Glucocerebrosidase Mutations and Synucleinopathies: Toward a Model of Precision Medicine

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ABSTRACT: Glucocerebrosidase is a lysosomal enzyme. The characterization of a direct link between mutations in the gene coding for glucocerebrosidase (GBA1) with the development of Parkinson’s disease and dementia with Lewy bodies has heightened interest in this enzyme. Although the mechanisms through which glucocerebrosidase regulates the homeostasis of α-synuclein remains poorly understood, the identification of reduced glucocerebrosidase activity in the brains of patients with PD and dementia with Lewy bodies has paved the way for the development of novel therapeutic strategies directed at enhancing glucocerebrosidase activity and reducing α-synuclein burden, thereby slowing down or even preventing neuronal death. Here we reviewed the current literature relating to the mechanisms underlying the cross talk between glucocerebrosidase and α-synuclein, the GBA1 mutation-associated clinical phenotypes, and ongoing therapeutic approaches targeting glucocerebrosidase. © 2018 International Parkinson and Movement Disorder Society

Key Words: α-synuclein; GBA1; GCase; Lewy bodies; Parkinson’s disease

Glucocerebrosidase (GCase) is a lysosomal enzyme encoded by the GBA1 beta-glucosylceramidase gene (GBA1). Homozygous mutations in the GBA1 gene cause Gaucher’s disease (GD), of which 3 subtypes have been described based on clinical progression and the presence/absence of neurological symptoms and signs.1,2 GCase has recently attracted strong interest in the field of synucleinopathies following the demonstration of a close association between homo- and heterozygous GBA1 mutations and an increased incidence of Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). Initially a cohort of 6 GD patients was reported to “showing typical extrapyramidal symptoms.”3 Genetic case-control and prospective cohort studies of GD patients subsequently confirmed the link.4-8 These studies revealed that GBA1 mutations are the main genetic risk factor for PD, the association with DLB being even stronger than for PD.9 Although mutations in several genes —LRRK2, α-synuclein (α-syn), PINK1, and DJ-1—are also known to play a role in the
pathophysiology of synucleinopathies, GBA1 mutations are the most numerous.\textsuperscript{10}

Here we review the current understanding of the role of GBA1 mutations and GCase in the development of PD and DLB. Numerous recent developments have taken place, for which there is now increased interest in the therapeutic potential of targeting the GCase-\(\alpha\)-syn pathway given the possible association with sporadic forms of PD and DLB. This has led to the appointment of GCase as a validated target for disease modification by the Michael J. Fox Foundation for Parkinson’s Research.

**Mechanisms Underlying the Cross Talk Between GCase and \(\alpha\)-Syn**

Increasing evidence indicates that impaired GCase trafficking, sphingolipid accumulation, and protein quality control are driving forces underlying the pathological relationship between GCase dysfunction and \(\alpha\)-syn aggregation (Fig. 1).

**Disruptions in Lipid Metabolism**

The hypothesis that alterations in lipid membrane metabolism might explain the pathological cross talk between GCase and \(\alpha\)-syn derives from studies conducted in the brains of patients with GD.\textsuperscript{11-13}

Available evidence has shown that \(\alpha\)-syn interacts with membrane lipids and influences \(\alpha\)-syn structure, triggering formation of neurotoxic oligomeric or \(\beta\)-sheet conformers.\textsuperscript{14} Two mechanisms by which membrane lipids promote the formation of \(\alpha\)-syn aggregates have been proposed. On the one hand, the membrane surface might facilitate a local increase in \(\alpha\)-syn concentration stimulating aggregation.\textsuperscript{15} Conversely, changes in protein conformation could be induced directly by membrane binding.

GCase is involved in sphingolipid metabolism, as it hydrolyzes the glycolipids glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph). GCase catabolizes GlcCer to glucose and ceramide, which is recycled to generate new glycosphingolipids and sphingomyelins.\textsuperscript{16} GBA1 mutations reduce the enzymatic function of GCase,\textsuperscript{17} leading to accumulation of undigested substrate GlcCer and other lipids in lysosomes, thereby compromising lysosomal function.

In vitro experiments have suggested that the direct interaction between accumulating GlcCer and \(\alpha\)-syn promotes the toxic conversion of \(\alpha\)-syn into its insoluble form.\textsuperscript{18,19} Similarly, GlcCer accumulation stabilizes \(\alpha\)-syn oligomeric intermediates and induces rapid polymerization of fibrils.\textsuperscript{20} Moreover, a significant increase in \(\alpha\)-syn dimers has been observed on incubation with GlcCer-containing liposomes.\textsuperscript{19} The effects of GlcCer could be secondary to that exerted by GlcSph, which triggers the formation of oligomeric \(\alpha\)-syn in young GD/PD mouse brains, thus potentially increasing PD.

![Mechanisms involved in pathological GCase-synuclein cross talk: blue, GCase-induced changes in membrane lipid composition promote toxic \(\alpha\)-syn conversion; red, \(\alpha\)-syn accumulation impairs GCase trafficking; green, excessive/defective \(\alpha\)-syn and GCase deficiency impair quality control systems, exacerbating \(\alpha\)-syn accumulation. Boxes, potential pharmacological strategies.](wileyonlinelibrary.com)
risk in GD patients and carriers. Furthermore, it has been recently demonstrated that the reduction of ceramide species associated with GCase deficiency may contribute to the impaired secretion and intracellular accumulation of α-syn. Interestingly, the toxic conversion of physiological α-syn conformers by glycosphingolipids (GSLs) may be reversible. Accordingly, the use of agents able to reduce intracellular GSL production or accumulation may have potential as a therapeutic neuroprotective strategy. Indeed, it has been demonstrated that oligomeric α-syn, extracted from symptomatic patient midbrain neurons, reverts back to its native synapse-associated form when GSL levels are reduced. Equally, glucosylceramide synthase inhibitor GZ667161 appears to decrease α-syn pathology and improves behavioral outcomes in animal models of synucleinopathies. Interestingly, in vitro studies have demonstrated that overexpression of lysosomal integral membrane protein type 2 (LIMP-2; the receptor for lysosomal transport of GCase) has beneficial effects on α-syn clearance, probably related to the reduction of GlcCer levels, suggesting that manipulation of LIMP-2 expression could be another strategy for the treatment of synucleinopathies. Analogously, the increase of ceramide levels in GCase-deficient cells decreased oxidized and ubiquitinated species of α-syn.

Disruptions in Protein Trafficking

It has been shown that GCase binds the C terminus of α-syn in a pH-dependent manner, suggesting that under physiological conditions α-syn can directly interact with GCase within the lysosome. Conversely, α-syn accumulation results in GCase retention in the endoplasmic reticulum, thereby impairing GCase intracellular trafficking and activity. This emphasizes the bidirectional relationship between GCase and α-syn, with the loss of LIMP-2 presumed to reduce GCase trafficking, leading to α-syn accumulation in dopaminergic neurons. Accordingly, therapeutic strategies intended to restore or improve GCase trafficking have recently emerged for the management of synucleinopathies. Small molecular chaperones such as ambroxol and isofagomine, which target misfolded GCase and increase GCase trafficking to lysosomes, reduce α-syn burden, in both in vitro and in vivo disease models and may have utility as diseasemodifying agents. Furthermore, the use of peptides targeting helix 5 of LIMP-2 has been able to reduce α-syn levels by activating endogenous wild-type and mutant GCase. These findings open a window for the design of small molecules targeting this domain to enhance LIMP-2/GCase interaction.

Impairment of Protein Quality Control Systems

Defects in autophagic clearance represent another potential link between GCase and α-syn pathology. Degradation of excessive or defective α-syn involves 2 different pathways: the ubiquitin proteasome system and the autophagic system. Although it is difficult to determine which system is impaired initially in the synucleinopathies, it has been hypothesized that when α-syn is not degraded by the proteasome, it can be shuttled to the autophagy-lysosomal system, where it is catabolized by chaperone-mediated autophagy, microautophagy, and macroautophagy. A defective autophagic/lysosomal system has been observed in iPSC-derived neurons from GD and PD individuals carrying GBA1 mutations. This may account for increased levels of α-syn in these neurons. Moreover, lysosomal reformation is compromised in GCase-deficient fibroblasts and is accompanied by an increase in total and phosphorylated α-syn, oligomer deposition, and enhanced α-syn release. This indicates that accumulation of defective lysosomes contributes to impaired autophagy and α-syn buildup. It has also been suggested that protein phosphatase 2A inactivation could represent the potential mechanism through which GCase deficiency inhibits autophagy and promotes α-syn aggregation.

Pharmacological upregulation of autophagy by the mTOR blocker rapamycin and polyphenols showed beneficial effects in cellular and animal models of synucleinopathies by reducing intracytoplasmic proteinaceous aggregates and subsequent cell death. Interestingly, these mechanisms appear to underlie the cross talk between GCase and α-syn and may have an impact on disease propagation. In vitro studies showed that lysosomal dysfunction secondary to GCase loss of function promotes the extracellular propagation of α-syn aggregates, which can be reversed by the ectopic expression of wild-type GCase. More recently, these results have been replicated in an animal model of a GCase-deficient synucleinopathy, providing the in vivo evidence that either a decrease of GCase or overexpression of mutant GCase can increase α-syn secretion by exosomes.

The aforementioned experimental evidence supports the existence of an inverse relationship between GCase deficiency and α-syn aggregation. However, it should be considered that such a relationship may only create favorable conditions for the development of the disease in the absence of a direct pathogenic link between GCase defect and PD. Accordingly, by comparing iPSC-derived dopaminergic neurons from 2 sibling GD patients carrying the same homozygous GBA1 mutation variant N370S but discordant for PD, it has been observed that α-syn levels were elevated only in neurons from the sibling with PD, thereby suggesting that additional factors beyond GCase dysfunction can contribute to α-syn accumulation and PD development.
Clinical and Neuroimaging Features of GBA-Related Synucleinopathies

Although GD is categorized as a rare disease, up to 1% of individuals in the general population are heterozygous GBA1 mutation carriers, increasing to 8% in the Ashkenazi Jewish (AJ) population. GD may present with 3 clinical types: nonneuronopathic (type I), acute neuronopathic (type II), and chronic neuronopathic (type III). Accordingly, GD-causing mutations have been categorized as “mild” or “severe.” “Mild” mutations (eg, N370S) leave residual GCase enzymatic activity of 32%-38% and cause nonneuropathic GD (type II), whereas neuronopathic GD (types II and III) are caused by “severe” mutations (eg, L444P), which leave residual GCase activity of 13%-24%.

Approximately 7%-10% of patients with PD worldwide carry a GBA1 mutation; the odds ratio (OR) has been estimated to be 5.4 overall, with a 5- to 6-fold difference between carriers of mild versus severe mutations (Table 1). The age-specific cumulative risk of PD among GBA1 heterozygotes is relatively low, initially estimated to range up to 30% by 80 years of age. However, this value of 30% has been challenged recently and considered overestimated (likely because of ascertainment bias, as GBA1 carriers were recruited from a proband of familial PD cases), so that a lower average cumulative risk of PD of 1.5%-2.2% by ages 60-65 years and 7.7%-10.9% by ages 80-85 years among heterozygotes has been suggested. Nonetheless, as the 2 prospective studies to date were performed among family members of GD cases, the actual penetrance of GBA1 mutations in the general population remains to be established conclusively. A positive familial history can be identified in 21.5%-31% of PD carriers of GBA1 mutations, suggesting that more than two-thirds of PD carriers of GBA1 mutations are sporadic. The age-specific risk for PD among homoygotes is not significantly different from heterozygotes, being 4.7% by age 60 years and 9.1% by age 80 years. Hence, further study is needed to elucidate whether being a heterozygote versus a homozygote mutation carrier influences PD risk.

The likelihood of carrying a GBA1 mutation is higher in DLB than in PD, with an overall OR of 8.3, a relative 3-fold increased risk of developing DLB compared with PD. Comparing non-AJ versus AJ populations, the frequency of GBA1 mutations in patients with DLB ranges from 7.5%-15% to 31%, respectively.

Age at Onset

GBA1 heterozygotes have an earlier age at onset compared with noncarriers of 3-6 years for PD and 5-7 years for DLB respectively. Mean age at onset in heterozygous PD carriers is about 50-55 years. Among GBA1 carriers who develop PD, homozygotes have a 6- to 11-year earlier onset than heterozygotes. In PD, the onset occurs at a younger age among heterozygous carriers of severe versus mild GBA1 mutations, ranging from 2 to 13 years (Table 1).

Survival

The status of being a GBA1 mutation carrier appears to be associated with a more aggressive disease course and increased risk of mortality. Death occurs at an earlier age in carriers than in noncarriers, with survival reduced 2-fold compared with noncarriers. Reduced survival in heterozygote PD carriers seems to be independent of the presence of dementia. In general PD populations, the major predictors for mortality include age, sex, motor impairment, dementia, dysphagia, and orthostatic hypotension. GBA1 mutations increase the risk of several predictors of mortality in addition to dementia, including motor disability, dysphagia, and autonomic dysfunction. Considering that orthostatic hypotension is a strong predictor of mortality in PD, these data emphasize the importance of its assessment in all GBA1 carriers during routine clinical visits. This is especially pertinent given the substantial pipeline of putative neuroprotective compounds genetically targeted to the GCase pathway.

Clinical Features

Despite that GBA1-associated PD is clinically indistinguishable from idiopathic PD, it is recognized that PD carriers of GBA1 mutations are more likely to present with a more aggressive disease course. However, it is still controversial whether PD carriers of homozygous GBA1 mutations have a more aggressive clinical course than heterozygotes (Table 1).

Nonmotor Symptoms

Dementia. GBA1 mutations greatly increase the risk of incident dementia. The risk of cognitive impairment in GBA1 mutation carriers is 2.4- to 3-fold higher than in noncarriers. In turn, the risk for dementia in PD carriers of severe mutations is 2- to 3-fold higher than that in carriers of mild mutations. Interestingly, the GD-unrelated E326K variant is a predictor of progression to cognitive impairment in PD and PD dementia, suggesting that other factors independent of GD-related lysosomal dysfunction are implicated in the pathogenesis of synucleinopathies. In PD carriers of heterozygous GBA1 mutations, dementia occurs at an earlier age and the pattern of
cognitive impairment largely overlaps with the characteristic pattern of DLB.\textsuperscript{10,77,80,85} In PD subjects carrying homozygous mutations, cognitive performance is extremely variable, ranging from mild cognitive impairment to dementia with the typical features of DLB.\textsuperscript{68,76,84,86,87}

### Table 1. Clinical features of GBA-associated synucleinopathies

<table>
<thead>
<tr>
<th>Feature</th>
<th>GBA in PD</th>
<th>GBA in DLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers versus noncarriers</td>
<td>Severe versus mild GBA mutations</td>
<td>Homozygotes versus heterozygotes</td>
</tr>
<tr>
<td><strong>1. General features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Mutation frequency</td>
<td>7%-10% (25% in AJ)</td>
<td>—</td>
</tr>
<tr>
<td>b. Relative risk/odds ratio</td>
<td>OR, 5.4RR, 7.2</td>
<td>OR, 10.3 vs 2.2</td>
</tr>
<tr>
<td>c. Age-specific risk at 80 years</td>
<td>Higher risk (7.7%-10.9%)</td>
<td>Higher risk</td>
</tr>
<tr>
<td>d. Family history</td>
<td>Greater (21.5%-31%)</td>
<td>Comparable</td>
</tr>
<tr>
<td>e. Age at onset</td>
<td>Earlier (3 to 6 years)</td>
<td>Earlier (2.5 to 5 years)</td>
</tr>
<tr>
<td>f. Survival</td>
<td>Reduced (HR, 1.85)</td>
<td>Comparable</td>
</tr>
<tr>
<td><strong>2. Nonmotor features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Dementia</td>
<td>Higher risk (2.4- to 3-fold)</td>
<td>Higher risk (2- to 3-fold)</td>
</tr>
<tr>
<td>b. Visual hallucinations</td>
<td>Higher risk (1.8-fold)</td>
<td>Higher risk</td>
</tr>
<tr>
<td>c. Depression/anxiety</td>
<td>Higher risk (2.2-fold)</td>
<td>More frequent</td>
</tr>
<tr>
<td>d. Autonomic dysfunction</td>
<td>More frequent</td>
<td>More frequent</td>
</tr>
<tr>
<td>• Orthostatic Hypotension</td>
<td>More frequent</td>
<td>More frequent</td>
</tr>
<tr>
<td>• Urinary urge/ incontinence</td>
<td>More frequent</td>
<td>—</td>
</tr>
<tr>
<td>• Sexual dysfunction</td>
<td>Comparable</td>
<td>—</td>
</tr>
<tr>
<td>• Constipation</td>
<td>More frequent</td>
<td>—</td>
</tr>
<tr>
<td>e. REM sleep behavior disorder</td>
<td>More frequent (OR, 3.13)</td>
<td>More frequent</td>
</tr>
<tr>
<td>f. Olfactory dysfunction</td>
<td>Lower</td>
<td>Lower</td>
</tr>
<tr>
<td><strong>3. Motor features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Motor phenotype</td>
<td>Akinetic-rigid &gt; tremor-dominant</td>
<td>—</td>
</tr>
<tr>
<td>b. Motor symptom severity</td>
<td>Greater</td>
<td>Greater</td>
</tr>
<tr>
<td>c. Response to levodopa</td>
<td>Comparable (excellent)</td>
<td>Similar</td>
</tr>
<tr>
<td>d. Motor fluctuations/ dyskinesias</td>
<td>Conflicting data (likely comparable\textsuperscript{a})</td>
<td>Comparable</td>
</tr>
<tr>
<td>e. Dysphagia</td>
<td>More frequent</td>
<td>Comparable</td>
</tr>
<tr>
<td>f. Dysarthria</td>
<td>More frequent</td>
<td>More frequent</td>
</tr>
<tr>
<td>g. Freezing of gait</td>
<td>More frequent</td>
<td>Comparable</td>
</tr>
<tr>
<td>h. Progression to Hoehn and Yahr stage 3</td>
<td>Earlier</td>
<td>Earlier</td>
</tr>
<tr>
<td><strong>4. Imaging features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Cortical activity (blood perfusion)</td>
<td>Reduced in parieto-occipital areas, including precuneus</td>
<td>Reduced in parieto-occipital areas, including precuneus</td>
</tr>
<tr>
<td>b. Dopamine transporter SPECT</td>
<td>Greater reduction, greater asymmetry index</td>
<td>Greater reduction</td>
</tr>
<tr>
<td>c. MIBG SPECT</td>
<td>Controversial</td>
<td>—</td>
</tr>
</tbody>
</table>

AJ, Ashkenazi Jewish population; DLB, dementia with Lewy bodies; GBA, glucocerebrosidase; HR, hazard ratio; MCI, mild cognitive impairment; MIBG, \textsuperscript{123}I-metaiodobenzylguanidine; OR, odds ratio; PD, Parkinson’s disease.

\textsuperscript{a}After adjusting by age at onset.
Neuropsychiatric features. PD carriers of both heterozygous and homozygous GBA1 mutations are at a 1.8-fold higher risk for developing psychotic symptoms.83 Visual hallucinations are the most common features and may be present in up to 45% of carriers,10,49,60,66,68,74,79 occurring earlier during the disease course and at a younger age than is seen in patients with sporadic PD.10,49,79 The risk of visual hallucinations seems to be higher in GBA1 homozygotes than heterozygotes,68 and in carriers of severe versus mild mutations,49,60 supporting the hypothesis that patients with GBA1-associated PD may exhibit clinical features typical of DLB. In a DLB cohort, GBA1 carriers were at increased risk of visual hallucinations compared with DLB noncarriers.65 Concerning other neuropsychiatric symptoms, GBA1-associated PD patients have a 2.2-fold increased risk of developing trait anxiety and depression compared with noncarriers,10,49,66,83,88 as well as increased prevalence of apathy.58,66

Autonomic dysfunction. GBA1 carriers are at increased risk of autonomic dysfunction, both before the onset of clinical parkinsonism75 and during disease progression.66 In particular, GBA1-associated PD patients are at increased risk for orthostatic hypotension, as well as sexual and urinary dysfunction.49,66 Furthermore, PD carriers of severe mutations have more frequent autonomic dysfunction than noncarriers, whereas those with mild mutations do not differ from those with sporadic PD.49 As DLB is associated with a greater risk of autonomic dysfunction than sporadic PD,89 these data support the hypothesis of a close interrelationship between GBA1 mutations and the tendency for more widespread α-syn pathology diffusion to the brain stem, the sympathetic ganglia, and the spinal cord.90

Other nonmotor symptoms. GBA1 mutations are more frequent in subjects with idiopathic REM sleep behavior disorder (RBD) than in healthy controls91 and are predictive of conversion into DLB and PD.75,91 Sleep disturbances including RBD,60,66,68,92 olfactory dysfunction,68,86,87 constipation,60,66 and pain,93 may be present in both GBA1 homozygotes and heterozygotes, although their frequency is yet to be established.

Motor Features

Levodopa-responsive. Some research data suggest that GBA1 mutation carriers present with more symmetrical symptoms and signs2 and a non-tremor-dominant phenotype54,60,63,94,95 than sporadic PD. Although most GBA1 PD carriers are levodopa responsive,10,49,60,76,77 the progression of motor disability is faster in carriers of GBA1 mutations49,68,69 and some GD-unrelated variants (eg, E326K69). This difference might be driven by nondopaminergic features, because non-levodopa-responsive axial features progress faster than tremor in GBA1 carriers.49,69,74 The risk of developing levodopa-related motor fluctuations and dyskinesia is uncertain in GBA1 mutation carriers.49,54,60,79,96 Up to 17% of PD patients that underwent deep brain stimulation (DBS) were GBA1 mutation carriers,97 and the proportion of subjects fulfilling eligibility criteria for surgery or infusion therapies is similar to that of sporadic PD.49 As the principle indications for DBS are levodopa responsiveness with dyskinesia/fluctuations that are poorly controlled by medication alone, this suggests that, in terms of these 3 criteria, the presentation of PD in mutation carriers is similar to that of those with sporadic PD. Conversely, in those receiving DBS, GBA1 status is associated with worse long-term outcomes, predominantly because of a higher incidence and severity of cognitive impairment and other nonmotor symptoms.98 Moreover, it is worth noting that PD carriers of GBA1 mutations display poorer dual-task performance, supporting the presence of enhanced motor and cognitive dysfunction compared with noncarriers.99

Non-levodopa-responsive. The 2 principle drivers of the poor prognosis of GBA1 PD subjects appear to be progression to cognitive impairment and progression to non-levodopa-responsive motor symptoms and signs.49,69 Available evidence has shown that GBA1 carriers are characterized by faster progression to Hoehn and Yahr stage 3,49,63,68,69,74,79 with the type of mutation (including the GD-unrelated E326K mutation) determining the extent of this progression.49,69,72 GBA1-associated PD has a higher prevalence of other disabling motor features that are poorly responsive to levodopa, such as freezing of gait, dysarthria, and dysphagia.49

Neuroimaging Features

Cortical Activity

Significant reduction in resting activity in posterolateral parieto-occipital cortical regions including the precuneus has been consistently reported to differentiate PD carriers of either heterozygous49,79,100 or homozygous8,86 GBA1 mutations from those with sporadic PD. This pattern of cortical dysfunction is characteristic of DLB101 and consistent with the larger proportion of neocortical Lewy body pathology described in GBA1 mutation carriers.8,10,58 The impairment of lateral parieto-occipital association areas underlies the higher prevalence of visual hallucinations102 and the increased visuospatial dysfunction associated with GBA1 mutations.77 Carriers of severe mutations have a more extensive and pronounced reduction in activity in posterolateral parieto-occipital cortical areas than carriers of mild mutations.49 Notably, PD carriers of severe GBA1 mutations have a pattern of cortical dysfunction similar to those with
sporadic DLB without GBA1 mutations, whereas PD carriers of mild mutations did not significantly differ from PD noncarriers.49 The prognostic relevance of these data is fully consistent with the faster progression to dementia in carriers of severe versus mild GBA1 mutations.

**Nigrostriatal Function**

Heterozygous and homozygous carriers of GBA1 mutations exhibit more pronounced reduction of presynaptic nigrostriatal dopaminergic terminals than those with sporadic PD and are more similar to sporadic DLB.8,49,86,103 Although the pattern of presynaptic nigrostriatal dysfunction is similar to that in sporadic PD,8 GBA1 mutation carriers display greater striatal asymmetry index than those with both sporadic PD8 and PD carriers of α-syn, PINK1, and Parkin mutations.104 Furthermore, carriers of severe GBA1 mutations have more pronounced reduction of terminal density than carriers of mild mutations at a similar age and disease duration.49 Finally, patients with GD lacking PD-related symptoms have reduced putaminal dopamine synthesis,8 further supporting the greater risk for synucleinopathies among GBA1 mutation carriers.75

**Other Neuroimaging Studies**

The role of 123I-cardiac metaiodobenzylguanidine in GBA1-associated PD remains controversial, as the typical PD-related uptake reduction was reported in 1 study48 but not replicated in another.79 Compared with healthy control subjects, transcranial sonography of heterozygote PD GBA1 carriers showed a greater median maximal area of substantia nigra echogenicity93,105 without differences related to the severity of GBA1 mutations105 and reduced echogenicity of the brain stem raphe nuclei106 that was similar to sporadic PD.

**Approaches Targeting GCase for the Treatment of Synucleinopathies**

It is hoped that approaches targeting the GCase pathway will have direct applicability not only to GBA-associated PD but also to idiopathic PD as a whole. Indeed, GCase deficiency has been described in the brain,106 cerebrospinal fluid,107 and blood108 of sporadic PD patients without GBA1 mutations and correlates with increased α-syn levels.109

**GCase Replacement Therapy**

Direct supplementation of recombinant GCase enzyme has been a successful treatment in GD and has extended the life expectancy of these patients.110,111 Systemically delivered recombinant GCase has been shown to localize to the lysosome and upregulate enzyme activity. The limitation of this approach in the context of PD is that GCase (60 kDa) cannot cross the blood-brain barrier (BBB) in sufficient quantities to modify CNS GCase activity.112,113 A variety of approaches to enhance CNS GCase uptake have been suggested114 without any evidence of relevance to clinical practice. An alternative approach is direct intrathecal administration of recombinant GCase.113,115 A prototype exists in the lysosomal storage disorders Hurler’s and Hunter’s disease (mucopolysaccharidoses type I and II, respectively).116,117 However, doubt remains over the ability of intrathecally administered GCase to provide a sufficient concentration gradient to penetrate deeply into neuronal tissues. Accordingly, the use of enzyme replacement therapy is at best some years away in the context of PD.

**Substrate Reduction**

Glucosylceramide accumulation has been demonstrated in both neuronopathic and nonneuronopathic forms of GD, with microglial activation demonstrated in human brains and mouse models.118-126 This mechanism (and CNS immune dysregulation overall) has been postulated as pathogenic in PD.119,121-126 Substrate accumulation has been suggested as a pathogenic mechanism of PD in GBA1 mutation carriers.127 To date, substrate accumulation has not been demonstrated in PD, although 2 studies have found a reduction in ceramide.128,129 Nonetheless, there is significant interest in its use as a neuroprotective strategy in PD. For example, GCase substrate inhibition appears to reduce α-syn levels in synuclein-overexpressing cell lines.127 Furthermore, miglustat, a reversible inhibitor of glucosylceramide synthase, has been used in the context of GD type III, although concerns exist over its efficacy and side effect profile, particularly the incidence of peripheral neuropathy.130-132 Two other glucosylceramide synthase inhibitors, eliglustat and venglustat, are currently undergoing evaluation in clinical trials of GD (ClinicalTrials.gov identifier: NCT00891202) and PD (ClinicalTrials.gov identifier: NCT02906020).

**Small Molecular Chaperones of GCase**

Relying on the observation that mutant GCase is sequestered within the endoplasmic reticulum, small molecular chaperones act as molecular cofactors that aid physiological post translational folding and, in turn, upregulate trafficking of mutant GCase to the lysosomal compartment of the cell.134,136 The principle that small molecular chaperones can penetrate the BBB was first confirmed in a murine model of GM1 gangliosidosis, in which a small molecular chaperone of B-galactosidase was shown to reduce GM1 substrate levels.137,138 Two types of chaperones exist, the prototype inhibitory chaperone (which binds to the active site of the GCase protein) and noninhibitory chaperones, which bind to
alternate parts of the structure yet are still able to modulate folding sufficiently to restore or partially restore posttranslational folding. The inhibitory tag refers to these chaperones binding directly to the active site of the protein to induce conformational changes, in turn antagonizing the binding of the enzyme substrate(s) and hence reducing enzyme activity. The chaperone is eluted on encountering the acidic conditions of the lysosome, leaving the active site available for enzyme catalysis. The degree of elution is dependent on the affinity of the chaperone for the substrate; if the affinity is too great, then the active site will remain bound to the chaperone and no catalysis will occur. Far from aiding enzyme catalysis, prolonged binding at low pH to the active site of the protein may inhibit its action within the lysosome. This was the case with isofagomine, which maintains a high-binding affinity to the active site up to pH 4. Clinical trials of the compound in the context of GD were unsuccessful, with this enhanced inhibition possibly being the culprit. Most chaperones discovered to date are inhibitory; hence, careful consideration must be given to whether a chaperone’s affinity to GCase is within the narrow therapeutic window that allows sufficient GCase trafficking to the lysosome and elution once the chaperone is within it. To circumvent this issue, there is significant interest in the development of novel noninhibitory small molecular chaperones as potential neuroprotective agents in PD, and it is likely that clinical trials of these compounds will commence in the coming years.

**Ambroxol**

At present, the most likely small molecular chaperone candidate for use as a neuroprotective agent in PD is ambroxol, a metabolite of bromhexine that has been used for more than 30 years as a mucolytic and in the treatment of hyaline membrane disease. It has an excellent safety profile with few side effects. A screen of some 10,000 Food and Drug Administration-approved compounds tested on fibroblast and lymphoblast cell lines generated from GD patients showed that ambroxol was a mixed-type inhibitor of GCase, with high binding at neutral pH but almost no inhibitory effect below pH 4.6. Subsequently ambroxol was confirmed to increase GCase activity in both GBA1 and wild-type fibroblasts, mouse models of GD, and in a neural crest stem cell model. The latter study also showed upregulation of a number of autophagic pathways in response to ambroxol. Several studies have confirmed the brain penetration of ambroxol in vivo. In L444P GBA1 mutations and wild-type and synuclein-overexpressing mice, GCase activity increased on treatment with ambroxol, and in the latter α-syn levels were reduced. The increase in GCase activity was mimicked in nonhuman primates but only at a higher dose. Moreover, a phase 2 trial of ambroxol in 5 neuronopathic GD patients achieved 10%-20% of the serum ambroxol concentration in cerebrospinal fluid. Two phase 2 clinical trials of ambroxol in PD are currently underway at this dose. The first, completed in May 2018, is a non-placebo-controlled proof-of-principle trial, with a primary objective to show tolerance, CNS penetration, and target acquisition in this population (ClinicalTrials.gov identifier: NCT02941822). The second, with a projected completion date of December 2018, is a double-blind, randomized, placebo-controlled trial with a primary end point of a reduced rate of deterioration of cognition in those with pre-existing PD dementia (ClinicalTrials.gov identifier: NCT02914366).

**Gene Therapy With GBA1**

The use of adeno-associated viral vectors (AAVs) coding for the GBA1 gene to enhance GCase enzymatic brain activity also holds great promise. It has been reported that the coinjection of AAVs coding for GBA1 and mutated α-syn in rats prevented dopaminergic neurons from neurodegeneration. Furthermore, the gene therapy field is rapidly changing, with new arrivals being constantly incorporated. The recent availability of BBB-penetrating AAVs known as AAV9-PHP.B1 has broadened the therapeutic options available. The systemic administration of AAV9-PHP.B1-GBA1 in α-syn transgenic mice resulted in almost complete clearance of α-syn throughout the brain. Although these approaches remain in early preclinical testing stages, they all share great potential as disease-modifying therapies.

**Early Detection of Conversion in GBA-Related PD**

GBA1 mutations are a genetic risk factor for PD and are distinct from other Mendelian forms of genetic PD. Crucially, GBA1 mutation carriers are more likely to develop PD. The issue surrounding the variable penetrance of GBA1-related PD accordingly remains a major unanswered point in our understanding of PD and an obstacle to the administration of neuroprotectives before the onset of motor symptoms. More specifically, a family history of PD is the single greatest risk factor for developing PD, although only a small minority of cases develop PD in a Mendelian manner. One could speculate that untangling the factors that contribute to the conversion of GBA1 carriers to PD may also allow us to reveal those who will develop idiopathic PD.

One consideration is the “potency” of the GBA1 mutations. To date, more than 300 GD-associated GBA1 mutations and many more polymorphisms have...
been identified in the context of the “severe”/“neuropathic” GBA1 allele. In recent years, it has become clear that severe GBA1 mutations are associated with a much greater risk of PD than are mild ones. In the case of the most common mutations (N370S and L444P), it is even possible to quantify the risk individually, with each mutation conveying an OR of developing PD of 3-4, and 6-12, respectively.

That said, the cause of the variable penetrance GBA1 alleles in PD remains unclear. Established epidemiologically derived factors influencing PD incidence such as smoking, nonsteroidal anti-inflammatory drug exposure, coffee, outdoor lifestyle, and head trauma may play a role. It seems likely that genetic or epigenetic cofactors may be implicated. An example of this includes the role of the homologous pseudogene GBAP1, which is 80% homologous with the main GBA1 gene. Although formerly considered a noncoding gene, recent data not only show evidence of mRNA expression but that genetic variation in GBAP1 expression could determine expression of the GBA1 gene by acting as a competing antagonist mRNA. Similarly, evidence that variation in upstream promoter regions of the GBA1 gene may modulate GCase activity is available. Interestingly, there is some evidence that the haplotype of the L444P mutation may alter the penetrance of GD-associated alleles, and this may play a significant role in the penetrance of other mutations.

It has been known for some years that a clinical pro-drome that includes hyposmia, constipation, cognitive impairment, and RBD precedes the onset of motor PD by up to 20 years. Analysis of longitudinal cohorts of GBA1 carriers without PD has established that some of these features are prominent. Equally, genotyping of those with electrophysiologically proven RBD shows a higher prevalence of GBA1 mutation carriers compared with age-matched controls. It may be that by using a combination of genetic, clinical, biochemical, and imaging biomarkers, we are able to stratify the risk of developing PD among GBA1 mutation carriers to enable genetically targeted neuroprotection to be achieved.

Concluding Remarks and Open Questions

Taken as a whole, available data support the hypothesis of a more extensive brain synucleinopathy in carriers of GBA1 mutations associated with earlier and more severe involvement of neocortical areas but also of subcortical regions and even the spinal cord. In the clinicopathological continuum of diseases with Lewy bodies, carriers of GBA1 mutations localize equidistant between PD and DLB, with carriers of mild mutations closer to sporadic PD and carriers of severe mutations closer to DLB.

Ongoing research is providing crucial insights into the reciprocal relationship between GCase and α-syn. In this regard, the most enriched baseline expression of GCase in the control nonhuman primate brain has been found in brain locations where neurons are typically characterized by the presence of misfolded protein aggregates.

To date, however, several questions still require definitive answers. First, despite carriers of severe mutations being more likely to develop a more aggressive PD phenotype and being at greater risk of developing DLB, most carriers do not convert to either PD or DLB. Second, the association between GBA1 mutations and multiple system atrophy (MSA) is still controversial, given that although several studies reported no association, other studies have suggested the opposite. This might be because of a differential localization of α-syn pathology, primarily involving oligodendrocytes in MSA, as opposed to neurons in PD and DLB. Third, a number of GBA1 variants not associated with GD have been described in patients with PD and DLB. Finally, a substantial proportion of GBA1 mutations identified in DLB patients are “mild” variants rather than expected “severe” mutations. Future prospective studies in large populations stratified by the type of GBA1 mutation are required to fully establish the differential effects of “mild” versus “severe” mutations and of GD-related versus GD-unrelated mutations.

Ongoing clinical trials deserve considerable attention. Although there is a clear consensus that targeting the GCase pathway within the brain may be a means to reduce α-syn burden, it is still unclear which strategy is best suited to the purpose in terms of safety, efficacy, and reproducibility.

References


