Chapter 1

Introduction

1 Introduction

Parkinson's disease (PD) is an irreversible, intricate, and incapacitating motor disorder caused by the progressive degeneration of the nigrostriatal dopaminergic pathway and toxic build-up of the oligomeric form of α-synuclein (Wood-Kaczmar, Gandhi et al. 2006). This nigrostriatal dopaminergic pathway comprises two key regions: the substantia nigra pars compacta (SNc) and the striatum. Within the SNc, dopaminergic neuronal cell bodies are situated, while their axons extend to the striatum (Chinta, Andersen et al. 2005, Vercammen, Van der Perren et al. 2006). The degeneration of dopaminergic neurons leads to a decrease in the production of dopamine (DA), an important neurotransmitter involved in the regulation of motivation, learning, and the encoding of movement (Chinta, Andersen et al. 2005, Real, Garcia et al. 2017). Dopamine (DA) deficiency is recognized as one of the contributing factors to the development of PD symptoms. In the biosynthesis of DA, an essential neurotransmitter, tyrosine hydroxylase (TH) plays a crucial role as the rate-limiting enzyme. Disruptions in the activity or regulation of TH can impact the production of DA and contribute to the symptoms of PD (Sayyaed, Saraswat et al. 2023, Wang, Chen et al. 2023). The stages and hallmark motor symptoms (Shahed and Jankovic 2007, Boix, Von Hieber et al. 2018) of PD encompass the following:

(I) **Tremor:** Tremors, especially at rest, are a characteristic feature of PD. These tremors typically manifest in the hands, fingers, or thumbs. They may diminish or cease during voluntary movements but become more pronounced during periods of rest.

(II) **Bradykinesia:** PD often leads to bradykinesia, which refers to a significant slowness in movement. Individuals with Parkinson's may encounter challenges in initiating and executing movements, resulting in a general

deceleration of motor function. Tasks requiring fine motor skills may become particularly difficult.

(III) Akinesia: Akinesia refers to the difficulty in initiating and executing voluntary movements. PD patients may have trouble starting movements or may experience freezing of movement, especially during gait.

Table 1.1 Several stages of Parkinson's disease

PD Stage		Symptoms
Early-Stage	**I**	• Mild symptoms - not interfering with daily activities. • Mild Tremors. • Changes in posture, walking and facial expressions.
	II	• Worsening of Stage 1 symptoms. • Patient is still able to live alone, but daily tasks are quite difficult and lengthy.
Mid-Stage	**III**	• Loss of balance and slowness of movements. • Falls are more common.
Advanced-Stage	**IV**	• Symptoms are severe and limiting. • Difficult independent movement; may require a walker. • Needs help with activities of daily living and is unable to live alone.
	V	• Impossible to stand or walk. • Bedridden patients. • Non-motor symptoms are also involved.

(IV) **Gait abnormalities:** PD can cause changes in gait, leading to a shuffling or festinating (quickening and shortening) gait pattern. Patients may have difficulty with coordination and may exhibit reduced arm swing while walking.

(V) **Rigidity:** Stiffness and resistance in muscles, make it difficult for individuals to initiate and control movements.

(VI) **Postural instability:** Postural instability is frequently observed in the later stages of PD. It involves difficulties with maintaining balance and an upright posture. Individuals with Parkinson's may experience a stooped posture, increased risk of falls, and instability when changing direction or turning.

1.1 Available treatments for PD

The currently no cure for PD, several therapeutic options are available to manage its symptoms and improve the quality of life for individuals with the condition ((Hauser and Zesiewicz 2007, Kalinderi, Fidani et al. 2011, Nadeem, Hosawi et al. 2023). These therapeutic options include:

1.1.1 Medications

1.1.1.1 Levodopa: The most effective medication for managing Parkinson's symptoms. It is converted to dopamine in the brain and helps alleviate motor symptoms**.**

1.1.1.2 Dopamine agonists: Mimic the effects of dopamine in the brain and can be used in combination with levodopa or as standalone therapy.

1.1.1.3 MAO-B inhibitors: Inhibit the breakdown of dopamine in the brain, increasing its availability.

1.1.1.4 COMT inhibitors: Inhibit an enzyme that breaks down levodopa, prolonging its effects.

1.1.1.5 Anticholinergic: Help control tremors and other movement symptoms.

1.1.1.6 Deep Brain Stimulation (DBS): Involves surgically implanting electrodes in specific areas of the brain to deliver electrical impulses. It helps alleviate motor symptoms and can be an option for those who have not responded well to medications.

1.1.1.7 Glutamate Antagonists: Agents that block glutamate receptors, such as amantadine, have been studied for their potential neuroprotective effects. Glutamate is an excitatory neurotransmitter that, in excess, can contribute to neuronal damage.

1.1.1.8 Antioxidants: Compounds with antioxidant properties, such as coenzyme Q10 and creatine, have been studied as potential neuroprotective agents. They help neutralize free radicals and reduce oxidative stress, which can be damaging to neurons.

1.2 Shortcoming and challenges of available treatments for PD

While there have been significant advancements in the treatment of PD, there are still several shortcomings and challenges that exist. These include:

1.2.1 Symptomatic treatments: Current therapies primarily focus on managing the symptoms of PD rather than targeting the underlying cause or slowing down disease progression. While medications like levodopa can provide substantial relief, they do not halt the neurodegenerative process.

1.2.2 Side effects and long-term complications: Many Parkinson's medications can cause side effects such as dyskinesias (involuntary movements), hallucinations, nausea, and sleep disturbances.

Prolonged use of levodopa can lead to motor fluctuations and dyskinesias. Balancing the benefits and side effects of medications can be challenging.

1.2.3 Surgical Interventions: Deep brain stimulation (DBS) is an effective treatment option for some individuals with PD. However, it is an invasive procedure that requires surgical expertise and is not suitable for everyone**.**

1.2.4 Lack of Disease-Modifying Treatments: Despite extensive research, there are currently no disease-modifying therapies available for PD. Neuroprotective agents that can slow or halt disease progression have not yet been successfully developed and validated.

Addressing these shortcomings requires continued research to develop more effective therapies, including neuroprotective agents, disease-modifying treatments, and strategies to manage non-motor symptoms. However, the lack of effective treatment for PD is indeed a significant factor contributing to the burden of the disease globally. The cases of PD are near to doubling after a gap of a few years (1990: 2.5 million; 2016: 6.1 million: 2020: 9.3 million, and by 2040, there will be expecting over 14 million cases worldwide) which puts significant emotional and financial burden on caregivers (Victorino, Guimaraes-Marques et al. 2020, Albarmawi, Zhou et al. 2022, Sadeghian, Eyvari-Brooshghalan et al. 2022). This growing global burden of PD can be reduced by

developing effective treatment strategies that slow the rate of neurodegeneration or stop the disease progression (Kalia, Kalia et al. 2015, Kip and Parr-Brownlie 2022, Chopade, Chopade et al. 2023). As of my knowledge cut-off in September 2022, no neuroprotective therapy has been proven to definitively slow or stop the progression of PD (Soni, Delvadia et al. 2023, Zagórska, Czopek et al. 2023). However, ongoing research continues to explore new approaches and potential treatments for neuroprotection in PD. Overall, addressing the lack of neuroprotective agents requires continued research and development efforts to identify novel therapeutic targets and interventions that can modify the disease course and protect neurons from degeneration. Interestingly, targeting the lysosomal therapy has emerged as a promising and effective approach in current clinical trials for developing disease-modifying therapies in PD (Chatterjee and Krainc 2023).

1.3 Lysosomal theory in PD

The Lysosomal Theory, also known as the "Lysosomal Dysfunction Hypothesis" is a proposed mechanism underlying PD. It suggests that impaired lysosomal function and the accumulation of toxic substances within the lysosomes contribute to the degeneration of dopamine-producing neurons in the brain (Audano, Schneider et al. 2018, Navarro-Romero, Montpeyó et al. 2020, Mächtel, Boros et al. 2022). The theory is based on several lines of evidence:

1.3.1 Alpha-Synuclein accumulation: Research suggests that lysosomes play a role in the degradation and clearance of alpha-synuclein. Dysfunction in lysosomal activity may lead to the accumulation of alpha-synuclein aggregates and subsequent neurodegeneration (Neumann, Bras et al. 2009, Goker-Alpan, Stubblefield et al. 2010, Blanz and Saftig 2016, Takahashi, Bhagwagar et al. 2024).

1.3.2 Genetic mutations: Mutations in genes associated with lysosomal function, such as glucocerebrosidase (GCase) and lysosomal-associated membrane protein 2 (LAMP2), have been linked to an increased risk of developing PD. These mutations can impair lysosomal function and contribute to the accumulation of toxic substances (McNeill 2013, Vieira, Schapira et al. 2021, Domenicale 2024, Rubilar, Outeiro et al. 2024).

1.3.3 Lysosomal enzyme dysfunction: Lysosomes contain enzymes responsible for breaking down various molecules, including proteins, lipids, and sugars. Defects in these lysosomal enzymes, such as glucocerebrosidase, can disrupt normal cellular processes and contribute to the degeneration of neurons (Schapira and neuroscience 2015, Avenali, Blandini et al. 2020, Menozzi and Schapira 2020).

1.3.4 Autophagy and lysosomal clearance: Lysosomes are essential components of the autophagy-lysosomal pathway, responsible for clearing damaged proteins and organelles in cells. Impaired autophagy and lysosomal function can lead to the accumulation of damaged components and the build-up of toxic substances, ultimately contributing to neuronal dysfunction and cell death (Magalhaes, Gegg et al. 2016, Mazzulli, Zunke et al. 2016, Kuo, Tasset et al. 2022, Kuo, Tasset et al. 2022).

These approaches highlight the growing interest in targeting lysosomal dysfunction as a potential disease-modifying therapy for PD. Among all the lysosomal theory, glucocerebrosidase (GCase), as a key enzyme within the lysosomal system, has gained significant attention in clinical trials as a prime therapeutic target for the development of disease-modifying therapies in PD (Audano, Schneider et al. 2018, Navarro-Romero, Montpeyó et al. 2020, Mächtel, Boros et al. 2022).

1.4 Significance of glucocerebrosidase in PD: Unveiling the importance

Glucocerebrosidase (GCase, EC: 3.2.1.45) is a lysosomal acid glycoside hydrolase (GH) that belongs to family 30 and is encoded by the GBA1 gene (Ketudat Cairns, Esen et al. 2010, Mazzulli, Zunke et al. 2016). The GBA1 gene is one of the genes linked to lysosomal storage disorders (LSDs), which account for 56% of instances of PD (Robak, Jansen et al. 2017). GCase is one of many enzymes found in lysosomes that involve in this process (Siebert, Sidransky et al. 2014, Gegg and Schapira 2018). If these enzymes are not functioning properly, the clearance of toxic substances may be impaired, contributing to the accumulation of damaging molecules. In the context of PD, a dysfunctional lysosomal GCase enzyme hinders the removal of abnormal proteins like alpha-synuclein. These proteins with time form aggregates called Lewy bodies, a key feature of PD. These protein aggregates are thought to contribute to neuronal dysfunction and cell death (Manning-Boğ, Schüle et al. 2009, Foltynie and Kahan 2013, Do, McKinney et al. 2019). GCase is involved in the metabolism of its substrate glucocerebroside (GC) (Bo, Li et al. 2022). GCase inhibition resulted in a build-up of GC in SNc, which serves as a scaffold or a surface to facilitate the stabilization of α- synuclein oligomers (Robak, Jansen et al. 2017, Gatto, Da Prat et al. 2019, Munoz, Petersen et al. 2021). In sporadic PD brains, there is a loss of GCase activity along with an increase in α- synuclein (Poewe, Mahlknecht et al. 2009, Gegg, Burke et al. 2012). Reduced GCase

activity has been observed in the brains of individuals with PD and animal models of the disease (Mishra, Chandravanshi et al. 2018, Silveira, MacKinley et al. 2019, Menozzi and Schapira 2020). In GBA cells and animal models, a *vice-versa* relationship between the level of oligomeric α-synuclein and GCase activity has been reported (Richter, Fleming et al. 2014, Migdalska-Richards, Daly et al. 2016, Stojkovska, Krainc et al. 2018). A decrease in about half of the GCase activity has been analysed in the substantia nigra (SN) of PD patients who carried mutant or non-mutant GBA (Gegg, Burke et al. 2012, Mullin, Smith et al. 2020). GCase activity in the cerebellum and putamen of sporadic PD brains was also found to be decreased (Gegg, Burke et al. 2012). Even in healthy older brains, decreased GCase activity by 30-50 % makes individuals more susceptible to PD (Rocha, Smith et al. 2015). Literature evidence has also supported reduced GBA protein expression found in non-mutant PD brains (Gegg, Burke et al. 2012, Berger, Perkins et al. 2015, Chiasserini, Paciotti et al. 2015). The precise mechanism by which GCase is involved in the pathogenesis of PD remains largely unexplored (Mullin, Smith et al. 2020). However, in studies conducted on GBA mutant human cells and fly model lines, an increase in ER–stress markers has been observed (Menozzi and Schapira 2020). This suggests a potential link between GCase dysfunction and ER – stress in PD, although further research is needed to fully understand the underlying mechanisms.

1.5 Significance of alpha-synuclein in the lysosomal theory of PD

The lysosomal theory of Parkinson's disease (PD) proposes that dysfunction in lysosomal pathways contributes to the development and progression of PD. Lysosomes are cellular organelles responsible for degrading and recycling cellular waste, including alpha-synuclein proteins. Dysfunction in lysosomal pathways can impair the clearance of alpha-synuclein aggregates, leading to their accumulation within neuron (Navarro-Romero, Montpeyó et al. 2020, Mächtel, Boros et al. 2023). Alpha-synuclein, a protein abundant

in the brain, plays a crucial role in this theory. In PD, alpha-synuclein tends to misfold and aggregate into insoluble structures called Lewy bodies. These Lewy bodies are a hallmark pathological feature of PD. GCase is one of lysosomal enzymes found in lysosomes that involve in this process (Siebert, Sidransky et al. 2014, Gegg and Schapira 2018). If these enzymesare not functioning properly, the clearance of toxic substances may be impaired,contributing to the accumulation of damaging molecules. Emerging evidence suggests that mutations in *GBA1* are the most common genetic risk factor for synucleinopathies, such as PD and dementia with Lewy bodies (DLB) (Granek, Barczuk et al. 2023). In cell and animal studies, decreased wild-type glucocerebrosidase leads to α-synuclein accumulation, and increased α-synuclein inhibits normal glucocerebrosidase function (Manning-Boğ, Schüle et al. 2009, Chiasserini, Paciotti et al. 2015). In post-mortem brain tissue from patients with PD and *GBA1* mutations, glucocerebrosidase and α-synuclein colocalize in Lewy bodies (Goker-Alpan, Lopez et al. 2008) and aggregated forms of α-synuclein are present (Choi, Kim et al. 2018). Recent reports show reduced glucocerebrosidase protein levels and enzyme activity in a range of affected brain regions in patients with PD (Gegg, Burke et al. 2012). Immunofluorescence studies on brain tissue samples from patients with parkinsonism associated with glucocerebrosidase mutations showed that glucocerebrosidase was present in 32–90% of Lewy bodies (Goker-Alpan, Stubblefield et al. 2010). Moreover, GCase deficit PD patients were even more frequently affected by cognitive deficits whose onset and presence was correlated with the severity of GBA1 mutations (Gan-Or et al. 2009; Neumann et al. 2009). This data demonstrates that glucocerebrosidase can be an important component of α-synuclein-positive pathological inclusions.

1.6 Shortcoming challenges of lysosomal therapy in PD

1.6.1 Limited Blood-Brain Barrier (BBB) Penetration: One major challenge is delivering therapeutic agents across the blood-brain barrier to reach the affected neurons in the brain. Ambroxol is one of such lysosomal agent. The disease-modifying therapy for PD with dementia is currently being developed with GCasechaperone (Ambroxol, AMB) (ClinicalTrials.gov Identifier, NCT02914366) (Silveira, MacKinley et al. 2019). However, all these effects were only seen at higher doses of AMB (800-1000 mg/kg *p.o*) due to issues with BBB.

1.6.2 Limitation of lysosomal Enzyme replacement therapy (ERT): ERT for glucocerebrosidase (GCase) deficiency in Parkinson's disease (PD) has shown promise, but it also comes with its own set of challenges and limitations:

I. ERT typically requires regular and long-term administration of the enzyme to maintain therapeutic effects. This necessitates frequent infusions or injections, which may not be convenient for all patients and can increase the risk of treatment non-compliance.

II. Some patients may develop immune responses against the exogenous enzyme, leading to reduced efficacy over time or even adverse reactions such as allergic responses or infusion reactions. The blood-brain barrier restricts the entry of large molecules like enzymes into the brain. Therefore, delivering sufficient amounts of the enzyme to the affected areas of the brain poses a significant challenge.

III. ERT can be expensive, potentially limiting access for patients, particularly in regions with limited healthcare resources or inadequate insurance coverage.

IV. Response to ERT can vary among individuals. Some patients may experience significant improvements in symptoms, while others may see only modest benefits or no improvement at all.

PD is a heterogeneous disorder with variability in symptoms, progression, and response to treatment among individuals. Tailoring lysosomal therapy to the specific needs of each patient presents a challenge.

1.7 Significance of targeting endoplasmic reticulum and GCase in PD

ER – stress plays a significant role in PD by causing an abnormal build-up of damaged or misfolded proteins, including GCase (Wang, Takahashi et al. 2007, Li, Liu et al. 2020, Wang, Dou et al. 2020). The close association between GCase and ER – stress may be from the fact that GCase is synthesized and folded in ER-associated polyribosomes (Bendikov-Bar, Maor et al. 2013). After undergoing proper folding, the GCase protein is transported to the lysosome to perform its designated function. However, dysfunctional or unstable GCase molecules that cannot fold correctly can accumulate in the ER, leading to decreased levels of functional GCase in the lysosome (Schapira and Gegg 2013, Nakagome, Kato et al. 2018). To counteract this protein-folding imbalance, the unfolded protein response (UPR) is activated as a cellular stress response mechanism triggered by ER – stress (Motawi, Al-Kady et al. 2022). The UPR aims to enhance the capacity for protein folding and alleviate the burden of non-functional proteins in the ER (Gaballah, Zakaria et al. 2016, Kang, Piao et al. 2022). Since PD is a chronic disorder, ER stress is constantly compromised. As a result, apoptotic pathways linked to ER – stress, such as CHOP/GADD153 and caspases 12, are activated (Hitomi, Katayama et al. 2004, Gaballah, Zakaria et al. 2016, Zhou, Wu et al. 2017). These pathways have been implicated in the onset and progression of PD. The initial detection of ER stress-induced apoptosis is mediated by GRP78/BiP, a glucose-regulated protein. Once activated, the neuronal apoptotic pathway is facilitated by both CHOP and caspases -12 [(Cai, Ye et al. 2016, Zhou, Wu et al. 2017, Ning, Zhang et al. 2019). Studies have reported dopaminergic cell death in PD through the ER – stress-induced activation of CHOP *via* the

GRP78/PERK pathway (Silva, Ries et al. 2005, Shrivastava, Vaibhav et al. 2013). Additionally, caspase-12, an ER-specific apoptotic protein, directly activates caspase-3 and caspase-9 in the cytosol (Hitomi, Katayama et al. 2004, Gaballah, Zakaria et al. 2016. Elevated levels of these caspases, along with CHOP, have been observed in the preclinical PD model (Hitomi, Katayama et al. 2004, Wang, Dou et al. 2020). Animal models of PD with increased levels of α-synuclein oligomers also exhibit overexpression of these caspases, highlighting the potential role of ER – stress in PD development [(Guardia-Laguarta, Area-Gomez et al. 2015, Wang, Dou et al. 2020). Recent studies have suggested a link between GCase deficiency and ER – stress in PD (Migdalska-Richards and Schapira 2016). Unstable GCase is more prone to misfolding and aggregation. This accumulation of misfolded GCase in the ER can trigger ER – stress and activate the unfolded protein response (UPR), which is a cellular pathway that aims to restore ER homeostasis (Costa, Manaa et al. 2020) . ER–stress markers, such as GRP78/BiP and CHOP, are increased in the brains of individuals with PD, particularly in brain regions affected by the disease (Baek, Mamula et al. 2019, Ning, Zhang et al. 2019). These markers indicate the presence of ER – stress and activation of the UPR. Furthermore, studies have shown that the accumulation of misfolded GCase in the ER can impair ER-associated degradation (ERAD), a process responsible for eliminating misfolded proteins from the ER (Ron, Rapaport et al. 2010, Westbroek, Gustafson et al. 2011, Kuo, Tasset et al. 2022). Moreover, GCase deficit *drosophila* resulted in increased levels of the ER–stress markers including other PD markers such as dopaminergic neuronal loss and locomotor defect (Sanchez-Martinez, Beavan et al. 2016). The molecular mechanisms by which GBA deficiency increases the risk of PD due to ER – stress are still largely unknown; however, inhibiting the ER – stress by stabilizing or enhancing GCase activity may be a crucially effective strategy for PD treatment.

1.8 Chaperone-based treatment strategies in PD: Importance and implications

Chaperones, which are small molecules, have gained attention in clinical trials for PD due to their potential to stabilize GCase and restore its activity (Do, McKinney et al. 2019, Mullin, Smith et al. 2020, Kuo, Tasset et al. 2022, Menozzi, Toffoli et al. 2023). **Figure 1** explained the binding mechanism of chaperones with GCase. The pH-dependent binding and stabilizing properties of chaperones offer a mechanism to enhance the function of GCase and restore its activity within the lysosomes (Nakagome, Kato et al. 2018, Menozzi, Toffoli et al. 2023). By facilitating the correct folding and trafficking of GCase, chaperones have the potential to address the deficiency in enzyme activity observed in PD patients (Bendikov-Bar, Maor et al. 2013). Studies have shown that AMB can increase GCase activity by acting as a chaperone molecule that helps stabilize the enzyme and facilitate its proper folding and trafficking (Jung, Patnaik et al. 2016, Kopytova, Rychkov et al. 2021). The disease-modifying therapy for PD with dementia is currently being developed with GCase chaperone (Ambroxol, AMB) (ClinicalTrials.gov Identifier, NCT02914366) (Silveira, MacKinley et al. 2019, Chwiszczuk, Breitve et al. 2023). Many reports support that chaperone increases wild-type GCase activity and decreases the levels of α-synuclein aggregation in preclinical and clinical PD models (Mazzulli, Zunke et al. 2016, Migdalska-Richards, Daly et al. 2016, Mishra, Chandravanshi et al. 2018). In cell culture models, AMB has been shown to increase wild-type GCase activity (Bendikov-Bar, Maor et al. 2013, McNeill, Magalhaes et al. 2014, Magalhaes, Gegg et al. 2018). Studies have shown that AMB treatment leads to increased GCase activity in the mouse brain. In a preclinical PD model, overexpressing human α-synuclein showed decreased GCase activity levels (Tayebi, Parisiadou et al. 2017, Nikolaev, Kopytova et al. 2022). Additionally, AMB therapy in the preclinical PD model overexpressing α-synuclein led to lower levels of α-synuclein protein (Migdalska-

Richards, Daly et al. 2016, Kuo, Tasset et al. 2022). These findings, along with the decreased GCase activity observed in aged and sporadic PD brains, suggest that AMB could be a potential disease-modifying therapy for PD (Mullin, Smith et al. 2020) . Studies have shown that AMB treatment leads to increased GCase activity in the mouse brain. In a preclinical PD model, overexpressing human α-synuclein showed decreased GCase activity levels (Tayebi, Parisiadou et al. 2017, Nikolaev, Kopytova et al. 2022). Additionally, AMB therapy in the preclinical PD model overexpressing α-synuclein led to lower levels of α-synuclein protein (Migdalska-Richards, Daly et al. 2016, Kuo, Tasset et al. 2022).

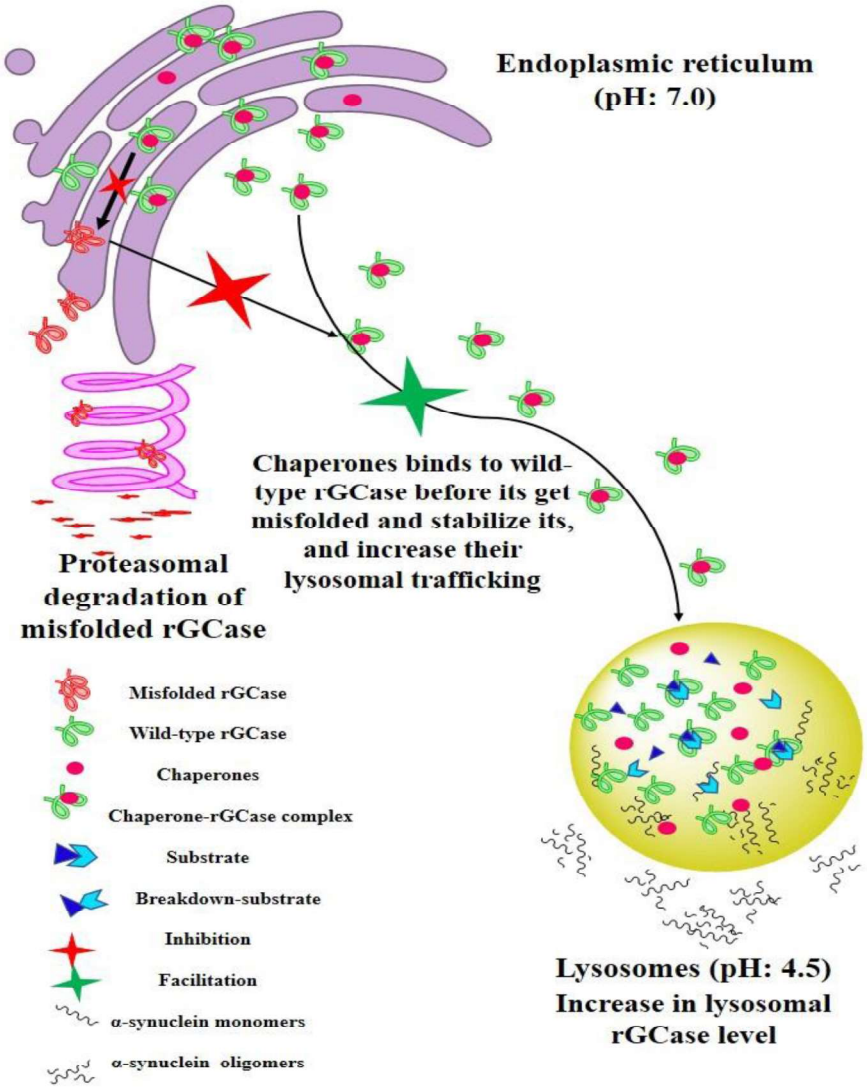

Figure 1.1 Therapeutic strategies to enhance glucocerebrosidase. In healthy cells, wild-type glucocerebrosidase is sorted to the lysosome via the endoplasmic reticulum, where glucocerebrosidase interacts with its substrate glucocerebroside (GC) as well as monomers of α-synuclein, facilitating the breakdown of both at acidic pH. Non-functional glucocerebrosidase is stuck in the endoplasmic reticulum, and undergoes proteasomal degradation, resulting decrease in lysosomal GCase activity. Decreased levels of Glucocerebrosidase will result in a slowdown of α-synuclein degradation and a gradual build-up of glucocerebroside substrate, with the eventual formation of a-synuclein oligomers and fibrils. Chaperones can bind to both wild-type and misfolded forms of GCase, helping to stabilize or refold the enzyme in the endoplasmic reticulum (ER). By doing so, they facilitate the transport of GCase to lysosomes, where it can function properly and increase its enzymatic activity.

These findings, along with the decreased GCase activity observed in aged and sporadic PD brains, suggest that AMB could be a potential disease-modifying therapy for PD (Mullin, Smith et al. 2020) . Notably, studies in our lab on the sub-chronic administration of chaperones have shown a disease-modifying effect by increasing GCase activity in the rat-PD model (Mishra and Krishnamurthy 2020). Another study of our lab in a wild-type rat model of PD demonstrated that the reduction in GCase activity induced by the neurotoxin 6-Hydroxydopamine hydrochloride (6-OHDA) is enhanced by a chaperone (Mishra, Chandravanshi et al. 2018). Chaperone AMB's neuroprotective effects have already been documented (Bhardwaj, Arunachalam et al. 2016). However, these effects were observed only at higher doses of AMB (800-1000 mg/kg *p.o*) due to limitations in blood-brain barrier penetration. Currently, there are no approved drugs available for neuroprotection or neurorestoration in PD patients with GCase deficiency, despite the potential role of GCase in PD pathogenesis. This highlights the need to discover novel GCase chaperones with disease-modifying potential for PD treatment.

While GBA1 mutations in the GCase encoding gene are a significant risk factor for early-onset PD, they only account for 5-10% of PD cases (Beavan and Schapira 2013, Yang, Wu et al. 2023). Reduced GCase activity is also found in sporadic PD cases, which comprise the majority (90-95%) of PD cases (Braak, Müller et al. 2006). Thus, wild-type GCase deficiency can be emerging as a serious risk factor in sporadic PD (Gegg and Schapira 2018). The wild-type rat model is widely used in the initial stages of drug discovery for PD (Blandini, Armentero et al. 2008, Wu, Hung et al. 2023), and chaperones are being developed as potential anti-PD drugs. However, there is currently a lack of *in silico* and *in vitro* screening of chaperones on rat GCase (rGCase) due to the unavailability of commercial rGCase and its 3D structure. Moreover, the process of selecting suitable chaperones with more protein stabilising potential through virtual screening during drug

discovery is still unexplored. This limitation may be a reason for the absence of disease-modifying chaperones in preclinical and later clinical trials. To address these gaps, there is a need for further research and exploration in the selection and evaluation of chaperones with protein stabilizing potential, as well as understanding their precise mechanisms in PD with GCase deficiency using preclinical PD models. Hence, our specific objectives are to develop *in silico* screening techniques, investigate rGCase-chaperone interactions, assess structure stability, and elucidate their molecular mechanism in an *in vivo* PD model. Therefore, we have developed the following objective to address these gaps:

Objective 1: Exploring wild-type rat glucocerebrosidase: Insights into chaperone interactions and mechanism through *in silico* and *in vitro* studies.

Objective 2: To identify the novel GCase chaperones for PD treatment: *In silico* screening and *in vitro* investigation.

Objective 3: Oral acute toxicity study of a selected chaperone in rats.

Objective 4: Neuroprotective effect and mechanism evaluation of a top-screened chaperone in 6-OHDA-induced rat PD Model.

Objective 5: Development and validation of HPLC method for quantification of GCase chaperone in rat plasma and brain.