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## Pathological Mechanisms and Clinical Aspects of *GBA1* Mutation-Associated Parkinson's Disease

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**Abstract:** Parkinson's disease (PD) is a common neurodegenerative disorder, characterized by a motor syndrome consisting of bradykinesia, rigidity, resting tremor, and postural instability. Mutation in the *GBA1* gene, which encodes the lysosomal enzyme glucocerebrosidase, has recently emerged as the most common genetic abnormality associated with PD. Approximately 5% of PD patients carry a *GBA1* mutation, in comparison to <1% of the healthy population.

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Heterozygous or homozygous *GBA1* mutations increase the risk of PD 20–30 fold. The pathogenic mechanisms of *GBA1* mutation-associated PD are not fully understood. Several studies suggest loss of enzyme activity underlies the pathogenicity of *GBA1* mutations, while others suggest that a gain of function due to enzyme misfolding is important. Lysosomal-autophagic and mitochondrial dysfunction, as well as endoplasmic reticulum stress and alpha-synuclein accumulation, have all been demonstrated in association with *GBA1* mutation. The clinical features of *GBA1* mutation-associated PD are similar to that of sporadic disease but with an earlier age of onset and a more rapid cognitive and motor decline. Given this impact of clinical course and its relatively high frequency, understanding the pathogenic mechanisms of *GBA1* mutations will allow for the development of targeted, potentially disease-modifying treatments that will have implications for many patients with PD.

**Keywords:**  $\alpha$ -synuclein; Gaucher disease; *GBA1*; Glucocerebrosidase; Parkinson's disease

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## INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder, characterized clinically by a typical motor syndrome consisting of bradykinesia, rigidity, resting tremor, and postural instability (1). The motor disorder in part results from the loss of striatal dopamine, as a consequence of degeneration of the dopaminergic neurons of the substantia nigra (1). Most individuals also develop neuropsychiatric manifestations (such as anxiety, depression, and hallucinations), sleep disturbances, autonomic dysfunction, and cognitive decline and dementia, due to involvement of extra-nigral brain regions including the cortex and multiple brainstem sites (2).

The natural history of PD may follow a more benign motor-predominant course in some patients, while in others the disabling non-motor features predominate. The underlying basis of the clinical heterogeneity is poorly understood, but it is becoming clear that this is, at least in part, due to genetic factors (1, 3, 4). One of these genetic risk factors is mutation in the *GBA1* gene, which has emerged numerically as the most important genetic abnormality associated with PD (5, 6), being found in about 5% of patients with the so-called sporadic PD. In this chapter, we discuss the epidemiology, pathogenic mechanisms, clinical features, and implications for treatment of *GBA1* mutation-associated PD.

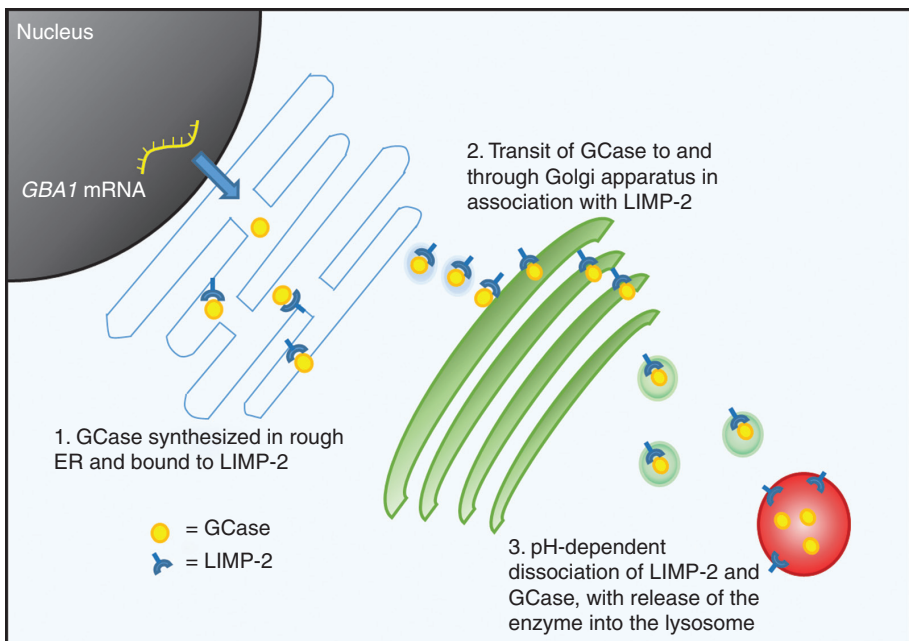
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## THE GBA1 GENE AND GLUCOCEREBROSIDASE

*GBA1* encodes the lysosomal enzyme glucocerebrosidase (GCase). The gene consists of a 7.6-kb sequence, with 11 exons and 10 introns, and is sited on the long arm of chromosome 1 (7). *GBA1* gives rise to at least two mRNA sequences, resulting from alternate polyadenylation sites, and the resultant amino acid peptides are processed to form the 496 amino acid mature protein (6); 16 kb downstream of

the *GBA1* gene lies a 5.7-kb sequence, which is almost identical to the *GBA1* gene (6, 7). This pseudogene has complicated genetic sequencing and the identification of novel pathogenic mutations.

GCCase is responsible for the hydrolysis of glucosylceramide (also termed glucocerebroside) glycosphingolipids to ceramide and glucose, in the lysosome (8). It consists of three domains, with the catalytic site residing in the third domain, which has the structure of a triose phosphate isomerase barrel (9). The enzyme is synthesized in the rough endoplasmic reticulum (ER) and traverses the Golgi apparatus via a phosphatidylinositol-4-kinase (PI4K)-dependent pathway to the lysosome. Transit is mediated by a specific transporter, lysosomal membrane protein-2 (LIMP-2) (10, 11). This differs from the majority of other lysosomal proteins, where delivery to the lysosome is dependent on mannose-6-phosphate receptors (12). GCCase and LIMP-2 are bound in the ER, and they pass through the Golgi apparatus in complex. On delivery to the lysosome, the low pH results in the dissociation of LIMP-2 from GCCase (Figure 1) (11). Enzyme activity is dependent on the substrate-presenting co-factor saposin C, which is cleaved from prosaposin (13).



**Figure 1** Synthesis and processing of GCCase. GCCase is synthesized in the rough ER where it becomes associated with LIMP-2. These two proteins transit in complex to the Golgi apparatus for processing. The GCCase-LIMP-2 complex then migrates to the lysosome, where GCCase is released into the lysosomal compartment, facilitated by pH-dependent dissociation from LIMP-2. ER, endoplasmic reticulum; GCCase, glucocerebroside; LIMP-2, lysosomal integral membrane protein-2; mRNA, messenger ribonucleic acid.

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## GBA1 MUTATIONS AND GAUCHER DISEASE

Over 300 mutations in the *GBA1* gene have been identified, which include point mutations, insertions, deletions, splice-site mutations, and recombinations (14, 15). Bi-allelic mutations result in negligible GCase enzyme activity and the autosomal recessive lysosomal storage disorder, Gaucher disease (GD), in which glucosylceramide accumulates in various organs and cells (particularly macrophages) (14). GD is classified based on the presence or absence of neurodegenerative features and the rate of clinical progression (16). Type 1 (non-neuronopathic) GD is characterized by hepatosplenomegaly, anemia and thrombocytopenia, and bone lesions. Types 2 and 3 (neuronopathic) GD are also associated with progressive neurological decline, which is rapid in type 3 GD (6, 14).

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## ASSOCIATION BETWEEN *GBA1* MUTATION AND PARKINSON'S DISEASE

At the end of the 20th century, reports of an association between PD and GD began to emerge. A proportion of patients with type 1 GD were observed to develop a parkinsonian syndrome in adult life (17–19), and it was subsequently noted that first-degree relatives of GD patients (who were obligate or confirmed carriers of a single *GBA1* mutation) also carried an increased risk of developing PD (20, 21). PD occurs in about 10% of patients with type 1 GD before the age of 80 years, compared to about 3–4% in the normal population (22).

A number of studies have since reported on the prevalence of *GBA1* mutation in the PD population, with estimates ranging up to 21% (23). The prevalence of *GBA1* mutations in PD is likely to have been overestimated in postmortem studies, in which cohorts may be more likely to include “atypical” or early-onset PD. It is generally accepted that about 5% of PD patients carry a *GBA1* mutation (24–28). In PD patients of Ashkenazi Jewish origin, the carrier frequency of *GBA1* mutation is much higher at 15–20% (5, 27). In contrast, <1% of the general healthy population carries a mutation (3% in the healthy Ashkenazi Jewish population) (27). This makes it numerically the most important known genetic risk factor for PD (6). Additionally, *GBA1* mutations have been associated with dementia with Lewy bodies, providing further support that there is a pathogenic link between this gene and  $\alpha$ -synucleinopathies (29–31). It is, however, important to understand that *GBA1* mutations do not cause a Mendelian form of PD, but are considered a genetic risk factor, increasing the risk 20–30 fold (6, 15, 27, 32). In absolute terms, the penetrance of PD in association with this mutation is relatively low, with about 30% of carriers developing the disease before the age of 80 (6).

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## PATHOGENIC MECHANISMS

The hallmark of PD pathology is the presence of Lewy bodies and Lewy neurites which consist predominantly of  $\alpha$ -synuclein (33, 34). Abnormalities in a number

of intracellular processes have now been implicated in *GBA1* mutation-associated PD, of which the one with greatest evidence base is dysfunction of the lysosome-autophagy system—a system known to be important in the clearance of  $\alpha$ -synuclein (35–38).

### Loss of function or gain of function?

A growing number of studies have linked loss of GCase activity to PD pathology (35, 39–42). Some studies have found that loss of enzyme function results in accumulation of sphingolipid substrates, which potentially play a pathogenic role, as discussed below (35, 39, 42). The loss-of-function hypothesis offers therapeutic targets for putative disease-modifying agents, which may act by augmenting GCase activity or removing substrates through other pathways.

GCase activity is reduced in several brain regions of individuals with sporadic and *GBA1* mutation-associated PD, and indeed in association with normal aging (43). Chemical suppression of GCase activity using conduritol- $\beta$ -epoxide increases  $\alpha$ -synuclein levels in wild-type mice and in neuroblastoma cells (39, 40). Higher levels of  $\alpha$ -synuclein have also been observed in the olfactory bulb (44) and substantia nigra (40) in GD mice. Additionally, *GBA1* knockout in wild-type mice results in aberrations in the lysosome-autophagy system and mitochondrial function, suggesting that loss of enzyme activity can negatively impact on other systems relevant to PD pathogenesis (45). Conversely, enhancing GCase activity can reduce  $\alpha$ -synuclein pathology in animal models (46, 47). While these studies have correlated reduced GCase activity with PD-relevant pathology, the mechanism by which this happens is yet to be clearly demonstrated. One possibility is that reduction in GCase activity results in accumulation of its sphingolipid substrates (42–44), which in turn stabilize the pathogenic species of  $\alpha$ -synuclein, contributing to its accumulation and aggregation (35, 48).

However, only a minority of GD patients, in whom GCase activity is negligible, develop PD (22). If the increased risk of PD in heterozygous *GBA1* mutation carriers relates to a loss of enzyme function, then one would expect a much higher incidence of PD within the GD population. Additionally, *GBA1* mutations which do not significantly reduce enzyme activity have been associated with PD (49). It appears, therefore, that the pathogenic mechanisms of *GBA1* mutation-associated PD are more complex than a simple reduction of enzyme activity and accumulation of substrates. Additionally, there are *GBA1* mutations that do not cause GD, but that increase the risk of PD, further suggesting that the pathogenesis of *GBA1* mutation-associated PD differs from that of GD (49).

The majority of pathogenic mutations in the *GBA1* gene are missense mutations that result in misfolding of the enzyme, and thus, an alternative hypothesis is that the pathogenicity of these mutations is conveyed by the gain of a toxic function of the misfolded enzyme. Misfolded GCase may be retained in the ER, leading to activation of the unfolded protein response (UPR) and ER-associated protein degradation (ERAD—see below). Prolonged activation of these systems and ongoing retention of misfolded enzyme may ultimately lead to activation of apoptotic pathways and neuronal loss. However, there are null mutations associated with PD risk, and as discussed above, knocking down *GBA1* or inhibition of GCase activity in animal and cell models can recapitulate relevant  $\alpha$ -synuclein

and PD pathology (35, 39–42). Gain of function therefore does not fully account for the increased risk of PD.

The mechanisms by which *GBA1* mutations predispose to PD pathology are multifaceted, with several elements potentially being important. A further degree of complexity is added by the fact that different *GBA1* mutations increase the risk of PD to different extents, and (at least in GD) there is a degree of genotype–phenotype correlation. For example, the common N370S mutation is rarely associated with neuronopathic GD, while homozygous L444P mutations result in type 3 GD, and some known mutations carry a much greater risk of PD development (e.g., L444P and D409H) than others (e.g., N370S) (5, 50). It is therefore conceivable that different pathogenic mechanisms are more or less pronounced in carriers of different mutations. The remainder of this section will outline what is currently known about the intracellular pathogenic mechanisms of *GBA1* mutation-associated PD.

### Lysosome-autophagy dysfunction

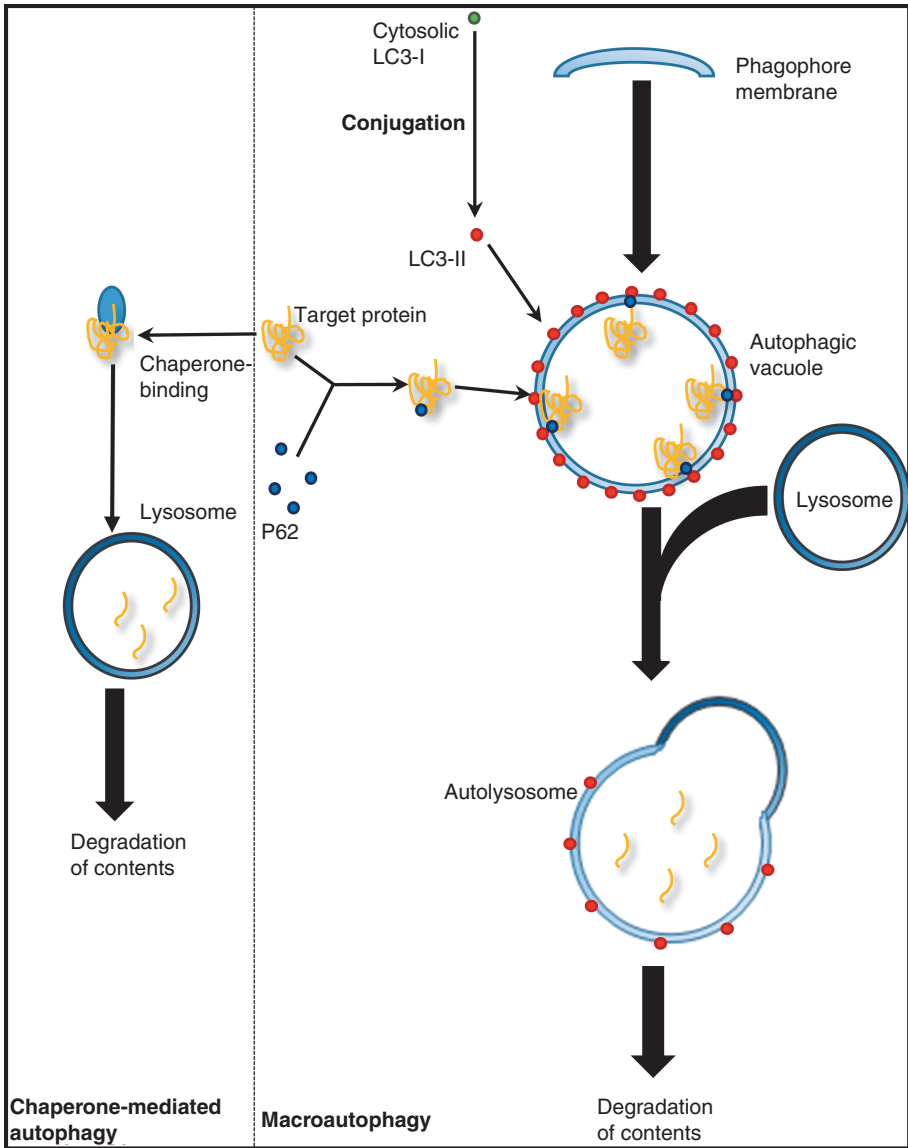
Autophagy is an umbrella term for some of the cell's major mechanisms of protein clearance and organelle turnover (51). Macroautophagy and chaperone-mediated autophagy are important in the degradation of  $\alpha$ -synuclein, and therefore, it is feasible that dysfunction of these systems can potentiate PD pathology. In chaperone-mediated autophagy, soluble proteins are directed to lysosomes by chaperone proteins for degradation by lysosomal enzymes (52, 53). In contrast, macroautophagy involves the formation of vacuolar structures called autophagosomes from cytosolic membrane fragments (phagophore). These autophagosomes take up proteins and other cellular waste before fusing with lysosomes to form an autolysosome. The contents are then degraded by the lysosomal compartment and recycled (Figure 2) (51, 53).

Dysfunction of the lysosome-autophagy system (in particular, macroautophagy) appears to be central to the pathogenic role of *GBA1* mutations, with a growing number of studies reporting autophagy dysfunction in association with *GBA1* mutation (35, 36, 42, 54). For example, increased numbers of lysosomes have been reported in neuroblastoma cells carrying *GBA1* mutations (54) and induced-pluripotent stem cell (iPSC)-derived dopaminergic neurons from homozygous or heterozygous *GBA1* mutation carriers (36, 42). Higher autophagosome numbers have also been reported in these studies (42, 54). Additionally, increased numbers of autophagosomes and lysosomes have been detected in neurons from wild-type mice treated with conduritol- $\beta$ -epoxide, suggesting that these changes can occur due to reduced GCase enzyme activity (39).

While the lysosome-autophagy system is clearly implicated in PD associated with *GBA1* mutations, the specific nature of the dysfunction is not understood. The possible sites of perturbation in macroautophagy could be at the level of:

- i. biosynthesis of autophagosomes and/or lysosomes
- ii. uptake of the targets of degradation into autophagosomes
- iii. fusion of autophagosomes with lysosomes
- iv. enzymatic degradation of the contents of the autolysosome (Figure 2).

The aforementioned observations of increased numbers of lysosomes and autophagosomes in *in vitro* and *in vivo* models of *GBA1* mutation-associated disease



**Figure 2** Macroautophagy and chaperone-mediated autophagy pathways. During macroautophagy, phagophore membrane fragments form autophagosomes. During this process, the protein LC-3 is conjugated. Proteins are targeted to the autophagosome, which involves the protein P62. The autophagosome fuses with the lysosome to form an autolysosome, and the contents are degraded by lysosomal enzymes. In chaperone-mediated autophagy, proteins for removal bind to protein chaperones, which help to target them directly to the lysosome for enzymatic degradation. Together, these mechanisms constitute important pathways for  $\alpha$ -synuclein clearance.

suggest that the site of dysfunction is not in the generation of these structures, but that it lies in the later stages of the pathway (36, 39, 42, 54). One possibility is that the increased number of these structures is due to increased activation of macroautophagy, perhaps as a compensatory response to other intracellular stresses. Alternatively, it could reflect an impairment in the fusion step of macroautophagy, which is supported by the observation that iPSC-derived neurons from *GBA1* mutation carriers have reduced co-localization of autophagosomes and lysosomes compared to isogenic controls (42).

Other studies, however, suggest that the problem lies in the enzymatic function within the lysosome. Mazzulli and colleagues reported reduced lysosomal proteolysis in primary mouse cortical neurons with sh-RNA-mediated knock-down of *GBA1* (35). Reduced lysosomal proteolysis may also be inferred by the observation that lysosomes in iPSC-derived dopaminergic neurons from *GBA1* mutation carriers are enlarged, with increased electron dense material, suggesting that their contents are not cleared appropriately (36). Therefore, while alterations in the function of the lysosome-autophagy system associated *GBA1*-mutation appear to occur, especially in the later stages of the macroautophagy pathway, the precise site of the dysfunction has yet to be conclusively shown.

Accepting that there is a problem with the lysosome-autophagy system in *GBA1* mutation carriers, a question remains as to whether this is relevant to the increased risk of PD or whether it is an incidental observation associated with the mutation (taking into account the fact that only a minority of GD patients develop PD).  $\alpha$ -synuclein clearance is dependent on multiple pathways, but both macroautophagy and chaperone-mediated autophagy (both dependent on lysosomal hydrolysis) have been shown to be fundamentally important in its degradation (38, 55). In particular, a growing number of *in vitro* and *in vivo* studies have identified increased levels of  $\alpha$ -synuclein in association with lysosome-autophagy dysfunction in *GBA1* mutation or GCase suppression (35, 39–41, 44, 54, 56–58). Therefore, for example, *GBA1* knock-down in mouse cortical neurons has been shown to lead to reduced lysosomal proteolysis associated with reduced clearance of soluble and insoluble  $\alpha$ -synuclein (35). Lysosomal dysfunction has also been shown to increase exosomal release of  $\alpha$ -synuclein from the cell (59, 60), which may in turn then be taken up by adjacent neurons, resulting in aggregation of endogenous  $\alpha$ -synuclein in surrounding cells, all of which could account for the more accelerated clinical course seen in GBA-PD.

Taking into account the above discussion, it is clear that lysosome-autophagy system dysfunction in *GBA1* is complex. A bidirectional loop has been proposed in which reduced GCase activity leads to perturbation in lysosomal function and accumulation of glycosphingolipid substrates, and accumulation of pathogenic oligomers of  $\alpha$ -synuclein (35). The GCase substrate glucosylceramide has been shown *in vitro* to lead to stabilization of pathogenic high molecular weight recombinant  $\alpha$ -synuclein oligomers, before accelerated aggregation (35, 48). The bidirectional loop is completed by the observation that  $\alpha$ -synuclein appears to impede transit and processing of GCase between the ER and the lysosome, resulting in a further reduction in lysosomal GCase activity (35). Further evidence for this comes from the observation that overexpression of  $\alpha$ -synuclein in cortical neurons results in an increase in the ER form of the enzyme and a reduction in the post-ER, glycosylated form of the enzyme (35), which fits with the



finding of reduced GCase activity in some PD patients with wild-type *GBA1* in some studies (39).

### Mitochondrial dysfunction and the role of oxidative stress

Mitochondrial dysfunction is clearly important in PD pathogenesis, illustrated by the fact that mitochondrial toxins such as rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induce death of dopaminergic neurons (61–63) and the fact that some hereditary forms of PD occur due to mutations in genes related to mitochondrial function and health (e.g., *PINK-1* and *DJ-1*) (1). Few studies have commented on mitochondrial dysfunction in *GBA1* mutation-associated disease, but there is some evidence that it plays a role (41, 45, 57, 64).

Suppression of GCase activity using conduritol- $\beta$ -epoxide in neuroblastoma cells (41) and wild-type neurons in culture (57) results in a reduction in ATP production and oxygen consumption, demonstrating that mitochondrial dysfunction can occur as a result of reduced GCase enzyme activity. Additionally, a progressive reduction in mitochondrial membrane potential has been observed *in vitro* with prolonged GCase suppression, further supporting this idea (41). Furthermore, mitochondrial morphology and function are abnormal in *GBA1* knockout mice, with the degree of abnormality greatest in homozygous compared to heterozygous knockout animals (45). In addition, reactive oxygen species are found in higher levels in the fibroblasts of PD patients carrying *GBA1* mutations in comparison with those with wild-type *GBA1* (65). These cells also had increased levels of the antioxidant, NQ01—a potential compensatory mechanism (65). Finally, protein aggregates including  $\alpha$ -synuclein have been noted to be localized to mitochondria in the hypomorphic prosaposin murine model of GD (57). While all these studies support a potential role for mitochondrial dysfunction in *GBA1* mutation-associated PD, evidence is sparse in this area, and it is not clear whether these aberrations are directly related to abnormalities in GCase and the *GBA1* gene, or alternatively are incidental markers of neurodegeneration.

It is possible that perturbations in mitochondrial function arise downstream of *GBA1* mutation-induced  $\alpha$ -synuclein accumulation and aggregation. However, it has recently been suggested that oxidative stress is an earlier component of the pathogenic process in PD (66). Mitochondrial oxidant stress has recently been shown to be associated with *DJ-1* knockout or dysfunction in dopaminergic neurons generated from patient-derived iPSCs, and after prolonged culture, this results in reduced GCase enzyme activity and impaired lysosomal proteolysis (66). While this process occurs in cells from patients with sporadic PD, it was accelerated in neurons derived from patients with homozygous *DJ-1* mutations, suggesting that mitochondrial dysfunction is an important aspect of PD pathogenesis, potentially through inducing lysosomal dysfunction (66). Furthermore, mitochondrial antioxidants reduce the levels of soluble  $\alpha$ -synuclein, suggesting that oxidant stress contributes to  $\alpha$ -synuclein accumulation (66). This important study suggests that a sequential pathogenic pathway exists in PD, in which mitochondrial oxidant stress leads to reduced GCase activity and lysosomal function, which potentially leads to  $\alpha$ -synuclein accumulation. *GBA1* mutation may feed into this by lowering the threshold for, or altogether bypassing the need for, mitochondrial oxidant stress.

## ER-stress

As is discussed above, GCase is produced in the rough ER before it is processed in the Golgi apparatus and transported to the lysosome (10, 11). If proteins are not folded correctly in the ER, the misfolded protein may be retained for re-folding, mediated by ER chaperones (67, 68). Failure to correct the protein structure results in removal of the offending proteins via ERAD and the ubiquitin-proteasome system (67–69). *GBA1* mutations, which result in structural change and misfolding of the protein, may result in activation of these systems (70, 71). Once their capacity to remove the misfolded protein is exceeded, activation of the UPR ensues (36, 72). If ER-stress is prolonged and the UPR fails to restore normal function, the cell may be directed to an apoptotic cell death fate. In support of this, increased levels of chaperone markers of ER-stress have been found in iPSC-derived neurons from PD patients carrying *GBA1* mutations compared to controls, along with increased levels of markers of the UPR (36).

## Interaction with $\alpha$ -synuclein

An increasing number of studies have identified  $\alpha$ -synuclein accumulation in the setting of *GBA1* mutation (36, 46, 56). This may occur due to reduced clearance, secondary to dysfunction of the lysosome-autophagy system as discussed above (35, 36, 66). However, GCase also appears to interact directly with  $\alpha$ -synuclein, and the significance of this is not known (73, 74). In one postmortem study, GCase was identified in 32–90% of Lewy bodies of PD patients with *GBA1* mutation, and in 10% of those with wild-type *GBA1* (75). It is possible that this co-localization is a manifestation of the cell's attempt to clear these aggregates, but alternatively it may be that mutant GCase stabilizes  $\alpha$ -synuclein species or serves as a platform for fibrillization to occur. As well as these postmortem findings, recombinant GCase and  $\alpha$ -synuclein have also been shown to interact *in vitro* (73, 74). At lysosomal pH, membrane-bound  $\alpha$ -synuclein forms a complex with GCase, resulting in reduced enzymatic function (47, 73, 74). This interaction potentially explains the alterations in GCase activity that has been reported in PD with wild-type *GBA1* (50, 76).

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## CLINICAL ASPECTS OF *GBA1* MUTATION-ASSOCIATED PD

The characteristic motor features of PD include bradykinesia, rigidity, and resting tremor. Onset is usually asymmetric, and the disease progresses slowly over years. Other motor manifestations include postural instability, hypomimia, dysphonia, and altered posture. There are often additional non-motor features, such as anosmia, neuropsychiatric symptoms including depression and anxiety, sleep disturbance, autonomic dysfunction, and cognitive decline and dementia (1). At an individual level, PD patients carrying *GBA1* mutations are clinically indistinguishable from those with sporadic PD. However, at a population level, *GBA1* mutations are associated with differences in the age of onset, and incidence of cognitive involvement, in comparison with PD patients with wild-type *GBA1* (24, 77–81).

In this section, the clinical and imaging aspects of *GBA1* mutation-associated PD will be discussed.

## Clinical features

Age of onset is earlier in *GBA1* mutation-associated PD, with multiple studies suggesting that motor manifestations occur between 2 and 10 years earlier than in sporadic PD patients (77–83). Additionally, *GBA1* mutations are twice as common in those with PD of onset before age 50 than in later-onset cases (81, 82).

The clinical syndrome in PD associated with *GBA1* mutations is similar to that seen in sporadic PD. However, motor progression to Hoehn and Yahr stage 3 (bilateral disease with development of postural instability) is accelerated in patients with *GBA1* mutation, with an associated increase in mortality (24, 77, 84). One study reported that *GBA1* mutation carriers are more likely to present with tremor compared to patients with sporadic PD and that they have an increased risk of development of levodopa-induced dyskinesia (82). However, it should be noted that this group of patients were on significantly higher doses of levodopa compared to the sporadic PD patients which may explain the increased incidence of dyskinesia (82). Non-motor features, including REM-sleep behavior disorder, depression, anxiety, and hallucinations, are also said to be more prevalent in PD patients with *GBA1* mutations compared to those without (81, 85–88). Depression and REM-sleep behavior disorder are also more common in individuals with heterozygous *GBA1* mutations without a diagnosis of PD, compared to individuals with no mutation, which may reflect a prodromal phase of PD (85). Olfactory dysfunction is considered to be a prodromal non-motor feature of PD, and hyposmia has been identified at a greater incidence in individuals with heterozygous and homozygous *GBA1* mutations compared to those with wild-type *GBA1* (85, 86, 89). The greater burden of non-motor symptoms in *GBA1* mutation carriers has been associated with lower quality-of-life scores compared to individuals with wild-type *GBA1* (87). While autonomic features including constipation, bladder and erectile dysfunction, and orthostatic hypotension occur in *GBA1* mutation-associated PD, they do not appear to be any more prevalent than in sporadic PD (86, 88, 89).

The most significant difference in the clinical features of *GBA1* mutation-associated PD compared to sporadic PD is the increased incidence and rate of cognitive decline (24, 77, 84, 86, 88). The prevalence of dementia in individuals with sporadic PD who are over 65 years old is estimated to be between 24 and 31% (90). One retrospective longitudinal study found that 56% of *GBA1* mutation carriers had dementia at age 70, compared with 15% of sporadic PD patients (84). Carriers of *GBA1* mutations had a threefold increased risk for dementia and approximately twofold increased risk of mortality, which was not entirely attributable to the dementia (84). This is consistent with findings from other studies (77, 86, 88, 91). Cognitive decline occurs more frequently in *GBA1* mutation-associated PD compared to sporadic PD, while the rate of progression is accelerated. In a community incident cohort of PD patients, those with *GBA1* mutations had a median time to diagnosis of dementia of approximately four years, compared to eight years for those without *GBA1* mutations (24).

## Neuroimaging findings

Imaging studies, using dopamine transporter imaging scans, magnetic resonance imaging (MRI) scans, transcranial sonography, and positron emission tomography (PET) scans, have all been used to look for differences between sporadic PD and PD associated with *GBA1* mutation.

Dopamine transporter PET imaging (DaTSCAN) using the dopamine transporter ligands 18F-fluoropropylcarbomethoxyiodophenylnortropane (18F-FP-CIT) and 123-iodoflupane (123I-FP-CIT) have shown a greater degree of dopaminergic neuronal loss in PD associated with *GBA1* mutation than in patients with wild-type *GBA1* (84, 92–94). Asymmetry of radio ligand uptake is more pronounced in PD associated with *GBA1* mutations than in some of the genetic forms of PD associated with a Mendelian inheritance pattern (*SNCA*, *PINK1*, or *Parkin* mutations) (92). However, a larger study in which F18-fluorodopa PET imaging was used to investigate patients with *GBA1* mutation did not find any significant difference in dopamine synthesis, when compared with sporadic PD patients (95). Magnetic resonance diffusion tensor imaging (DTI) demonstrates reduced fractional anisotropy in a number of white matter tracts (corpus callosum, anterior limb of internal capsule bilaterally, right anterior external capsule, and olfactory tracts) in PD patients with *GBA1* mutations compared to those without mutations (96, 97). Although some subtle differences in the imaging features of *GBA1* mutation-associated PD and sporadic PD have been described, the utility of these imaging techniques is largely limited to research at present. However, as refined treatments are developed, imaging findings may be useful in guiding genetic testing in PD, particularly when seeking mutations in the *GBA1* gene, as sequencing is complicated by the presence of a pseudogene in close proximity to the gene (as is discussed above).

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## THERAPEUTIC PROSPECTS AND IMPLICATIONS

Current treatment of *GBA1* mutation-associated PD is the same as for sporadic PD. There are no specific treatments that modify the disease course in PD, with or without *GBA1* mutation, and the current treatment involves the use of dopaminergic agents to improve motor symptoms. Understanding the mechanisms by which *GBA1* mutations predispose to PD pathogenesis may offer novel therapeutic avenues and a greater prospect of identifying disease-modifying agents for this group of patients. Furthermore, given that some patients with sporadic PD also show a significant reduction in GCase activity (see above), it may also be that therapies targeting GCase dysfunction and its associated pathology are useful in other groups of PD patients as well (43).

As is discussed above, many studies suggest that reduced GCase enzyme activity is important in the pathogenesis of *GBA1* mutation-associated PD (35, 39–42). One approach, therefore, may be to augment enzyme activity, as is done in the treatment of non-neuronopathic GD (98). Enzyme replacement therapy and small molecule substrate reduction therapies are useful for the peripheral manifestations of GD, but do not cross the blood–brain barrier, therefore are unlikely to provide any meaningful improvement in the course of PD (99).

A number of small molecule chaperones have recently been investigated for their ability to restore GCase function by binding to the misfolded enzyme that is retained in the ER and facilitating its correct re-folding, thus increasing lysosomal GCase levels and reducing ER stress. One of these compounds, isofagamine, yields an improvement in motor function, reduction in microglial activation, and a reduction in  $\alpha$ -synuclein aggregation in  $\alpha$ -synuclein over-expressing mice (100). This demonstrates that these compounds have the potential to alter central nervous system pathology. Another chaperone, ambroxol hydrochloride, is currently being investigated as a potential treatment for *GBA1* mutation-associated PD. Ambroxol has been used as an expectorant since the 1980s and has been shown to increase lysosomal GCase levels and activity in several *in vitro* models (65, 71, 101, 102). Binding of ambroxol to GCase is pH-dependent, being strongest at the neutral pH of the ER, and allowing dissociation to occur in the acidic pH of the lysosome (103). Additionally, it seems that this increase in GCase activity enhances  $\alpha$ -synuclein clearance (65,102). However, ambroxol does not appear to cross the blood–brain barrier, at least at the doses that are used clinically, and it remains to be seen whether this approach can be useful in *GBA1* mutation-associated PD or GD (104).

As well as investigating GCase enhancement as a therapeutic option for *GBA1* mutation-associated PD, it may be possible to slow disease progression by targeting the downstream mechanisms by which *GBA1* mutations increase PD risk, such as lysosome-autophagy system dysfunction. Rapamycin, an immunosuppressant agent, is a well-established inducer of macroautophagy (105), which has been shown to reduce  $\alpha$ -synuclein accumulation in a *GBA1* mutation model *in vitro* (56). While rapamycin is unlikely to be used as a treatment for PD in view of its adverse effect profile, other drugs that are able to correct the lysosome-autophagy defect may be useful in *GBA1* mutation-associated PD. This may involve repurposing of drugs that are already established as treatments for other conditions, which are found to have a beneficial effect on autophagy. Potential examples of these agents include the tricyclic antidepressant nortriptyline, the disaccharide trehalose, and the antihypertensive rilmenidine (106–113).

Gene therapy, in which the *GBA1* gene is introduced using a viral vector, has also been investigated in rodent models (46, 114). Memory impairment in a GD mouse model was reversed using this approach (114). However, given the widespread nature of pathology in *GBA1* mutation-associated PD, reflected by the increased incidence and degree of cognitive impairment, it will be challenging to achieve effective delivery of the gene to the relevant population of cells, and much more work is required before this approach is considered suitable for the clinic.

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## CONCLUSION

As we enter the era of genomic sequencing, our understanding of genetic risk factors for conditions such as PD is becoming increasingly important. There are currently no disease-modifying therapies available for PD, and in view of the heterogeneous pathogenic mechanisms present in PD patients, it is likely that different treatment approaches will be necessary for different groups.

Establishing the processes by which genetic susceptibility factors increase the risk of PD pathology provides the opportunity to develop specific therapies that target the relevant pathways in individual patients. *GBA1* is now recognized as one of the most important genetic risk factors for PD, both numerically and clinically, with it leading to a greater degree of cognitive impairment and other non-motor features, all of which lead to a reduction in the quality of life in this population. Identification of drugs that are able to attenuate *GBA1* mutation-induced pathology can therefore provide significant clinical benefit, and investigations toward this are under way.

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