Base and translation of β-glucocerebrosidase and its transporter LIMP-2 in neuropathies

Philipp Arnold*, Friederike Zunke*

The lysosomal enzyme β-glucocerebrosidase (GCase) belongs to the family of glycosidases and hydrolyses the glycosphingolipid glucosylceramide (GluCer) into glucose and ceramide. The enzyme is of central importance for two pathologies: (1) the lysosomal storage disorder Gaucher's disease (GD) and (2) the neurodegenerative disorder Parkinson's disease (PD). GCase is encoded by the gene *GBA1* and mutations within *GBA1* are the monogenetic cause for GD. This rare lysosomal storage disorder (overall global incidence 0.45–25.0 per 100,000 with high geographical and ethnical variations being more common in the Eastern world and among the Ashkenazi Jewish community with an estimated frequency of 1 in 1000) presents with high heterogeneity (Goker-Alpan and Ivanova, 2024). Due to the accumulation of GluCer, the substrate of GCase, common manifestations include enlarged liver and/or spleen (hepato-/splenomegaly), anemia, thrombocytopenia as well as skeletal malformations. GD is classified in three types, which are distinguished by the presence and extent of neurological symptoms, including seizures, cognitive impairment, and spasticity (type I: non-neuronopathic; type II: acute neuronopathic; type III: chronic neuronopathic) (Beutler, 2001). Most GD cases (~90%) are related to type I caused by mild *GBA1* variants (e.g., N370S). Severe variants (e.g., L444P) are more likely to lead to GD type II or III. All forms of GD are inherited in an autosomal recessive manner. The gold standard for treatment of GD type I is enzyme replacement therapy by regular intravenous administration of recombinant GCase (e.g., Imiglucerase) to overcome peripheral symptoms, such as organomegaly.

Mutations within *GBA1* exhibit one of the highest genetic risk factors to develop PD and different studies estimate (depending on ethnicity) that 3%–25% of PD patients carry *GBA1* mutations (Hertz et al., 2024). The presence of heterozygous *GBA1* variants, whether mild or severe, might differentially impact the risk, age of onset, and disease course of PD. Interestingly, the *GBA1* variant E326K is only described in PD, but absent in GD patients. The molecular mechanisms of *GBA1* variants resulting in dysfunctional GCase and PD pathology are not well understood to date. However, it has been shown that the disease-causing protein α-synuclein strongly interacts with various classes of lipids, which modify its native structure, favoring the formation of pathology-associated conformers and subsequent aggregation of the protein. In cell models with lysosomal GCase dysfunction and GluCer accumulation, changes in the structure and stability of oligomeric α-synuclein conformers were present, resulting in neurotoxic aggregation of the protein (Zunke et al., 2018). Importantly, α-synuclein aggregation is roughly 17 times faster at lysosomal pH, when compared to cytosolic pH (Eymsh et al., 2020). Within the lysosome, α-synuclein is proteolytically degraded by different proteases under physiological as well as pathological conditions. Thus, α-synuclein is exposed to various factors triggering its aggregation such as low pH, GluCer, and crowding within the lysosome. Conclusively, efficient lysosomal function (including GCase activity) is pivotal to obtain healthy proteostasis preventing protein/α-synuclein aggregation. Hence, activating GCase enzymatic function within the lysosome is a strategic goal in many pre-clinical and clinical studies focusing on GD and PD.

Boosting lysosomal GCase activity can be obtained in two ways: (1) enhancement of its lysosomal trafficking and (2) direct activation of the enzyme within the lysosome. Importantly, strategies for functional GCase recovery depend on the genetic *GBA1* variant, hence the site of amino acid exchange within the GCase protein. Interestingly, GCase belongs to the few enzymes that are transported to the lysosome in a mannose-6-phosphate-independent manner. It solely relies on the lysosomal integral membrane protein type 2 (LIMP-2) as a transport protein (Reczek et al., 2007). LIMP-2 is a type III transmembrane glycoprotein encoded by the *SCARB2* gene and has recently gained more attention for its important role in proper GCase sorting as well as a modifier and risk gene for GD and PD, respectively. Further, mutations within *SCARB2* are causative for action myoclonus-renal failure syndrome, presenting with progressive myoclonic epilepsy and severe renal dysfunction. Besides its role as a GCase receptor, trafficking the enzyme from the endoplasmic reticulum (ER) to the lysosome, it has been described to be involved in lipid trafficking and as receptor for enterovirus 71 and coxsackievirus A16 at the cell surface causing hand foot mouth disease (Yamayoshi et al., 2009; Heybrock et al., 2019).

Surprisingly, little is known about the molecular and structural interaction modalities of GCase and LIMP-2, although individual crystal structures and hence structural information of both individual proteins are available for years (Liou et al., 2006; Neculai et al., 2013) (see overview **Figure 1**). LIMP-2 and GCase first interact in the ER, potentially co-maturating and co-folding, as a lysosome-targeted protein complex. After further maturation (including glycosylation) and joint trafficking through the Golgi apparatus, diffusion of the enzyme from its receptor within the late endosome/lysosome has been described. A pH-dependent diffusion mechanism was suggested. However, molecular properties of this dissociation process as well as the organization and structure of the lysosomal GCase/LIMP-2 complex (with and without other lysosomal components) are still lacking to date. A better understanding of the overall GCase/LIMP-2 protein structure and the interaction interface in particular is of importance for two main reasons: First, it provides a fundamental understanding of mannose 6-phosphate receptor-independent lysosomal transport mechanisms. Second, the function of LIMP-2 as an enhancer for GCase enzymatic function as shown previously could be exploited for GCase-activating strategies (Zunke et al., 2016; Dobert et al., 2024b).

In a recent study, we solved the structure of the GCase/LIMP-2 protein complex to 3.7Å resolution utilizing cryo-electron microscopy (Dobert et al., 2024a). We can now allocate the interaction interface to residues from helices 1 and 3 and loops A–D from GCase and helices 5 and 7 from LIMP-2. The core of the interaction interface is formed by hydrophobic residues that are shielded by a rim of hydrophilic amino acids. Additionally, two salt-bridges form between GCase and LIMP-2 that could stabilize the complex. Mutation of one of the salt-bridge forming residues was reported in a GD type III patient, arguing for the importance of this residue for proper GCase transport to the lysosome.

In previous studies, we could show that a LIMP-2-derived helix 5 peptide is sufficient to increase GCase enzymatic function (Zunke et al., 2016; Dobert et al., 2024b). With the molecular details on the interaction interface at hand, we can now capitalize on this knowledge and design activating peptides or small molecules that mimic the interaction interface. Importantly, we would like to point out that these compounds have to be delivered directly into the lysosome to not interfere with lysosomal GCase transport. To test such compounds, cell systems of different complexity can be applied. We introduced a HEK293T-based cell system, which enables to discriminate between transport-deficient and activity-diminished GCase variants. In this system, we could show that the PD-associated variant GCase E326K can be targeted to the lysosome by LIMP-2 and exerts enzymatic function there. Furthermore, GCase L444P resides in the ER and cannot be rescued by LIMP-2. Genetic engineering of LMIP-2 (depletion of transmembrane helices and exchange of the signal peptide) yielded a LIMP-2 variant that binds GCase in the ER and

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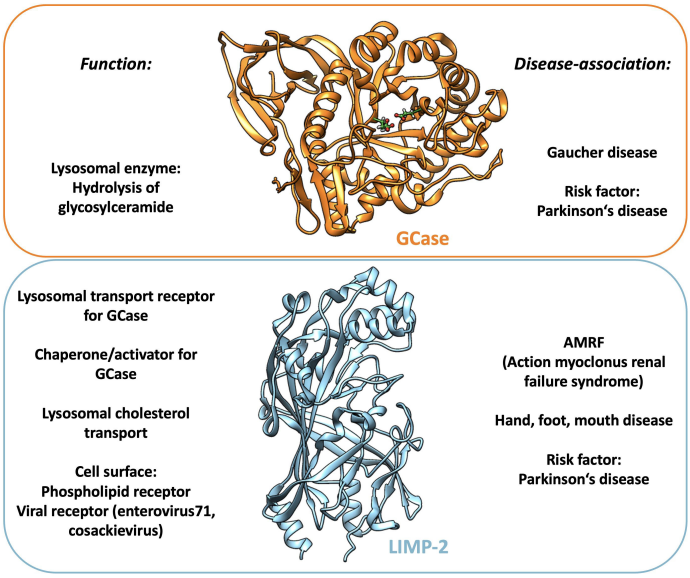

Figure 1 | Overview of structure, function, and disease-association of the lysosomal proteins β-glucocerebrosidase (GCase; PDB: 6TN1) and the lysosomal integral membrane protein type-2 (LIMP-2; PDB: 4Q4F).
Together they form a trafficking complex from the endoplasmic reticulum via the Golgi to the lysosomal compartment where GCase exhibits its enzymatic activity. Dysfunction of both proteins is associated with monogenetic disorders such as the lysosomal storage disorder Gaucher's disease and action myoclonus-renal failure syndrome (AMRF), but they are also described as risk genes for Parkinson's disease. Visualization of PDB files (6TN1, 4Q4F) using UCSF Chimera1.16 (https://www.cgl.ucsf.edu/chimera).

shuttles it into the cell culture supernatant (Dobert et al., 2024b). We could show that this shuttle enables to test and precipitate untagged PD-associated GCase variants via LIMP-2, which is an important advantage over directly affinity-tag labeled GCase. We showed that the addition of an affinity-tag (His-tag or STREP-tag) reduces the enzymatic activity of wt GCase significantly when compared to a non-tagged variant (Dobert et al., 2024b). All PD-associated GCase variants show reduced enzymatic activity in our system. Addition of an affinity-tag to such GCase variants could lead to additive effects and the sole influence of the underlying variant might be impossible to determine.

We are convinced that the recent progress in cellular and structural understanding of GCase biology will open ample opportunities for fundamental and translational research efforts in the upcoming years. Advancing GCase activators based on the GCase/LIMP-2 structure will open new avenues that could ultimately benefit PD patients and lead to a first disease modifying treatment option. Further efforts should focus on defining the state of GCase and LIMP-2 in the lysosome together with all physiological and naturally occurring activators. These studies should also include disease-associated GCase variants, which will further enhance our knowledge of GCase regulation and activity that might help in identifying novel disease-modifying treatment strategies for GD as well as PD. We feel that there are many exciting findings ahead of us with regard to GCase and its role in (patho-)physiological processes.

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Philipp Arnold*, Friederike Zunke*
Institute of Functional and Clinical Anatomy, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany (Arnold P)
Department of Molecular Neurology, Uniklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany (Zunke F)
***Correspondence to:** Friederike Zunke, PhD, friederike.zunke@fau.de; Philipp Arnold, PhD, philipp.arnold@fau.de.
https://orcid.org/0000-0002-0408-6388 (Friederike Zunke)
https://orcid.org/0000-0003-3273-9865 (Philipp Arnold)
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