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Biochemical consequences of *glucocerebrosidase 1* mutations in Parkinson's disease

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Parkinson's disease (PD, OMIM #168600) is a common neurodegenerative disorder with a global prevalence of approximately 8.5 million. PD is characterized by four cardinal motor symptoms: bradykinesia, rigidity, resting tremor, and subsequently by postural instability. It usually involves non-motor symptoms such as rapid eye movement sleep disorder, dementia, anosmia, and autonomic dysfunction. The gene *glucocerebrosidase 1* (*GBA1*), which encodes the lysosomal enzyme glucocerebrosidase (GCase) (IUBMB: EC 3.2.1.45), shows strong linkage with PD; variants of *GBA1* are the commonest genetic association with PD (Sidransky et al., 2009). Several mechanisms may underlie the relationship between *GBA1* mutations/variants and the molecular pathology of PD (**Figure 1A** and **B**).

***GBA1* – Structure & function:** *GBA1* is located on chromosome 1 and has 11 exons with a pseudogene (*GBAP*) that is highly homologous 16 kb downstream, making it prone to recombination and rearrangements. The *GBA1* gene product, GCase, functions primarily in lysosomes especially in autophagy by recycling cerebrosides, a group of sugar lipids found in the brain. GCase converts two cerebrosides, glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph) to glucose and ceramide, and glucose and sphingosine, respectively. Mutations in GCase are known to be implicated in both metabolic and neurodegenerative disorders, causing deficient GCase activity and lysosomal dysfunction. Possessing two copies of mutated *GBA1* alleles causes a rare autosomal recessive lysosomal storage disorder, Gaucher disease (GD). Having one mutated allele of *GBA1* can increase the risk of PD.

GCase has 3 functional domains with an active site containing key catalytic residues: Glu235, Glu340, and Cys342. There are five N-linked glycosylation modification sites which are Asn19, Asn59, Asn146, Asn270, and Asn462. GCase is synthesized in the rough endoplasmic reticulum, transported to lysosomes via LIMP2, then gains full function in the acidic pH of lysosomes following the interaction with its activator saposin C (Sap C). As can be seen in the structural analysis of GCase (Protein Data Bank: 3GXI), the sites of *GBA* mutations are scattered in the structure and not necessarily located at or in very close proximity to the active sites nor the modification sites. This suggests that the distance of the mutations from the active sites and the modification sites does not correlate with the severity of GCase dysfunction, hence cannot act as a reliable marker for genotype-phenotype correlation (Smith and Schapira, 2022). Other sites to be considered are the interface with Sap C and the receptor interaction site with its transporter.

Gaucher disease and Relationship with PD: Since *GBA1* is expressed in all cells, the phenotypic presentations of GD arise from failure to recycle cerebrosides in multiple organs, including the brain, eyes, lungs, blood, and bones, causing pancytopenia, splenomegaly, and hepatomegaly. GD is classified into three types; Type 1, 2, and 3 (T1,2,3GD), depending on their severity and neurological involvement. T1GD is known as the non-neuropathic type and the other two, T2GD and T3GD are known as the neuropathic type with T2GD having the most severe symptoms and rapid disease progression. The prevalence of *GBA1* variants in the PD populations is 10–15%, rising to 31% in the Ashkenazi-Jewish (AJ) population, which has the highest GD incidence in the world (Smith and Schapira, 2022). Carrying one or two copies of *GBA1* mutant alleles increases the risk of PD. Interestingly, despite having two dysfunctional *GBA1* alleles, GD patients have the same increased risk of PD as single variant carriers, even though GCase activity is absent or markedly decreased in GD compared to PD. The only observed difference between PD in GD patients, and GBA-PD (PD in *GBA1* mutation carriers), is that GD patients have earlier onset of PD than GBA-PD patients (Liu et al., 2016). GBA-PD patients present similarly to idiopathic PD patients but again with earlier age at onset on average by 5 years, more frequent cognitive impairment with greater non-motor symptom presentation, and faster disease progression (Smith and Schapira, 2022). This suggests that GCase activity levels do not correlate directly with symptom severity, but there is a broad correlation with age at onset for PD.

Pathophysiology of GBA-PD: Five main cellular processes are most likely to be involved in PD pathology. The affected pathways include autophagy-lysosomal pathway, alpha-synuclein (αSyn) metabolism, protein clearance pathways endoplasmic reticulum-associated degradation and unfolded protein response (UPR), mitochondrial function and quality control pathway, and neuroinflammation. Amongst these, αSyn metabolism is thought to be the central pathway where all other biochemical pathways converge. αSyn is mainly cleared via autophagy-lysosomal pathway, particularly through chaperone-mediated autophagy, a type of autophagy where soluble proteins are selectively transported to lysosomes for degradation via chaperone proteins. Autophagy is a cellular quality control mechanism that entails the removal and recycling of cellular components that are redundant or impaired. The process of autophagy starts with the formation of autophagosomes, a double-membrane vesicle that encapsulates redundant cellular materials. They then fuse with lysosomes, forming autolysosomes so that the encapsulated cellular components undergo degradation via lysosomal enzymes such as GCase. Lysosomal dysfunction is thought to contribute to PD pathogenesis; sequence variants of genes encoding lysosomal proteins are associated with an increased risk for PD, and abnormalities of lysosomal function are seen in PD brain (Alvarez-Erviti et al., 2010; Straniero et al., 2022).

There is an inverse relationship between GCase activity and αSyn levels. There are two main hypotheses suggesting how GCase dysfunction could lead to αSyn accumulation and subsequently, increase the risk of PD: loss-of-function and gain-of-function hypotheses (**Figure 1A**). The loss-of-function hypothesis suggests that *GBA1* mutations cause a reduction in GCase activity leading to substrate accumulation and resulting in altered glycosphingolipid homeostasis. GCase activity is naturally reduced with aging, but a more extreme reduction is observed in PD patients (Rocha et al., 2015). Growing evidence shows that the loss of GCase activity causes significant downstream effects on lysosomal function, especially through changing intra-lysosomal pH, leading to chaperone-mediated autophagy dysfunction and contributing to αSyn pathology. The change in intra-lysosomal pH promotes the accumulation of sphingolipids like GlcCer and GlcSph, and cholesterol in the lysosomes (Navarro-Romero et al., 2022). Membrane-bound αSyn is found to form complexes with GCase at the lysosomal pH of 5.4, limiting the enzymatic function of GCase. The interface of GCase and αSyn binding is the same interface used by its activator, Sap C-GCase binding. Thus, αSyn can act as a competitive inhibitor of Sap C. GlcCer is known to directly interact and stabilize protofibrils of αSyn and GlcSph is found to trigger the conversion of αSyn to fibrils, exacerbating αSyn pathology (Surface et al., 2022). Recently, it was found that in GCase mutant fibroblasts, GlcSph and cholesterol species exist in excess in lysosomes and GlcSph accumulates more markedly than GlcCer (Navarro-Romero et al., 2022). In cells expressing the E326K *GBA1* variant, GCase activity is unaffected or only mildly depressed, but there is an accumulation of lipid droplets (Smith et al., 2022). These findings suggest a pathogenic loop between αSyn and GCase through GCase inhibition, resulting in αSyn stabilization and accumulation.

The gain-of-function hypothesis argues that expression of misfolded GCase due to mutations impairs the cell's protein clearance pathways, leading to reduced clearance of aberrant

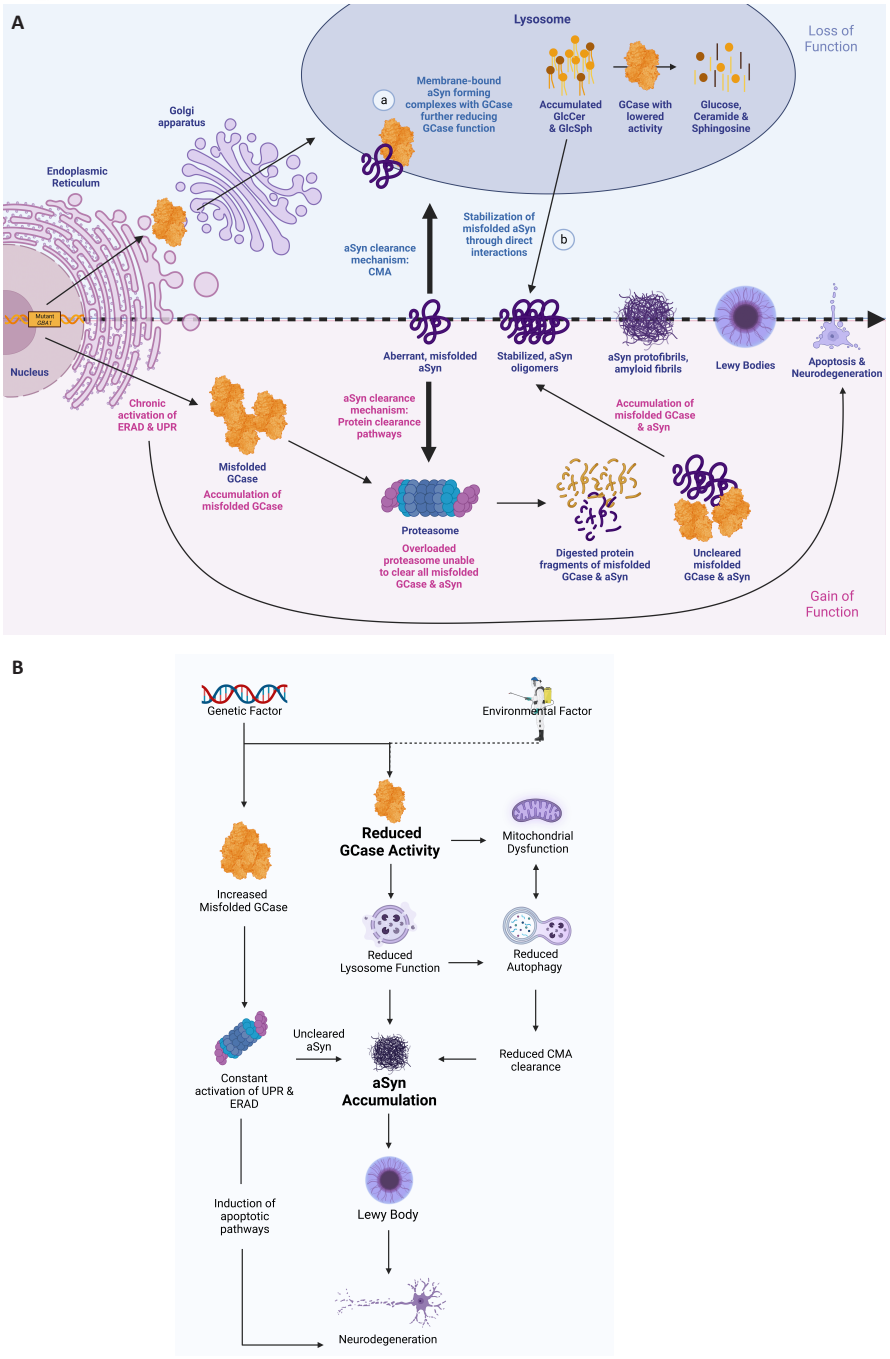

Figure 1 | **Potential pathophysiology of GBA-PD.**
(A) Potential pathways in the pathophysiology of GBA-PD. The figure schematically represents both the loss-of-function hypothesis (top–blue) and gain-of-function hypothesis (bottom–pink), the two suggested hypotheses for *GBA1* mutation and GCase dysfunction leading to alpha-synuclein accumulation and Lewy body formation, the central hallmarks of Parkinson's disease. (B) Simplified diagram of possible GBA-PD pathophysiology. The diagram depicts the possible contributing factors to reduced GCase activities and leads to alpha-synuclein accumulation and Lewy body formation, the hallmarks of Parkinson's disease. The dashed line indicates yet to be fully confirmed relationship between environmental factors and reduced GCase activity. Created with BioRender.com. αSyn: Alpha-synuclein; CMA: chaperone-mediated autophagy; CMA: chaperone-mediated autophagy; ERAD: endoplasmic reticulum-associated protein degradation; GBA: glucocerebrosidase; GCase: glucocerebrosidase; GlcCer: glucosylceramide; GlcSph: glucosylsphingosine; PD: Parkinson's disease; UPR: unfolded protein response.

αSyn and αSyn accumulation. When UPR and endoplasmic reticulum-associated degradation are chronically activated, the endoplasmic reticulum, where GCase is post-translationally modified, undergoes a stress response. This inhibits the trafficking of GCase to lysosomes, causing the mutant GCase to be retained in the endoplasmic reticulum, activating further stress response and apoptotic pathways. In GBA-PD, GCase constitutes approximately 32–90% of Lewy body compared to < 10% in idiopathic PD; this probably reflects the function of Lewy bodies as sites of aberrant protein accumulation (Goker-Alpan et al., 2010). This hypothesis, however, cannot explain the "risk" variants e.g., E326K, which elevate the risk of PD. It is likely that more than one of the above mechanisms operate at any one time to result in the increased risk of PD in *GBA1* variant carriers, the relevance of each being determined by the effects of the change in GCase structure and function.

Classification & epidemiology of GBA1 variants: The current classification of *GBA1* mutations is based on the three types of GD. The neuronopathic type-causing *GBA1* mutations (e.g., L444P, 84GG & D409H) are collectively called "severe" mutations. The T1GD-causing mutations (e.g., N370S) are known as "mild" mutations. *GBA1* mutations which are found to increase the risk of PD in carriers but do not cause GD when present bi-allelically (e.g., E326K, T369M) are called "risk" mutations or variants. Severe mutations tend to present with a more aggressive form of PD with earlier symptom onset and rapid symptom progression. They also lead to higher PD risk with an OR of 14.6–19.3 compared to mild mutations (OR: 3.0–4.7) with less GCase activity.

L444P (c1448T>C; p. L483P): L444P is the most common severe *GBA1* mutation. The structural deformity caused by L444P significantly reduces GCase activity by 35% in L444P/wt human fibroblasts (Sanchez-Martinez et al., 2016). The mutation is located near the Sap C binding site of GCase, suggesting a possible interaction with activator binding, reducing the activity of the protein. L444P is carried by 1.15% of AJ-PD patients and is also the most common *GBA1* mutation in the Asian population (Sidransky et al., 2009). The age at onset is earlier in L444P patients at around 47.0 years compared to the average age of 60 years. L444P/+ mice show a significant increase in pathological αSyn deposits and human fibroblasts show sphingolipid changes and the promotion of αSyn aggregation (Migdalska-Richards et al., 2020; Galvagnion et al., 2022). This further enhances the contribution of sphingolipid species to αSyn pathology. Activation of UPR and endoplasmic reticulum retention has also been observed in L444P mice and PD patient-derived cell lines suggesting L444P's exacerbation of αSyn pathology via the gain-of-function route.

N370S (c1226A>G; p. N409S): N370S is a mild mutation and is the most common *GBA1* mutation seen in the AJ population, its prevalence reaches about 14.1% in AJ-PD patients and 1.5% in the non-AJ PD population (Sidransky et al., 2009). It reduces GCase activity by 32% in N370S/wt patient-derived fibroblasts (Sanchez-Martinez et al., 2016). However, N370S conveys a lower risk of developing PD compared to L444P, although it causes misfolding of GCase and is also located in close vicinity to the Sap C binding site of GCase. It is also in close proximity to Glu340, one of the key residues forming the GCase active site. This is reported to change the behavior of GCase, making it moderately rigid, and disabling subtle structural modifications upon pH changes.

E326K (c1093G>A; p. E364K): E326K is a risk variant that causes almost no change in GCase level nor activity, but elevates the risk of PD. Its location in GCase is distant from the active site. Therefore, there is currently no structural basis as to why E326K elevates the risk of PD but does not cause GD when present bi-allelically. A recent report demonstrated lipid droplet accumulation in E326K mutant neuroblastoma cells simultaneously with increased insoluble αSyn (Smith et al., 2022). This implicates the role of E326K in lipid dysregulation. However, further studies are required to determine the exact components of these lipid droplets. In terms of pathophysiology, there were no significant data to suggest that UPR, lysosomal quality reduction nor mitochondrial defects, contribute to pathogenesis.

Other less significant *GBA1* mutations are beyond the scope of this perspective. 84GG, D409H and IVS2+1 are three severe mutations of *GBA1*. Both 84GG and IVS2+1 are founder null mutations of the AJ population causing significantly reduced production of GCase (Gan-Or et al., 2015).

Final remarks and perspectives: application of lipidomics in GBA-PD: As highlighted in E326K research and the discussion of the loss-of-function hypothesis, more reports are being published on the observation of accumulation of GCase substrates and lipid droplets. Thus, there is a recent focus on the importance of GCase enzyme substrates, GlcCer and GlcSph, especially GlcSph, and their dyshomeostasis contributing to αSyn pathology. Since both GlcCer and GlcSph are glycolipids, to gain further insight, lipidomics, the study of lipids, in particular glycolipidomics, will be necessary. The main analytical technique currently used in the field of lipidomics is mass spectrometry which only allows for the identification and quantification of lipids, limiting the application of lipidomics in pathway analysis. Better functional techniques will be required to allow easy localization and tracking of particular lipid species in cellular systems. Currently, there are techniques that can track the movement of lipid droplets in cells (Ren et al., 2021). For lipidomics to be relevant to PD research, ideally, we need to be able to track the movement of a particular lipid in real time within the cells and identify the structural interactions between lipids, proteins, and sugars.

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