substrate accumulation in *GBA1*-PD brains. Altered lipid homeostasis secondary to loss of GCase activity could also contribute to PD pathology.

Treatments that target the *GBA1* pathway could reverse these pathological processes and halt/slow the progression of PD. These range from augmentation of GCase activity via *GBA1* gene therapy, restoration of normal intracellular GCase trafficking via molecular chaperones, and substrate reduction therapy. This review discusses the pathways associated with *GBA1*-PD and related novel *GBA1*-targeted interventions for PD treatment.



**Contents**



*Abbreviations:* BBB, blood–brain barrier; CBE, conduritol β-epoxide; CSF, cerebrospinal fluid; CTSD, cathepsin D; DLB, dementia with Lewy bodies; ER, endoplasmic reticulum; GCase, glucocerebrosidase; GD, Gaucher disease; GCS, glucosylceramide synthetase; GlcCer, glucosylceramide; GlcSph, glucosylsphingosine; iPSC, induced pluripotent stem cells; iRBD, idiopathic REM sleep behaviour disorder; LD, lipid droplets; PD, Parkinson disease; PSAP, prosaposin; SAPC, saposin C; SNpc, substantia nigra pars compacta; UPR, unfolded protein response.

* Corresponding author at: Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, London NW3 2PF, UK.
*E-mail address:* a.schapira@ucl.ac.uk (A.H.V. Schapira).

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## 1. Introduction

Parkinson disease (PD) is a progressive neurodegenerative condition characterised by loss of nigrostriatal dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of aggregates of α-synuclein in the form of Lewy bodies (LBs) and neurites in different brain regions. Typically, patients display motor features suggestive of PD, such as bradykinesia, tremor and rigidity, after 50% to 80% of SNpc dopaminergic neurons have been lost (Cheng, Ulane, & Burke, 2010). This supports the need to find treatments that can be provided to individuals in the very early stage of their condition and, preferably, in the preclinical phase. Epidemiologically, PD is the second most common neurodegenerative disease, affecting 1% of the global population over 60 years of age. Between 1990 and 2016, the number of people affected by PD has doubled to over 6 million worldwide (GBD, 2018), and this number is predicted to reach over 12 million by 2040 (Dorsey & Bloem, 2018). Cumulative lifetime risk of PD has been estimated to be 4 to 6.6% in the overall population, and up to 7.8% in non-smokers (Driver, Logroscino, Gaziano, & Kurth, 2009; Elbaz et al., 2002; Licher et al., 2019). Several different factors, alone or in combination, have been considered responsible of this increased prevalence, including increased longevity and decreased smoking rates (Dorsey, Sherer, Okun, & Bloem, 2018).

Recent advances in our understanding of the genetic causes and pathophysiology of PD have identified novel therapeutic targets to change the course of PD. Amongst these, genetic variants in the *GBA1* gene have attracted considerable attention and *GBA1* now represents one of the most exciting targets for new drug development in PD. Understanding the biological consequences associated with different *GBA1* variants, and how these may also contribute to pathological changes in individuals not carrying variants in *GBA1*, represents a major challenge.

In this review, we discuss the epidemiological and clinical features associated with *GBA1*-PD and present the biological mechanisms supporting a direct role of *GBA1* in PD pathogenesis. We then summarize the range of possible *GBA1*-targets for PD and their translation into pharmacological treatments.

## 2. *GBA1* variants in PD: Classification and epidemiological aspects

The *GBA1* gene is located on chromosome 1q22 and is made up of 11 exons and 10 introns spanning a sequence of 7.6 kb. Located 6.9 kb downstream, an untranslated pseudogene (*GBAP1*) shares an overall 96% homology in the coding region (Horowitz et al., 1989), and up to 98% in the region from intron 8 to the 3′-UTR (Zampieri, Cattarossi, Bembi, & Dardis, 2017). *GBA1* encodes a 60 kDa lysosomal hydrolase enzyme, glucocerebrosidase (GCase, or acid-β-glucosidase, EC 3.2.1.45), which hydrolyses glucosylceramide (GlcCer) to ceramide and glucose. Biallelic pathogenic variants in *GBA1* are responsible for the autosomal recessive lysosomal storage disorder Gaucher disease (GD). GD is a rare condition in the general population (affecting 1/40,000 to 1/50,000 births), but much more common in the Ashkenazi Jewish (AJ) population with 1/800 births being affected (Grabowski, 2008; Stirnemann et al., 2012). GD is the result of insufficient activity of GCase, and the lysosomal accumulation of its main substrate, GlcCer. The excessive accumulation of GlcCer in macrophages leads to a series of clinical manifestations affecting GD patients such as hepatosplenomegaly, anaemia, thrombocytopenia, and bone involvement. According to the degree of neurological involvement, GD can be categorised into three clinical subtypes: GD type I (OMIM 230800), which is the commonest form and is free from neurological symptoms (thus called 'non-neuronopathic variant'); and GD type II (OMIM 230900) and type III (OMIM 231000), the 'neuronopathic variants', characterised by different degrees of neurological involvement (Grabowski, 2008). *GBA1* variants can be classified according to their biochemical effect on GCase enzymatic activity as: mild variants (e.g., p.N370S, p.R496H), which result in *in-vitro* residual enzymatic activity of 32-38% and are associated with GD type I in homozygous/compound heterozygous carriers, and severe variants (e.g., p.L444P, p.84GG, IVS2_1, p.V394L, p.D409H), which cause *in-vitro* residual activity of 13-24% and are associated with GD type II and III in homozygous/compound heterozygous carriers (Alfonso et al., 2004; Malini et al., 2014). These genotype-phenotype correlations are not absolute, and exceptions do occur.

The association between *GBA1* variants and an increased risk for developing PD was first noticed more than two decades ago in the GD clinics, when physicians observed that both GD patients and their unaffected relatives showed some signs of parkinsonism (Goker-Alpan et al., 2004; Neudorfer et al., 1996). Large sample size studies conducted on PD populations followed this initial clinical observation and confirmed that variants in *GBA1* are the commonest genetic risk factor for PD (Sidransky et al., 2009). Overall, *GBA1* variants are found in 5-15% of European PD cases, but up to 20-30% of AJ PD cases (Zhang et al., 2018). The rate of *GBA1* variants is much lower in the Chinese PD population (only 5.4%-8.4%), and in the general population (less than 1%) (Chen et al., 2020; Yu et al., 2015; Zhang et al., 2018).

Over the years, the list of *GBA1* variants identified in PD cases has expanded and their classification modified (for an overview of *GBA1* variants classification, see Table 1). For PD, *GBA1* variants are classified as "severe" and "mild" still based on GD classification of variant severity; within the severe group, "complex" variants resulting from conversions, fusions, and insertions of parts of the pseudogene *GBAP1* into *GBA1* can be found. Moreover, some *GBA1* variants which do not cause GD in homozygous carriers but increase PD risk are now classified as "risk" variants (e.g., p.E326K, p.T369M), together with an increasing number of "unknown" variants whose significance still needs to be elucidated. A recent comprehensive list of all *GBA1* variants reported in PD has been published, together with an associated online browser to retrieve available data associated with genetic variants (Parlar, Grenn, Kim, Baluwendraat, & Gan-Or, 2023). More than 300 variants and gene rearrangements in the *GBA1* gene have been reported. Of these, 5.9% were identified as mild, 22.6% as severe, and 0.8% as risk variants for PD. The risk variants reported were biallelic and heterozygous forms of E326K, T369M, and E388K. The remaining 70.6% were classified as unknown because of lack of information on either their pathogenicity for GD or their association with PD risk (Parlar, Grenn, Kim, Baluwendraat, & Gan-Or, 2023), so there is still much to discover about the genetic background of *GBA1*-PD. Moreover, the reasons behind the incomplete penetrance of *GBA1* variants for PD, which has been shown to range from 10% to 30% (Anheim, et al., 2012; Rana, Balwani, Bier, & Alcalay, 2013), remain to be understood. Odds ratios (ORs) for PD risk are variant-specific, with N370S, L444P and E326K variants increasing the risk of PD by roughly 2.20-7.80, 6.40-30.40, and 1.57-5.50 respectively (Parlar, Grenn, Kim, Baluwendraat, & Gan-Or, 2023).

Some individuals with *GBA1* variants might also develop Dementia with Lewy Bodies (DLB), a more severe form of parkinsonism characterised by accumulation of α-synuclein and more aggressive disease course. An OR of 8.28 has been estimated for *GBA1* variants and DLB (Nalls et al., 2013). Recently, *GBA1* variants were also found to be more common in patients with idiopathic REM sleep behaviour disorder (iRBD; 9.5%) compared with controls (4.1%), and *GBA1*-positive iRBD patients showed increased rate of conversion to PD compared with *GBA1*-negative iRBD (Krohn et al., 2020). Which factors, genetic or environmental, ultimately determine the development of one condition or another are still unknown. Prospective studies evaluating large cohorts of unaffected *GBA1* carriers are needed.

## 3. Genotype-phenotype associations in *GBA1*-PD

The classification of *GBA1* variants according to type and severity is helpful in PD for different reasons. First, frequencies of specific *GBA1*

**Table 1**
Overview of current knowledge about *GBA1* variants and functional defects associated with bi-allelic variants, their classification as "severe", "mild" and "risk" in PD, related specific PD phenotypes and *GBA1* dependent metabolic pathways and therapeutic options.

| Variants class | | Severe | Mild | Risk |
|---|---|---|---|---|
| Most common variants in class | | L444P, RecNCI1, 84GG | N370S, R496H | E326K, T369M |
| Type of GD caused by bi-allelic variants in class | | Types II and III | Type I | Do not cause GD |
| OR for PD risk* | | 6.40–30.40 | 2.2–7.80 | 1.57–5.50 |
| GCase activity reduction | | Severe reduction | Mild reduction | Marginal reduction |
| Specific PD phenotype features | Age at onset | ↓↓↓ | ↓↓ | ↓ |
| | Motor symptoms severity | ↑↑↑ | ↑↑ | ↑ |
| | Non-motor symptoms severity | ↑↑ | ↑↑ | ↑↑ |
| | Small molecule chaperones | ✓ | ✓ | ✓ |
| | Gene therapy | ✓ | ✓ | ✓ |
| | Substrate reduction therapy | ✓ | ✓ | ✘ |
| | Acid ceramidase inhibition | ✓ | ✓ | ✘ |
| | Lipid droplets biosynthesis | ✓ | ✓ | ✓ |
| | CTSD, SAPC and PGRN | ✓ | ✓ | ✓ |
| Potential therapeutic targets | Cholesterol homeostasis | ✓ | ✓ | ✓ |

Legend: CTSD, cathepsin D; GCase, glucocerebrosidase; GD, Gaucher disease; OR, odds ratio; PD, Parkinson disease; PGRN, progranulin; SAPC, saposin C; *estimated on the variants L444P, N370S and E326K, respectively (Parlar, Grenn, Kim, Baluwendraat, & Gan-Or, 2023).

variants differ across PD populations, with the risk variants E326K or T369M being the commonest in European cases, the mild N370S in AJ cases and the severe L444P in some East and South Asian populations (Parlar, Grenn, Kim, Baluwendraat, & Gan-Or, 2023). Second, specific genotype-phenotype associations have been recognised for *GBA1*-PD. *GBA1*-PD cases were reported to develop symptoms at earlier age compared with non-carriers, especially those carrying severe variants as compared to mild or risk variants (Gan-Or et al., 2015; Lerche et al., 2020; Petrucci et al., 2020). In terms of motor profile, carriers of severe variants showed a more aggressive phenotype with faster development of axial symptoms, as opposed to carriers of mild or risk variants, the latter showing late occurrence of motor fluctuations (Petrucci et al., 2020; Stoker et al., 2020). When evaluating the non-motor profile, the difference between *GBA1*- and non-carrier PD cases is even more noticeable. *GBA1*-PD cases showed a higher burden of cognitive issues, with carriers of complex or severe variants displaying more severe and rapidly progressive cognitive decline than carriers of mild or risk variants (Cilia et al., 2016; Liu, et al., 2016; Petrucci et al., 2020). Similarly, psychiatric symptoms and hallucinations occurred more frequently in *GBA1*-PD than non-carrier PD patients (Brockmann et al., 2011; Petrucci et al., 2020; Wang, et al., 2014), again with carriers of severe and complex variants presenting the more severe phenotype (Petrucci et al., 2020; Thaler et al., 2018). Olfactory dysfunction was also more prominent in *GBA1*-PD (Malek, et al., 2018), with carriers of severe variants showing the worst profile (Thaler, Bregman, et al., 2018). A recent meta-analysis on sleep disorders in PD concluded that *GBA1*-PD patients showed higher risk of RBD compared with non-carriers, with carriers of mild N370S and severe L444P variants presenting the highest risk (Huang, Cheng, Li, & Shang, 2022). In terms of disease progression, several studies reported that *GBA1*-PD patients progressed faster than non-carriers (Brockmann et al., 2015; Stoker et al., 2020). When survival rates were compared with non-carriers, carriers of severe and mild variants showed reduced survival, whereas carriers of risk variants did not show any difference (Stoker et al., 2020). However, a longitudinal study conducted on AJ PD patients did not show any significant effect of *GBA1* variants (either mild or severe) on survival rates (Thaler et al., 2018), and the direct comparison of mild and severe variants carriers did not result in any differences either (Cilia et al., 2016).

Despite a few discrepancies across studies, *GBA1*-PD is now recognised as a multifaceted condition. At one end of the spectrum, there are carriers of risk and mild variants whose phenotype is closer to that of non-carriers, and at the other end, there are carriers of severe and complex variants whose phenotype is more severe and associated with faster progression. Stratification of patients according to their *GBA1* genotype is thus possible, however caution should be used as objective biomarkers able to predict disease trajectories on an individual basis are still missing. A few biomarkers, such as GCase activity or sphingolipids levels in blood, have been tested and seem promising (see below). Such biomarkers could not only be applied in clinical setting to improve patients' management and counselling, but also in clinical trials to achieve target engagement/modulation when testing GCase targeted therapies.

## 4. GCase structure

The mature GCase protein consists of a 497-amino-acid membrane associated protein with a 39-aminoacid leader sequence and five glycosylation sites (Do, McKinney, Sharma, & Sidransky, 2019). It comprises three non-continuous domains. Domain I (residues 1-27 and 383-414) consists of an antiparallel β-sheet and contains two disulfide bridges, which are essential for correct folding of the protein. Domain II (residues 30-75 and 431-497) is an immunoglobulin-like domain made up of 8 β-sheets. Domain III (residues 76-381 and 416-430) is a $(\beta/\alpha)_8$ triosephosphate isomerase (TIM) barrel with three free cysteines at residues 126, 248 and 342, which contains the active site (Dvir et al., 2003; Liou et al., 2006; Smith, Lee, Menozzi, & Schapira, 2022; Smith, Mullin, & Schapira, 2017). Three flexible loops are housed within the mature GCase protein, of which loop 3 can change conformation in an acidic environment to give substrates access to the active site (Lieberman et al., 2007; Lieberman, D'Aquino, Ringe, & Petsko, 2009).

Following translation, GCase protein is folded in the endoplasmic reticulum (ER) and passes along the secretory pathway from the ER to the Golgi. After several steps of glycosylation, the protein is then trafficked to the endosomes and the lysosomes. To traffic from the ER to the lysosome, GCase needs to bind to the transporter lysosomal integral membrane protein-2 (LIMP-2) which is encoded by the *SCARB2* gene (Reczek et al., 2007). Within the lysosome, GCase interacts with its activator protein saposin C (SAPC), derived from the cleavage of its precursor prosaposin (PSAP) (Tamargo, Velayati, Goldin, & Sidransky, 2012), and finally becomes active in the acidic environment.

As a result of *GBA1* variants, the structure of GCase protein can change with consequent loss of GCase activity. The residual catalytic activity of GCase differs according to *GBA1* variant severity. For the severe and mild variants such as L444P and N370S in bi-allelic form, GCase activity is reduced around 80-95% and 50-60% respectively compared to native GCase protein (Grace, Ashton-Prolla, Pastores, Soni, & Desnick, 1999; Liou et al., 2006), whereas the reduction is much lower or even absent for risk variants (Grace et al., 1999; Liou & Grabowski, 2012). Different mechanisms can lead to reduced GCase activity: a) loss of transcription/translation, b) misfolded GCase protein promoting ER stress and activation of the unfolded protein response (UPR), c) failure of GCase to exit the Golgi, or d) loss of critical amino acids in the catalytic domain (Do et al., 2019; Gegg, Menozzi, & Schapira, 2022). Once again, different *GBA1* variants might exert their effect on GCase activity

through different mechanisms (discussed below), for example the L444P or N370S variants being associated with UPR activation and ER stress (Fernandes et al., 2016; Ron & Horowitz, 2005; Sanchez-Martinez et al., 2016). Loss of GCase function could also occur in the absence of *GBA1* variants, as a result of incorrect lysosomal trafficking due to genetic variants in *SCARB2*, or SAPC defects (Do et al., 2019). Moreover, *in vitro* evidence suggests that loss of GCase activity can also be secondary to increased levels of α-synuclein (Gegg et al., 2012; Mazzulli et al., 2011), supporting the existence of a reciprocal effect between GCase and α-synuclein (see below).

## 5. The interactions between GCase and α-synuclein

There are several mechanisms by which *GBA1* variants and dysfunctional GCase can promote α-synuclein pathology.

Strong evidence from *in vitro* and *in vivo* models recapitulating GCase deficiency supports the hypothesis that reduced GCase function is sufficient to cause α-synuclein accumulation. *In vitro*, higher levels of α-synuclein were observed in human dopaminergic midbrain neurons differentiated from induced pluripotent stem cells (iPSC) of patients with GD and parkinsonism (Aflaki et al., 2016), and human midbrain organoids generated from GBA1$^{-/-}$ embryonic stem cells (Jo et al., 2021). Increased levels of α-synuclein were found in iPSC-derived neurons after treatment with the GCase inhibitor conduritol β-epoxide (CBE), and in *GBA1*-PD iPSC neurons (Schondorf et al., 2014; Woodard et al., 2014; Yang, Beavan, Chau, Taanman, & Schapira, 2017; Yang, Taanman, Gegg, & Schapira, 2022), suggesting that also heterozygous *GBA1* variants may predispose to PD via alterations in α-synuclein clearance (Baden, Yu, & Deleidi, 2019). In mice heterozygous for the L444P variant, GCase dysfunction was able to enhance neuronal vulnerability and dopaminergic cell loss triggered by increased α-synuclein expression (Migdalska-Richards et al., 2017).

Several mechanisms by which dysfunctional GCase can lead to α-synuclein accumulation have been proposed. Pathways involved in α-synuclein clearance were shown to be altered in the presence of GCase dysfunction. At the cellular level, two major pathways contribute to intracellular protein degradation, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway, with the latter further categorised into three subtypes – macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) – according to their selectivity and the mechanisms by which they deliver substrates to the lysosome (Campbell, Morris, & Schapira, 2018).

Macroautophagy relies on the formation of double-membraned autophagosomes, which ultimately fuse with lysosomes where the cytoplasmic contents, including misfolded or aggregated proteins, are degraded (Alvarez-Erviti et al., 2010). Markers of macroautophagy impairment have been reported in GCase deficient neurons (Fernandes et al., 2016; Magalhaes et al., 2016) and zebrafish, Drosophila, and mice *GBA1* KO models (Keatinge et al., 2015; Kinghorn et al., 2016; Osellame et al., 2013). Since reduction of GCase activity generally caused an increase in the number of autophagosomes in these models, macroautophagy impairment is likely to be secondary to decreased fusion of autophagosomes within the lysosome, resulting in increased lysosomal content and reduced lysosomal degradation of cargo proteins such as α-synuclein (Gegg et al., 2022). To note, autophagosome accumulation resulting from lysosomal impairment has also been shown in sporadic (non-*GBA1*) models of PD (Dehay et al., 2010).

CMA is dependent on the heat shock cognate 70 (hsc70) protein and its binding to the lysosomal-associated membrane protein 2A (LAMP2A), a lysosomal surface receptor. Previous reports showed that CMA was impaired in sporadic PD, as both LAMP2A and hsc70 levels were significantly decreased in the SNpc and amygdala of PD patients compared with controls (Alvarez-Erviti et al., 2010). Only recently, the association between GCase, CMA and PD has become evident. Lysosomal levels of LAMP2A were drastically reduced in *GBA1* KO, or *GBA1* N370S or L444P differentiated human dopaminergic-like neuroblastoma cells (Navarro-Romero et al., 2022). Decreased CMA activity was observed in brains of *GBA1*-PD patients following single-nucleus RNA sequencing and proteomic analyses. The presence of mutant GCase protein, but not the loss of enzymatic activity, was found to inhibit CMA (Kuo et al., 2022). Despite a very inefficient lysosomal internalization via the LAMP2A multimeric translocation complex, misfolded N370S and L444P GCase was shown to block the multimerization of LAMP2A and therefore prevent degradation of other CMA substrates including α-synuclein (Kuo, Tasset, Cheng, et al., 2022). Based on this evidence, it has been hypothesised that *GBA1* variants leading to misfolding and mislocalization of GCase into the cytosol, can result in a toxic gain-of-function due to blockage of CMA, followed by accumulation of CMA substrates. Replacing GCase enzyme may, thus, not be sufficient to normalize cellular dysfunction for those mutant GCase forms that inhibit CMA (Kuo, Tasset, Cuervo, & Sulzer, 2022).

Accumulation and aggregation of α-synuclein was also shown to be promoted by increased ER stress and activation of UPR secondary to misfolded GCase (Bellucci et al., 2011; Colla et al., 2012; Heman-Ackah et al., 2017).

Besides the effect of GCase protein/enzyme on α-synuclein turnover, increased levels of α-synuclein can negatively affect maturation and trafficking of GCase and other lysosomal hydrolases (Mazzulli et al., 2011). In human neuroblastoma SH-SY5Y over-expressing high levels of exogenous α-synuclein, GCase activity and protein levels were decreased by 70% and 87% respectively, whereas protein levels were decreased by 33% in cells expressing 10-fold lower levels of exogenous α-synuclein (Gegg et al., 2012). Differentiated dopaminergic SH-SY5Y or hippocampal and cortical neurons treated with α-synuclein preformed fibrils (PFF) showed decreased GCase activity (Gegg, Verona, & Schapira, 2020; Henderson et al., 2020). Similar findings were demonstrated also outside the CNS, specifically within the gut, where it has been proposed that α-synuclein pathology might start in a subgroup of PD subjects (Borghammer & Van Den Berge, 2019). The inoculation of PFF in the duodenal wall decreased GCase activity in animal models (Challis et al., 2020). Although the precise mechanisms behind the effect of α-synuclein on GCase remain unclear, this bidirectional loop between GCase and α-synuclein supports the hypothesis that pathological α-synuclein and GCase deficiency can spread in tandem, both in the CNS and in the periphery (Gegg et al., 2022).

Accumulation of GCase substrates may also lead to α-synuclein aggregation. As mentioned above, the result of GCase enzymatic dysfunction is the intracellular accumulation of its upstream substrate GlcCer. GlcCer can either exit the lysosome into the cytosol, or remain within the lysosome where it is processed to glucosylsphingosine (GlcSph) which then is secreted into the cytosol (Lerche et al., 2021). There are several *in vitro* and *in vivo* studies suggesting that GlcCer and GlcSph may promote α-synuclein aggregation, and thus substrate accumulation might be responsible for PD pathology build-up. GlcCer was shown to directly promote the conversion of physiological α-synuclein into insoluble forms in iPSC derived from GD patients and PD patients with triplication of *SNCA* gene, and this process was reversed by decreasing GlcCer levels (Zunke et al., 2018). Similarly, GlcSph was shown to promote the formation of oligomeric α-synuclein in young GD/PD mice brains (Taguchi et al., 2017). Mice carrying the severe *GBA1* L444P variant showed increased levels of GlcSph, but not GlcCer, in brain and serum, with increased α-synuclein inclusions in the hippocampus, which were not rescued though by treatment with a GlcCer synthase inhibitor (Mahoney-Crane et al., 2023).

Changes in GCase activity can also modulate the aggregation propensity of α-synuclein through its effect on lipid membrane composition (Galvagnion, 2017). An increase in short chain (C34) sphingomyelin, ceramide and hexosylceramide and a consistent reduction of long chain (C42) sphingomyelin, ceramide and hexosylceramide were seen in fibroblasts from PD patients with heterozygous L444P vs control or non-carrier PD. Increased C34:C42 sphingolipid ratio was correlated with decreased GCase activity (Galvagnion et al., 2022).

The kinetics of α-synuclein aggregation were significantly accelerated after addition of *GBA1*-PD lipid extracts when compared to control samples (Galvagnion et al., 2022). Interestingly, treatment with a GCase chaperone ambroxol (discussed below) restored GCase activity and sphingolipid composition of membranes and reversed the pro-aggregation effect on α-synuclein exerted by lipid extracts from *GBA1*-PD fibroblasts (Galvagnion et al., 2022).

In conclusion, several mechanisms could underlie the bidirectional crosstalk between GCase and α-synuclein. For the different *GBA1* variants, there is no "one-size-fits-all" pathway, and more likely several mechanisms may act simultaneously. Also, some of these pathways might play a role in non-*GBA1*-PD patients, where similar abnormalities to *GBA1*-PD patients have been observed. Both GCase and α-synuclein might, therefore, be appealing targets for therapeutic development in PD.

## 6. Translational neuroscience: GCase molecular targets and treatments

In this section, the most appealing GCase molecular targets evaluated so far will be presented. For each GCase target, we will summarise the rationale behind the choice of the selected molecular targets by focusing on human studies (preclinical studies on α-synuclein are summarised in Section 5), and then present the developed treatments and their application in clinical trials, when available. A detailed summary of the completed, currently active, and planned clinical trials targeting *GBA1* in PD is reported in Table 2.

### 6.1. Reduced GCase activity and protein levels

In post-mortem studies conducted on cohorts of PD patients carrying *GBA1* variants, GCase activity was found to be reduced in several brain areas, with the greatest deficiency (58%) in the SNpc (Gegg et al., 2012). Other studies conducted on cohorts of advanced PD cases and DLB patients carrying *GBA1* variants also showed reduced GCase activity in the SNpc and frontal cortex (Kurzawa-Akanbi et al., 2012; Moors et al., 2019). Due to limited sample size, no definite conclusions about genotype-GCase activity association could be drawn from these studies. Measurement of GCase activity in cerebrospinal fluid (CSF) and blood has confirmed the findings of the post-mortem studies. Lower GCase enzymatic activity was found in *GBA1*-PD patients compared with non-carriers in CSF (Lerche et al., 2021; Parnetti et al., 2017), dried blood spots (Alcalay et al., 2015; Huh et al., 2020; Omer et al., 2022), and peripheral blood mononuclear cells (PBMCs) (Avenali et al., 2021; Petrucci et al., 2020). A possible genotype effect was hypothesised since decreased GCase activity was associated with increased severity of *GBA1* variants (Huh et al., 2020; Lerche et al., 2021; Pchelina et al., 2017; Petrucci et al., 2020), and over a two years median follow-up *GBA1*-PD patients carrying severe variants showed steeper decline of enzymatic activity compared with non-carriers or carriers of mild or risk variants (Huh et al., 2020). However, other studies failed to identify differences in levels of GCase activity measured in dried blood spots between carriers of severe and mild variants, or any correlation with clinical features (Omer et al., 2022), so the applicability of peripheral GCase as a marker of disease severity/progression still needs to be established.

GCase dysfunction has been shown to contribute to PD pathogenesis even in individuals with PD or other synucleinopathies who do not carry any *GBA1* variants. Post-mortem studies conducted on non-carrier PD or DLB cohorts, showed reduced GCase activity in the SNpc, caudate, cerebellum, and other brain regions compared with matched controls (Chiasserini et al., 2015; Gegg et al., 2012; Moors et al., 2019; Murphy et al., 2014). Once again, reduced GCase activity was found in CSF and dried blood spots of non-carrier PD patients, with higher residual activity being associated with longer disease duration (Alcalay et al., 2015; Parnetti et al., 2017). In both *GBA1*-PD and non-carrier PD brains, the reduced GCase activity correlated with decreased protein expression (Gegg et al., 2012; Kurzawa-Akanbi et al., 2012; Murphy et al., 2014).

Overall, these lines of evidence suggest that loss of GCase enzymatic activity and protein can be related to the accumulation of α-synuclein, and contribute to PD pathogenesis and progression, in both carriers and non-carriers of *GBA1* variants. Therefore, treatments tailored to restore or enhance GCase activity might be an optimal strategy to slow down disease progression.

**Table 2**
Overview of completed, currently active and planned clinical trials targeting *GBA1* in PD.

| Recruitment status | Mechanism of action | Drug/Sponsor | Clinical Trial ID | Clinical Trial name | Phase | Study start date | N. participants | Primary outcome | Results |
|---|---|---|---|---|---|---|---|---|---|
| Completed | Molecular chaperone | Ambroxol/University College London | NCT02941822 | AiM-PD | 2a | December 2016 | 17 PD with and without *GBA1* variants | Changes in CSF ambroxol and GCase levels at days 186 compared to baseline | Ambroxol detected in CSF, increased CSF GCase protein levels |
| Early terminated | Substrate reduction therapy | Venglustat (GZ/SAR402671)/Genzyme (Sanofi) | NCT02906020 | MOVES-PD | 2 | December 15, 2016 | 221 PD with *GBA1* variants | Safety, tolerability, and changes in MDS-UPDRS part II+III score in OFF state at week 52 compared to baseline | Deterioration in motor function in venglustat-treated patients |
| Active, recruiting | Gene therapy | PR001 (LY3884961)/Prevail Therapeutics | NCT04127578 | PROPEL | 1/2a | January 3, 2020 | 20 [target] PD with pathogenic *GBA1* variants | Safety, tolerability, immunogenicity | NA |
| Active, not recruiting | Molecular chaperone | Ambroxol/Lawson Health Research Institute | NCT02914366 | NA | 2 | November 2015 | 55 [target] PDD | Changes in ADAS-cog and the CGIC at week 26 and 52 compared to baseline | NA |
| Active, recruiting | Molecular chaperone | Ambroxol/Fondazione I.R.C.C.S. Istituto Neurologico Carlo Besta | NCT05287503 | AMBITIOUS | 2 | February 15, 2022 | 60 [target] PD with *GBA1* variants | Changes in MOCA score and cognitive status at week 52 compared to baseline | NA |
| Planned, not yet recruiting | Molecular chaperone | Ambroxol/University College London | NCT05778617 | ASPro-PD | 3a | September 2023 | 330 [target] PD with and without *GBA1* variants | Changes in MDS-UPDRS part I-III score at week 104 compared to baseline | NA |

Legend: ADAS-Cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CGIC, ADCS-Clinician's Global Impression of Change; CSF, cerebrospinal fluid; GCase, glucocerebrosidase; MDS-UPDRS, MDS-Unified Parkinson's Disease Rating Scale; MOCA, Montreal Cognitive Assessment; NA, not available; PD: Parkinson disease; PDD, Parkinson disease dementia.

#### 6.1.1. Normalising GCase activity via gene therapy

To normalise enzymatic levels, enzyme replacement therapy has been used in GD since 1991 (Barton et al., 1991). However, recombinant GCase imiglucerase does not cross the blood–brain barrier (BBB) and therefore it cannot be used to treat neurological manifestations of GD (Stojkovska, Krainc, & Mazzulli, 2018). For the same reason, enzyme replacement therapy is not a suitable option for PD.

An alternative way to restore normal GCase protein activity and levels is via gene therapy. This has been proven to be protective in experimental models of neuronopathic GD (Massaro et al., 2018; Sardi et al., 2011) and synucleinopathies (Morabito et al., 2017; Rocha et al., 2015b; Rockenstein et al., 2016; Sardi et al., 2013). For instance, intravenous injection of adeno-associated virus (AAV)-PHP.B expressing *GBA1* in adult A53T-*SNCA* mice restored physiological levels of GCase enzyme, significantly reduced α-synuclein accumulation and improved behavioural features (Morabito et al., 2017). Again, co-injection of AAV-*GBA1* with AAV-A53T α-synuclein into the SN of rats prevented α-synuclein-mediated degeneration of nigrostriatal dopamine neurons (Rocha et al., 2015b). In a different model using transgenic mice overexpressing wild-type α-synuclein (ASO mice), intra-cerebral AAV-*GBA1* delivery increased GCase activity and reduced accumulation of α-synuclein in the SN and striatum (Rocha et al., 2015b). Given this promising evidence, three clinical trials testing *GBA1* gene therapy (LY3884961, formerly PR001) in humans have recently started, sponsored by Prevail Therapeutics. The PRV-PD101 (ClinicalTrials.gov ID: NCT04127578, PROPEL Study) is a phase 1/2a, multicenter, open-label, ascending dose study evaluating the safety, tolerability, immunogenicity, biomarkers, and clinical effects, of intracisternal high-dose and low-dose LY3884961 administration in patients with moderate to severe PD carrying at least one pathogenic *GBA1* variant. The other two studies, PRV-GD2-101 (ClinicalTrials.gov ID: NCT04411654, PROVIDE Trial), and J3Z-MC-OJAE (ClinicalTrials.gov ID: NCT05487599, PROCEED Trial), are open-label, phase 1/2, multicenter studies to evaluate safety, efficacy, immunogenicity, and biomarkers of single-dose intracisternal administration of LY3884961 in infants with GD type II, and single intravenous dose of LY3884961 in adults with GD type I, respectively.

### 6.2. Misfolded GCase

Next to the aforementioned loss-of-function hypothesis, *GBA1* variants could influence PD pathogenesis through a gain-of-function mechanism. Most *GBA1* variants are missense and thus lead to the production of misfolded proteins (Sidransky & Lopez, 2012). When unable to be refolded in the ER by chaperones, GCase undergoes ER-associated degradation (ERAD) and unfolded GCase proteins are re-directed to the proteasome to be degraded, thus promoting ER stress and UPR activation (Gegg et al., 2022). Different *GBA1* variants have shown variable degrees of ER retention and protein degradation (Ron & Horowitz, 2005), with a possible correlation with variant severity. For example, fibroblasts derived from patients carrying the L444P variant showed extensive ERAD (Bendikov-Bar, Ron, Filocamo, & Horowitz, 2011). Again, in fibroblasts or human midbrain neurons carrying L444P and N370S variants, activation of UPR caused dysregulation of intracellular calcium homeostasis which is an important mechanism favouring neurodegeneration (Kilpatrick et al., 2016; Schondorf et al., 2014). As reported in Section 5, misfolded GCase can also contribute to PD pathogenesis by blocking CMA (Kuo, Tasset, Cuervo, & Sulzer, 2022). Therefore, therapeutic strategies aimed to stabilize and refold GCase misfolded proteins, and thus alleviate ER stress may be another appealing option for PD.

#### 6.2.1. Molecular chaperones promote post-ER trafficking of mutant GCase

Small, brain-penetrant chemical chaperones can stabilize and refold GCase misfolded proteins, thus enabling trafficking of mutant GCase from the ER to the Golgi and then the lysosome (Blandini et al., 2019; Do et al., 2019). There are three groups of GCase molecular chaperones: inhibitory, non-inhibitory, and mixed-type.

The first class of GCase chaperones-the inhibitory, have the potential to antagonize the binding of the enzyme substrates and reduce GCase activity by binding to the active site of the protein (Blandini et al., 2019). However, if the chaperone affinity for the substrate is too high, the active site will remain bound to the chaperone, with consequent inhibition of lysosomal GCase activity (Blandini et al., 2019). Most of the GCase chaperones discovered so far are inhibitory. The first one to be described more than 20 years ago is N-nonyl-deoxynojirimycin, a chaperone belonging to the iminosugars family. In human fibroblasts with homozygous N370S variant, this compound increased GCase activity, promoting GCase transit from the ER to the lysosomes (Sawkar et al., 2002). Other iminosugars have also been identified for N370S but not for L444P variant, suggesting a variant-dependent chaperoning profile for these compounds (Sawkar et al., 2005). The iminosugar isofagomine (afegostat-tartrate, AT2101) also showed encouraging results in both *in vitro* and *in vivo* models. Isofagomine was shown to increase GCase activity in fibroblasts with homozygous N370S and L444P (Khanna et al., 2010; Steet et al., 2006), in GD animal models (Sun et al., 2012), fly models expressing human N370S and L444P (Sanchez-Martinez et al., 2016), and in mice overexpressing human wild-type α-synuclein (Thy1-aSyn mice) where treatment with isofagomine improved motor and nonmotor function, reduced neuroinflammation and α-synuclein aggregates, possibly suggesting its usefulness also in non-*GBA1* carrier PD (Richter et al., 2014). This molecular chaperone has been tested in patients with GD type I (ClinicalTrials.gov IDs: NCT00875160; NCT00813865; NCT00446550; NCT00433147; NCT00465062) without success, possibly due to the high-binding affinity to the active site up to pH 4 (Blandini et al., 2019).

The second class of pharmacological chaperones is composed by non-inhibitory chaperones. These compounds bind the enzyme outside of the active site, but still can restore post-translational folding of lysosomal mutant proteins (Jung, Patnaik, Marugan, Sidransky, & Westbroek, 2016). Through high-throughput screening of compound libraries using spleen-lysate and fibroblasts from GD patients homozygous for N370S, several GCase non-inhibitory chaperones were identified (Goldin et al., 2012; Patnaik et al., 2012). The compound NCGC00188758 was shown to enhance GCase lysosomal activity, and promote clearance of pathological α-synuclein in iPSC neuronal lines derived from PD patients with genetic variants in *SNCA* (triplication or A53T), *GBA1* or *PARK9*, and non-mutated PD patients (Mazzulli et al., 2016). Another non-inhibitory small molecular chaperone NCGC607 successfully promoted lysosomal trafficking of mutant Case, restored GCase enzymatic activity and protein levels, and reduced substrate accumulation in iPSC-derived macrophages and dopaminergic neurons from GD type I patients with or without parkinsonism (Aflaki et al., 2016). A GCase activator LTI-291 (also named BIA 28-6156) is a small molecule which has been tested in a randomized single and multiple ascending dose study on healthy volunteers. The data showed that LTI-291 had a positive safety, pharmacokinetic, and pharmacodynamic profile, with absent neurocognitive side effects but no dose-dependent changes in the levels of downstream products of GCase (den Heijer et al., 2021). The lack of clear target engagement could be secondary to patient selection (healthy subjects expected to have normal GCase enzymatic activity) (den Heijer et al., 2021), therefore further studies are warranted to test the benefit of these small molecules in *GBA1*-PD or non-carrier PD patients.

The third and final category of mixed-type small molecular chaperones includes one of the most promising therapeutic avenues targeting the *GBA1* pathway, ambroxol. This over-the-counter oral mucolytic drug was identified as enhancer of GCase activity in 2009 after screening a library of 1,040 Food and Drug Administration (FDA) approved drugs for compounds stabilizing GCase activity against thermal denaturation (Maegawa et al., 2009). Since then, preclinical evidence has accumulated to the point that ambroxol has now already been tested in phase

1-2 clinical trials in patients with PD and DLB and will soon enter a phase 3 study in patients with PD in the UK in late 2023. Chemically, ambroxol acts in a pH-dependent manner, with its inhibitory activity being maximal in the neutral pH of cytoplasm-where it inhibits the non-lysosomal *GBA2* activity (Bouscary et al., 2019), intermediate in the ER, and undetectable in the acidic pH of the lysosome (Maegawa et al., 2009). In fibroblasts from healthy controls, GD type I and heterozygous carriers of *GBA1* variants (both pathogenic and risk variants) with and without PD, treatment with ambroxol significantly increased GCase activity and reduced oxidative stress (McNeill et al., 2014). Besides acting as a GCase chaperone, it was shown that ambroxol impacts on other pathways, such as mitochondria, lysosomal biogenesis, and the secretory pathway in mouse cortical neurons. Upon ambroxol treatment, increased wild-type GCase mRNA, protein levels and activity were observed, together with increased levels of the GCase transporter LIMP2, and transcription factor EB (TFEB)-the latter involved in lysosomal biogenesis (Magalhaes, Gegg, Migdalska-Richards, & Schapira, 2018). In animal models, ambroxol increased brain levels of GCase activity in transgenic mice expressing the heterozygous L444P, or overexpressing human α-synuclein; in the latter, ambroxol effectively reduced both α-synuclein and phosphorylated α-synuclein protein levels (Migdalska-Richards, Daly, Bezard, & Schapira, 2016). Brain GCase activity was also increased in healthy nonhuman primates following ambroxol administration (Migdalska-Richards, Ko, Li, Bezard, & Schapira, 2017). Despite no significant changes in GCase activity, ambroxol was also able to prevent locomotor deficits in fly models expressing human N370S and L444P (Sanchez-Martinez et al., 2016).

In clinical studies, high-dose ambroxol was initially tested in small cohorts of neuronopathic GD patients, showing good safety and tolerability profile and ability to penetrate the BBB. Ambroxol was able to reduce accumulation of GCase substrates in the CSF, suggesting target engagement in the CNS, and improve neurological symptoms (Kim et al., 2020; Narita et al., 2016). In PD, ambroxol was firstly tested in the Aim-PD study, a phase 2a prospective, single-center, open-label, non-controlled clinical trial (ClinicalTrials.gov ID: NCT02941822). High-dose ambroxol (escalating dose up to 1,260 mg daily over 6 months) was evaluated in 23 patients with PD, with or without *GBA1* variants, of which 17 completed the study and entered the final analysis (the remainder were either excluded or withdrew because of lumbar puncture–related complications). In these 17 subjects (8 *GBA1*-PD and 9 non-*GBA1*-PD), ambroxol was shown to cross the BBB and increase GCase protein levels in the CSF, without serious adverse events. Moreover, ambroxol treatment was associated with increased α-synuclein levels in the CSF and reduction in the mean MDS-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III score, indicating an improvement of motor function in both *GBA1* carriers and non-carriers but in an open labelled study this may simply reflect a placebo response (Mullin et al., 2020). The encouraging results of the Aim-PD trial have paved the way for the development of a Phase 3a, UK, multi-centre, randomised, double-blind, placebo-controlled trial (ClinicalTrials.gov ID: NCT05778617, the Ambroxol to Slow Progression in Parkinson Disease study, ASPro-PD), which will start recruiting in September 2023. Recruitment target is 330 patients with early/moderate PD within 7 years from diagnosis, aged 40 to 75, with and without *GBA1* variants. During the 104-week blinded trial treatment period, patients will either be assigned to escalating dose of ambroxol (up to 1,260 mg/day) as add-on therapy or placebo. Primary outcome is the change in MDS-UPDRS part I-III score from baseline to week 104. Ambroxol has also been tested in a phase 2, single-center, double-blind, randomized placebo-controlled trial based in Canada (ClinicalTrials.gov ID: NCT02914366) targeting 55 individuals with mild to moderate PD-Dementia, randomly receiving ambroxol (1,050 mg/day), or placebo treatment. Primary outcome is comparison of cognitive scores between participants (Silveira et al., 2019). Another phase 2, multicenter, double-blind, randomized, placebo-controlled study based in Italy has recently started enrolment. Target population is 60 *GBA1*-PD patients, randomly allocated to either high-dose of ambroxol (1,200 mg/day) or placebo over 52 weeks (ClinicalTrials.gov ID: NCT05287503, AMBITIOUS). Primary outcome is comparison of cognitive performance between the two arms.

Ambroxol is also under evaluation in two trails for DLB. A phase 2a multicenter, randomized, controlled, double-blind trial is currently ongoing in Norway (ClinicalTrials.gov ID: NCT04588285, ANeED Study). A total of 172 patients with prodromal and mild DLB are expected to be recruited, with each participant receiving 5 dose escalations up to 1,260 mg/day or placebo for the duration of 18 months. Effects on cognitive and neuropsychiatric symptoms, and functional decline will be evaluated. Finally, a phase 1-2 randomized, placebo-controlled, double-blind study investigating safety, tolerability, and efficacy of ambroxol is on its way in Canada (ClinicalTrials.gov ID: NCT04405596). A total of 15 people with DLB will participate in the study for the duration of 52 weeks.

### 6.3. Disruption of sphingolipids metabolism: GlcCer and GlcSph

In recent years several clinical studies have addressed the question as to whether levels of GCase catabolic products, GlcCer and GlcSph, are altered in patients with PD, but the answer is still uncertain.

When measured by liquid chromatography (LC) coupled electrospray ionization tandem mass spectrometry (MS), plasma levels of GlcCer were found to be increased in cohorts of non-*GBA1*-PD, especially in individuals with more severe pain and cognitive dysfunction (Klatt-Schreiner et al., 2020; Mielke et al., 2013). Increased plasma or CSF levels of GlcSph or GlcCer were also detected in cohorts of *GBA1*-PD patients carrying pathogenic or risk variants compared with non-carrier PD in a few studies using LC-MS or MS (Guedes et al., 2017; Lerche et al., 2021; Surface et al., 2022). In one of them, plasma GlcSph levels were significantly higher also in asymptomatic heterozygotes carriers of N370S compared with non-carriers, suggesting that GlcSph could be a target engagement biomarker for interventions in asymptomatic individuals (Surface et al., 2022). However, the levels of plasma GlcSph reported in this study were within the normal range for healthy individuals, thus questioning the biological and clinical significance of these findings (Gleason, Tayebi, Lopez, & Sidransky, 2022). Moreover, whether the accumulation of these substrates truly plays a pathogenic role in PD has been challenged by equivocal data from post-mortem studies. On one hand, GlcCer and GlcSph were reported to accumulate in the SNpc of non-*GBA1*-PD brains above the age of 60 compared to matched controls, with increased GluSph levels accompanied by reduced GCase activity (Huebecker et al., 2019; Rocha et al., 2015a). On the other hand, no accumulation of GlcCer and GlcSph was observed in the putamen of *GBA1*-PD patients (Gegg et al., 2015), and no evidence of GlcCer accumulation was reported in a cohort of DLB patients with and without *GBA1* variants compared to controls (Kurzawa-Akanbi et al., 2021). Overall, the available clinical data do not fully support a pathogenic role of GlcSph or GlcCer in PD.

#### 6.3.1. Substrate reduction therapy

Despite the lack of strong evidence of substrate accumulation in the brain of PD patients, the strategy of directly inhibiting GlcCer synthase (GCS) enzyme, encoded by the UDP-glucose ceramide glucosyltransferase (*UGCG*) gene, to reduce substrate levels has been pursued in a number of preclinical and clinical studies in PD. Unfortunately, clinical trials showed negative outcomes.

The brain-penetrant GCS inhibitor, GZ667161, was reported to reduce GlcCer, GlcSph and the accumulation of α-synuclein in the brain and improved behavioural outcomes in both α-synuclein overexpressing mice and mice carrying homozygous *GBA1* variants (Sardi et al., 2017). Another brain-penetrant GCS inhibitor, venglustat, showed similar results in mouse models of *GBA1*- and GD-related synucleinopathy (Viel et al., 2021). These promising results led to the design of a phase 2, multicenter, randomized, double-blinded, placebo-controlled trial testing the effect of venglustat (GZ/SAR402671) in PD patients carrying

*GBA1* variants (ClinicalTrials.gov ID: NCT02906020, MOVES-PD). Venglustat showed an adequate safety and tolerability profile and reached a good target engagement in CSF determined by a dose-dependent reduction in CSF GlcCer (Peterschmitt et al., 2022). However, the study failed to demonstrate any disease-modifying effect of venglustat at week 52 compared to baseline and resulted in an early deterioration in motor function (changes in MDS-UPDRS part II+III score in OFF state) in the active arm (Peterschmitt et al., 2021). In the active arm, this trend was more prominent in patients carrying mild *GBA1* variants versus those carrying severe variants (Peterschmitt et al., 2021). Further developments of the compound for *GBA1*-PD have been discontinued.

### 6.4. Ceramides

Alteration in the homeostasis of sphingolipids other than GlcCer and GlcSph may also be implicated in PD pathogenesis. Among those, ceramides, the direct downstream products of GCase, have been the most studied. Ceramides are composed of a sphingosine and a fatty acid fraction, the latter determining the different isoforms (Lerche et al., 2021). In GCase-deficient HEK293-FT cells generated using CRISPR/Cas9-based genome editing, loss of GCase resulted in reduction of C18-ceramide species and impaired secretion of α-synuclein with accumulation of intracellular α-synuclein (Kim, Jeon, Burbulla, & Krainc, 2018). Initial reports analysing post-mortem brains of non-*GBA1*-PD patients showed that ceramide levels were reduced by 50-60% in the anterior cingulate cortex, and in those regions with reduced GCase activity and significant α-synuclein accumulation (Abbott et al., 2014; Murphy et al., 2014). Subsequent studies on DLB patients, though, reported increased ceramide levels in the brain of these patients compared to controls irrespective of their *GBA1* genotype status (Kurzawa-Akanbi et al., 2021). Measurements of ceramide in plasma or CSF also showed some discrepancies. Increased levels of ceramides were found in plasma of non-*GBA1*-PD (Mielke et al., 2013), as well as in *GBA1*-PD patients compared to non-carriers (Guedes et al., 2017). Considering carriers of pathogenic and risk variants together, no significant changes in CSF levels of ceramides were found between *GBA1*-PD patients and non-*GBA1*-PD; however, carriers of severe variants tended to have higher CSF ceramide levels (Lerche et al., 2021). Chemical inhibition of lysosomal enzyme acid ceramidase (with carmofur) increased ceramide levels and decreased α-synuclein levels in *GBA1*-PD patient-derived dopaminergic neurons, suggesting that acid ceramidase inhibition may be a potential therapeutic strategy to target *GBA1*-positive PD and DLB cases (Kim et al., 2018). It is possible, though, that ceramide could represent a potential therapeutic target only for specific *GBA1* variants. Notwithstanding, considering the disruption of lysosomal homeostasis secondary to acid ceramidase deficiency, the use of acid ceramidase inhibitors should be carefully evaluated (Park & Schuchman, 2006).

### 6.5. Lipid metabolism: Cholesterol and lipid droplets

In addition to dysregulation of sphingolipids metabolism, loss of GCase activity has been shown to affect levels of other lipids, and thus restoration of normal lipid metabolism could be a potential target within the *GBA1* pathway. Cholesterol is of special interest because of the availability of hyphocholesterolemic drugs. Both *in vitro* and *in vivo* models confirmed the association between *GBA1* and cholesterol, whereas convincing evidence in humans is still lacking (Gegg et al., 2022). *GBA1* knockdown SH-SY5Y cells and embryonic fibroblasts from Gba1$^{-/-}$ or Gba1$^{+/-}$ mice showed increased cholesterol accumulation, as well as mouse primary cortical cells treated with CBE (Magalhaes et al., 2016). Similarly, in human fibroblasts with the N370S variant, lysosomal cholesterol accumulation was observed with consequent altered autophagy-lysosome function and appearance of multilamellar bodies which increased cell vulnerability to cytotoxic stimuli (Garcia-Sanz et al., 2017; Garcia-Sanz, Orgaz, Fuentes, Vicario, & Moratalla, 2018). It has therefore been proposed that loss of GCase activity can lead to impairment of lysosomal recycling and endosome maturation, possibly due to alterations in cholesterol homeostasis (Magalhaes et al., 2016). Treatment with statin was able to lower cholesterol levels but not restore the lysosomal recycling in Gba1 KO and heterozygotes cells (Magalhaes et al., 2016). No changes in cholesterol levels were observed in brains of *GBA1*-PD patients (Gegg et al., 2015). Data regarding serum cholesterol in *GBA1*-PD are also controversial. Levels of total cholesterol and low density lipoproteins (LDL) were either reduced (Macias-Garcia et al., 2021), or similar (Grisanti et al., 2022; Guedes et al., 2017), in *GBA1*-PD patients compared to non-carrier PD patients and healthy individuals. Controversial results regarding serum cholesterol levels have also been reported in cohorts of non-*GBA1*-PD subjects (Guo et al., 2015; Hu, Antikainen, Jousilahti, Kivipelto, & Tuomilehto, 2008; Rozani et al., 2018; Wei et al., 2013). Altogether, current evidence could suggest a link between impaired cholesterol homeostasis and GCase dysfunction. It has been proposed that statin use is associated with reduced risk of PD (Bai et al., 2016). Whether this hypothetical benefit of statin is mediated by its effect on GCase activity should be explored.

At the cellular level, lipid metabolism is regulated through the turnover and recycling of lipids in membranes and organelles such as lipid droplets (LD) (Gegg et al., 2022). Since changes in the lipid composition of membranes can alter α-synuclein aggregation (Galvagnion, 2017), the role of LD homeostatis in *GBA1*-PD has been explored, with interesting findings. LDs store fatty acids (FAs) as triglycerides to prevent FAs accumulation and related cytotoxic effects (Gegg et al., 2022). In yeast models, blocking LD formation led to enhanced α-synuclein related cytotoxicity, suggesting a protective role for LD formation against α-synuclein toxicity (Fanning et al., 2019). In brains of mice injected with CBE, analysis of gene expression by RNA sequencing revealed upregulation of genes associated with LD formation (mainly the perilipin, *PLIN*, gene), which was reversed by treatment with a brain-penetrant GCS inhibitor (Blumenreich et al., 2021), supporting a direct effect of GCase on LD biosynthesis. However, the role of LD in *GBA1*-PD is more complex and could possibly be unrelated to GCase dysfunction, at least for specific genetic variants. In fact, it was recently reported that SH-SY5Y cells overexpressing the risk variant E326K and fibroblasts with heterozygous and homozygous E326K showed a significant increase in LD formation, despite no changes in GCase activity (Smith, Bolsinger, Chau, Gegg, & Schapira, 2023). Treatments targeting the LD biosynthesis pathway could therefore be useful for subgroups of *GBA1*-PD patients, irrespective of their effect on GCase activity.

### 6.6. Lysosomal proteins: Saposin C, progranulin and cathepsin D

There is increasing evidence supporting a role of the three key multifunctional lysosomal proteins, PSAP, progranulin (PGRN), and cathepsin D (CTSD), in PD. This unique intralysosomal network could not only be responsible for α-synuclein degradation but for modulating GCase activity, thus explaining why alteration of GCase activity can occur also in PD patients without *GBA1* variants (Tayebi, Lopez, Do, & Sidransky, 2020).

PSAP is the precursor of SAPC, one of the small nonenzymatic glycoproteins classically referred to as “sphingolipid activator proteins” (O'Brien & Kishimoto, 1991). SAPC is an activator of GCase (Ho & O'Brien, 1971), and therefore SAPC deficiency results in a disease phenotype that is similar to GD (Schnabel, Schroder, & Sandhoff, 1991). In SH-SY5Y cells, overexpression of PSAP reduced monomeric α-synuclein levels, whereas PSAP knockdown increased them, independently of GCase activity (Kojima et al., 2022). In humans, higher levels of α-synuclein and lower levels of SAPC protein characterised *GBA1*-PD ($n = 29$) from non-*GBA1*-PD ($n = 37$) (Avenali et al., 2021). Although the sample size was limited and these findings need replication in larger cohorts, restoring levels of SAPC could represent an attractive therapeutic avenue to explore for *GBA1*-PD patients. Recombinant

SAPC was shown to dislodge α-synuclein from artificial GlcCer-enriched lipid membranes at the lysosomal pH 5.4 (Kojima et al., 2022). Overall, these findings could suggest that PSAP and SAPC work as neuroprotective factors at least partially by promoting α-synuclein clearance via replacing it from the lysosomal membrane (Kojima et al., 2022). An interplay between SAPC and ceramide has also been detected. Through activation of cathepsin B maturation and activity, ceramide was shown to increase PSAP processing, thus increasing SAPC levels and GCase activity. This pathway was rescued upon treatment with carmofur (Kim, Jeong, & Krainc, 2022).

PGRN, encoded by *GRN*, is a ubiquitously expressed glycoprotein linked to multiple physiological processes, including tissue repair, tumorigenesis, anti-inflammation, and autoimmunity (Zhao et al., 2023). Genetic variants in *GRN* cause frontotemporal dementia but have also been associated with GD (Jian et al., 2016; Snowden et al., 2006; Tayebi et al., 2020). PGRN can act as a modifier of GCase by binding GCase to heat shock protein 70 (Hsp70) via the C-terminal domain of PGRN (Jian et al., 2016). Mice obtained by crossing $Grn^{-/-}$ mice into mice homozygous for the Gba1 D409V mutation, exhibited PD-like phenotype and severe neuroinflammation with microgliosis and astrogliosis; a brain-penetrant PGRN-derived peptide ameliorated PD pathology (Zhao et al., 2023). Similarly, arimoclomol, a small molecule that increases levels of the Hsp70 family, was shown to improve GCase refolding, maturation, and activity in fibroblasts derived from patients homozygous for L444P (Fog et al., 2018). Arimoclomol was under evaluation in a multicenter, double blinded, randomized placebo-controlled trial in patients with GD type I and III, prematurely terminated during the COVID-19 pandemic (ClinicalTrials.gov ID: NCT03746587). To date, there are no data available on arimoclomol in PD.

CTSD is an aspartyl protease that is ubiquitously expressed, but particularly abundant in the brain, and is involved in proteolytic degradation, cell invasion, and apoptosis. Genetic variants in the *CTSD* gene result in the lysosomal storage disorder neuronal ceroid lipofuscinosis-10, an early-onset progressive neurodegenerative condition (Steinfeld et al., 2006). The activation of pro-CTSD to mature CTSD is promoted by PGRN within the acidic environment of the lysosome (Tayebi et al., 2020). In midbrain dopaminergic neurons differentiated from neural crest stem cells of PD patients heterozygotes for N370S, decreased levels of CTSD protein and its enzymatic activity and higher levels of monomeric α-synuclein were observed suggesting that reduced CTSD occurred as a consequence of loss of GCase activity (Yang, Gegg, Chau, & Schapira, 2020). When *GBA1* mutant neurons were treated with ambroxol or recombinant GCase (cerezyme), GCase and CTSD protein levels and activity were increased, and monomeric α-synuclein decreased. When CTSD was inhibited, cerezyme failed to reduce monomeric α-synuclein levels in *GBA1* mutant neurons, indicating that the clearance of monomeric α-synuclein occurred through regulation of lysosomal CTSD (Yang et al., 2020). CTSD levels were also found to be significantly lower in N370S/WT cholinergic neurons compared with control (Yang et al., 2022). Application of recombinant human proform of CTSD (rHsCTSD) effectively reduced levels of insoluble α-synuclein in dopaminergic neurons derived from iPSC of PD patients harbouring the *SNCA* A53T mutation as well as in brain and primary neurons of a *CTSD*-deficient mouse model (Prieto Huarcaya et al., 2022). Taken together, these findings indicate that CTSD is critical for α-synuclein clearance and can be considered an interesting target for PD.

### 6.7. Miscellaneous

Histone deacetylase inhibitors (HIDACis) are another class of small molecules that may modulate GCase activity by stabilising mutant GCase. Through post-transcriptional modifications, histone deacetylase proteins can regulate the activity and expression of histones and chaperone proteins, such as heat shock protein 90 (hsp90) (Do et al.,

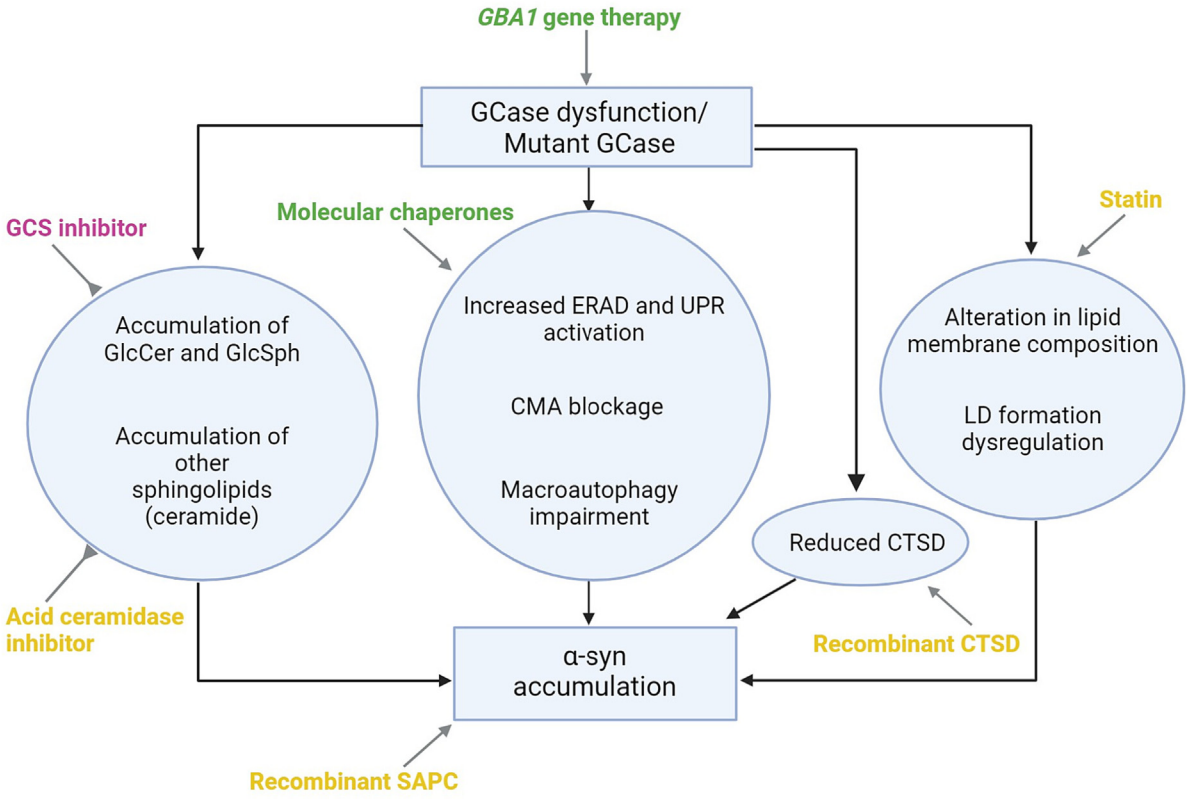


**Fig. 1.** Molecular targets and pathomechanisms associated with the *GBA1* pathway. *GBA1*-targeted interventions (grey arrows) are distinguished into treatments: currently tested in clinical trials (green), which have failed in clinical trials (purple), and only tested in preclinical studies (yellow).

2019). Hsp90 can direct proteins to the UPS. By reducing binding to chaperones such as hsp90, HIDACis were shown to prevent the recognition and degradation of mutant GCase, reduce the UPS degradation, and thus increase quantity and activity of GCase in fibroblast from patients with GD (Lu et al., 2011; Yang et al., 2013).

The antipsychotic quetiapine was recently shown to target GCase following a screen of 1,280 FDA-approved drugs. Quetiapine was shown to increase wild-type GCase protein levels and activity, and partially reduce α-synuclein accumulation in iPSC-derived dopaminergic neurons from patients with *GBA1*-PD and brains of *GBA1* mouse model (Burbulla et al., 2021).

## 7. Conclusions

The discovery of the association between *GBA1* variants and PD has provided novel insights into the pathogenic mechanisms underlying PD and novel therapeutics targeting the GCase pathway (Fig. 1). Patients with *GBA1*-PD tend to show faster progression, earlier cognitive impairment, and reduced survival, particularly those patients carrying severe or complex *GBA1* variants. Different clinical phenotypes likely reflect different molecular pathways by which *GBA1* variants promote neurodegenerative changes. Precision treatments aiming at slowing disease progression are therefore needed, especially in these subgroups characterised by an accelerated phenotype.

Several types of intervention have been explored so far, with varied outcomes. Among them, augmentation of GCase activity via molecular chaperones and *GBA1* gene therapy are currently under investigation in clinical trials. GCase-targeted treatments are also already under evaluation in other conditions such as DLB and could soon be extended to PD at-risk populations, such as asymptomatic *GBA1* carriers and iRBD. To increase chances of success of future clinical trials, we need to refine patients' selection at baseline, identify reliable markers of disease severity and progression (both clinical and biochemical) and achieve satisfactory target engagement. The goal of any future treatment for PD is to be precise and personalized, and the work performed in the *GBA1* field is the demonstration that this goal might soon be achieved.

## Data availability

No data was used for the research described in the article.

## Declaration of Competing Interest

AHVS has provided consultancy services to Astex, Auxilius, Coava, Desitin, Gain, Genilac, Prevail, Sanofi, Vivifi.

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