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Review

Insights into *GBA* Parkinson's disease pathology and therapy with induced pluripotent stem cell model systems

Pascale Baden[a,b], Cong Yu[a,b], Michela Deleidi[a,b,*]

[a] German Center for Neurodegenerative Diseases (DZNE), 72076 Tübingen, Germany
[b] Center of Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany

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ABSTRACT

While the link between *GBA* and Parkinson's disease (PD) was initially unexpected, it is now well established that *GBA* mutations are the most frequent genetic risk for PD. *GBA* has also been linked to sporadic PD, dementia with Lewy bodies, and ageing. Thus, *GBA* represents a promising target to counteract brain disease and the age-related decline of lysosomal function. The exact mechanisms involved in the risk of developing PD in *GBA* mutation carriers are still unclear and research in this field has faced the major challenge of a lack of proper modeling systems. Induced pluripotent stem cells (iPSCs) as well as advances in disease modeling and genome editing have facilitated studies of human brain disease. With regard to *GBA*-PD, iPSCs offer several advantages including the possibility of investigating sphingolipid (SPL) biology in relevant cells, the role of dopamine metabolism as well as non-cell autonomous mechanisms that are likely involved in the disease process. This review will summarize findings that emerged from iPSC-based studies in the context of *GBA*-PD pathology and therapy. We also highlight current advantages and challenges of stem cell models for neurological disease modeling and drug discovery.

1. Introduction

The discovery of a link between a rare inherited metabolic disorder such as Gaucher's disease (GD) and Parkinson's disease (PD), a common ageing brain disorder, was initially unexpected, in part because of the lack of obvious common symptoms between these diseases. GD is the most prevalent lysosomal storage disorder (LSD) caused by biallelic mutations in the *GBA* gene, which encodes β-glucocerebrosidase (GCase), a lysosomal enzyme that catalyzes the hydrolysis of glucosylceramide (GlcCer). In GD, the decreased activity of GCase causes substrate accumulation in several organs including the brain, leading to a chronic multi-organ disease (Nilsson et al., 1985). The clinical manifestations are extremely variable and have been classified into three different forms, based on the presence of neuronopathic symptoms and disease severity. GD types 2 and 3 are associated with neurological symptoms with different degrees of severity, often leading to death at a young age. The predominant form, GD type 1, has been historically defined as non-neuronopathic. However, this classification has been challenged by the clinical observation that these patients often develop parkinsonism (Neudorfer et al., 1996). Pathological evaluation of brains from GD patients with parkinsonism confirmed PD like features with Lewy body (LB) pathology (Goker-Alpan et al., 2010; Tayebi et al., 2003; Wong et al., 2004). Later on, multicenter studies identified *GBA* mutations as the most common genetic risk factor for PD (Sidransky et al., 2009). Although the clinical phenotype of *GBA*-PD is generally indistinguishable from idiopathic PD, motor progression and cognitive decline are faster and non-motor symptoms are more frequent in *GBA*-PD patients compared to idiopathic PD (Brockmann et al., 2011; Cilia et al., 2016; Gan-Or et al., 2015). Interestingly, studies conducted in GD patients and *GBA* heterozygotes suggest that the age of PD onset in mutation carriers is dose dependent (Alcalay et al., 2014). A decrease of GCase function has also been observed in sporadic PD patients as well as in aged individuals (Gegg et al., 2012; Hallett et al., 2018; Rocha et al., 2015). These findings strengthen the relevance of lysosomal genetic risk and the age-dependent impairment of lysosomal function (Cuervo, 2008) in PD aetiology. Interestingly, *GBA* mutations also lead to an increased risk for dementia with Lewy bodies (DLB) (Goker-Alpan et al., 2006; Nalls et al., 2013; Tsuang et al., 2012). These observations make *GBA* a very promising target to counteract the age-dependent decline of lysosomal function and neurodegenerative processes. Importantly, PD patients harboring *GBA* mutations represent an etiologically homogeneous cohort, therefore providing the ideal patient

* Corresponding author: German Center for Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tübingen, Otfried-Müller-Str. 23, 72076 Tübingen, Germany.
E-mail address: michela.deleidi@dzne.de (M. Deleidi).

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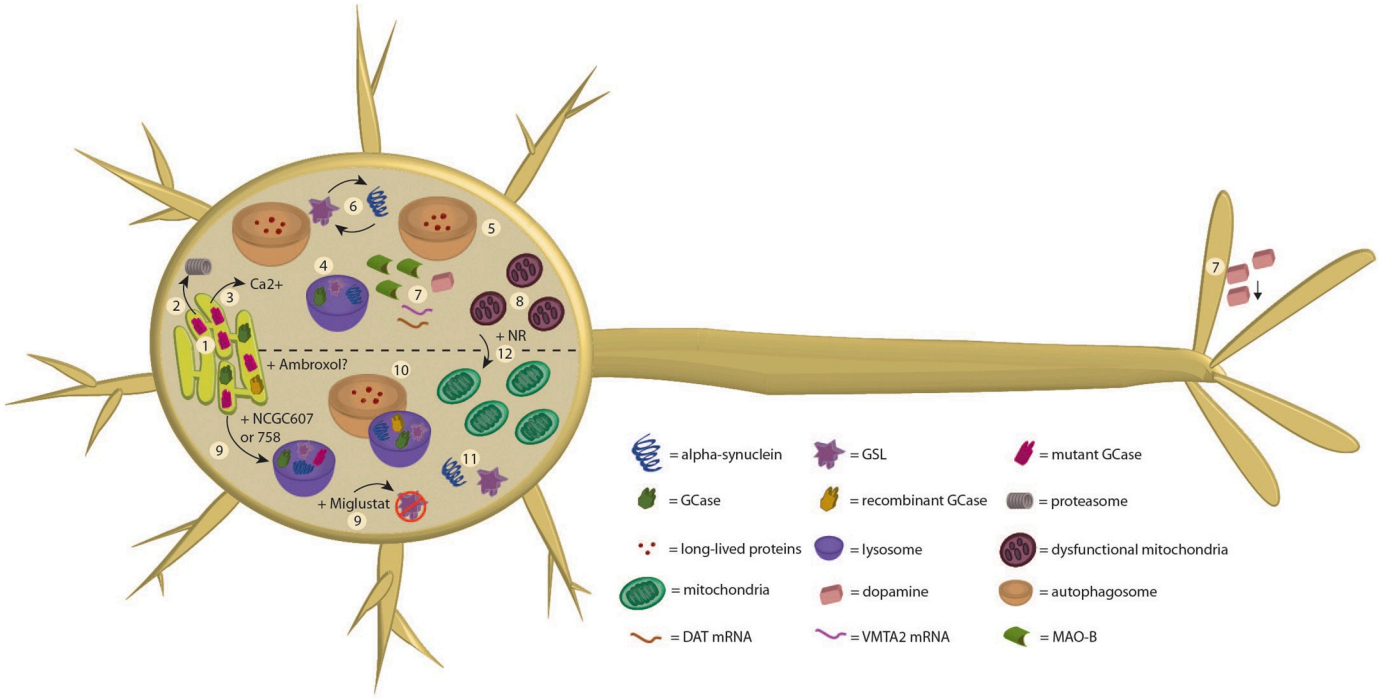

Fig. 1. Phenotypes observed in iPSC-derived neurons from Gaucher disease and *GBA*-related Parkinson's disease patients and therapeutic effects of different treatments. Misfolded mutant β-glucocerebrosidase (GCase) accumulates in the endoplasmic reticulum (ER) (1) and leads to ER stress, ER associated degradation (2) and calcium dyshomeostasis (3). Decreased GCase trafficking to the lysosome results in reduced GCase activity, lysosomal dysfunction (4) and accumulation of autophagosomes (5). GCase deficiency leads to glycosphingolipid (GSL) accumulation, which causes changes in α-synuclein conformation and aggregation. This in turn leads to reduced GCase trafficking and GSL-induced neurotoxicity (6). Dopamine levels and mRNA levels of dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) are decreased and levels of monoamine oxidase B (MAO-B) are elevated (7). Furthermore, GCase deficiency leads to mitochondrial morphology changes and dysfunction (8). Treatment with the small non-inhibitory molecules, NCGC607 and 758, or Miglustat, a glucosylceramide synthase inhibitor, improves GCase maturation and activity (9), promotes the fusion of autophagosomes with lysosomes (10), and decreases GSL accumulation as well as α-synuclein related pathology (11). Nicotinamide riboside (NR) rescues the mitochondrial morphology and function (12). The effect of Ambroxol is not yet investigated in iPSC-derived neurons.

population for precision medicine interventions. The exact mechanisms involved in developing GD, GD with parkinsonism, and PD with heterozygous *GBA* mutations are not completely understood. Both loss and gain of function mechanisms may contribute to disease. According to the loss-of-function hypothesis, GCase deficiency causes substrate accumulation that alters lysosomal function and promotes α-synuclein aggregation (Jo et al., 2000; Mazzulli et al., 2011; Velayati et al., 2010). According to the gain-of-function hypothesis, *GBA* mutations interfere with the folding process in the endoplasmic reticulum (ER), leading to ER-associated degradation, ER stress, and activation of the unfolded protein response (UPR) (Fernandes et al., 2016; Maor et al., 2013; Schondorf et al., 2018). Several *GBA*-related pathways have been described and linked to *GBA*-PD, including lysosomal dysfunction, α-synuclein related mechanisms, sphingolipid (SPL) dyshomeostasis, defects in autophagy and protein trafficking (Fig. 1). Moreover, non-cell autonomous mechanisms, namely immune pathways, may also contribute to disease onset and progression in these patients (Fig. 2). Research in the fields of GD and PD has faced the challenge of the lack of adequate model systems. In the last few years, induced pluripotent stem cells (iPSCs) and genome editing technologies have provided novel and relevant means for studying human disease mechanisms and tracking early disease-related molecular and cellular events. This is especially relevant for the investigation of human brain diseases, whose study has been hampered by the difficulty in having access to affected tissues. While *GBA*-related mechanisms have already been extensively reviewed in previous articles (Aflaki et al., 2017; Gegg and Schapira, 2018), here we will summarize findings that emerged from iPSC-based studies in the context of GD and *GBA*-PD pathology and therapy. We also highlight current advantages and challenges of stem cell models for neurological disease modeling and drug discovery.

2. Modeling *GBA*-PD: in vivo and in vitro experimental models

One of the major challenges facing research on neurological diseases has been the difficulty in having direct access to primary tissues and their limited growth *in vitro*. Thus, transgenic mice, primary neuronal cultures, and immortalized cell lines have long been used to study brain diseases. Similar to what has been observed in a variety of genetic PD mouse models, GCase deficient mice have failed to show selective nigrostriatal degeneration and PD related behavioral phenotype (Enquist et al., 2007). Immortalized cell lines and patient fibroblasts are valuable models for investigating *GBA* related mechanisms. However, immortalized cell lines often carry artifacts, whereas fibroblasts are not affected by the disease process and do not display substrate accumulation (Sillence et al., 2002). Even though neuroblastoma cell lines have been commonly used in LSD research, the SPL pattern of these cell lines differs from the pattern of differentiated neurons, limiting their use as a model system for the study of disturbed SPL metabolism (van Echten-Deckert and Herget, 2006). In the last few years, iPSC disease modeling and genome engineering have allowed the generation of relevant cells for human brain disease modeling. Patient-specific functional midbrain dopaminergic (mDA) neurons have been successfully generated from both sporadic and familial PD subjects (Cooper et al., 2012; Devine et al., 2011; Hargus et al., 2010; Jiang et al., 2012; Park et al., 2008; Reinhardt et al., 2013; Seibler et al., 2011). As discussed below, iPSC-derived neuronal modeling systems enable the analyses of key disease mechanisms, including lipid biology, DA homeostasis, and energy metabolism. Importantly, these aspects can be investigated within the

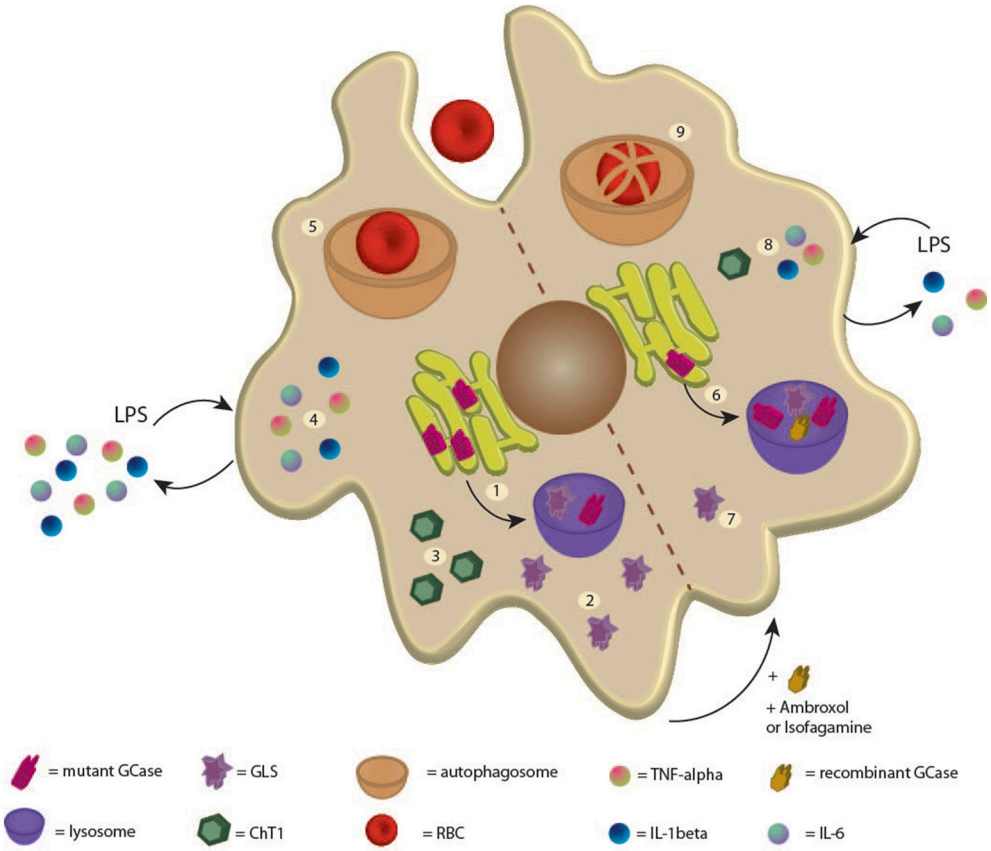

Fig. 2. Phenotypes observed in iPSC-derived macrophages from Gaucher disease (GD) patients and therapeutic effects of different treatments. Decreased glucocerebrosidase (GCase) trafficking to the lysosome and decreased enzymatic activity (1) result in glycosphingolipid (GLS) accumulation (2). Production of chitotriosidase (ChT1) is increased (3) and lipopolysaccharide (LPS) treatment leads to higher production of pro-inflammatory cytokines (4). Delayed red blood cell (RBC) clearance is observed in GD iPSC-macrophages (5). Treatment with recombinant GCase, Ambroxol or Isofagomine leads to increased GCase levels and improved trafficking to the lysosome (6), reduced substrate accumulation (7), decreased production of ChT1 and less pro-inflammatory cytokines upon LPS treatment (8), as well as improved RBC clearance (9). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

patient's genetic background, thus avoiding the confounding effects of overexpression systems. In addition to human stem cell models, powerful tools have been developed to manipulate the human genome, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) as well as the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system (Boch et al., 2009; Cong et al., 2013; Mali et al., 2013; Wood et al., 2011). Therefore, the phenotypic effects of candidate genes can be investigated by loss and gain of function strategies. CRISPR/Cas9 allows knocking out almost any gene of interest for functional studies in mammalian cells (Chen et al., 2015). Disease-associated genetic variants can be replaced with wild-type constructs by homologous recombination in patient-derived cell lines (Reinhardt et al., 2013; Schöndorf et al., 2014; Soldner et al., 2011). Alternatively, disease-associated genetic variants can be inserted into the endogenous wild-type sequence (Reinhardt et al., 2013; Soldner et al., 2011). Furthermore, functional genome-wide CRISPR/Cas9 screens can be performed to investigate gene function and physiological regulators of disease relevant pathways (Potting et al., 2018).

3. Stem cell-based models of *GBA*-PD

Stem cell-based models of *GBA*-PD are a valuable paradigm for mechanistic studies and drug research. In recent years, several pathogenic molecular mechanisms of *GBA*-PD have been identified using human iPSC models (Table 1). Successful efforts in this regard will be discussed below.

3.1. Autophagic-lysosomal pathway and alpha-synuclein pathology

Many PD genes participate in the autophagy-lysosomal pathway (ALP) either by encoding lysosomal enzymes, such as GCase, or by regulating cellular clearance pathways, pointing towards a key role of the ALP in the pathogenesis of PD (Dehay et al., 2013; Klein and Mazzulli, 2018; Robak et al., 2017). In line with this evidence, *GBA* mutations lead to ALP impairment (Gegg and Schapira, 2016; Pitcairn et al., 2018). Interestingly, reduced GCase activity has been observed in sporadic PD patient brain tissues affected by α-synuclein deposition and this has been related to lysosomal dysfunction (Murphy et al., 2014). Several studies conducted in stem cell model systems have addressed the link between *GBA* and ALP dysfunction. Initial work by Mazzulli et al. showed a disruption in long-lived protein degradation in iPSC-derived mDA neurons from GD patients, suggesting impairment of the ALP (Mazzulli et al., 2011). Enlargement of the lysosomal compartment and defects in the autophagic flux have also been shown in human *GBA*-PD mutant iPSC-derived DA neurons (Fernandes et al., 2016; Schöndorf et al., 2014). Down-regulation of the transcription factor EB (TFEB), the master regulator of lysosomal function and autophagy, and decreased lysosomal biogenesis have been described in neurons differentiated from GD patient iPSCs (Awad et al., 2015). Increasing evidence shows that *GBA* may affect different forms of autophagy, including microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA), contributing to PD development (Du et al., 2015; Li et al., 2018; Sanchez-Martinez et al., 2016). With regard to CMA, a recent study conducted in a variety of *GBA*-PD models, including patient-derived stem cells, has shown that mutant GCase binds the CMA lysosomal receptor but is poorly translocated into lysosomes, thus competing with α-synuclein for CMA (Sheng-Han et al., 2018). Since α-synuclein is degraded by macroautophagy and CMA (Cuervo et al., 2004), perturbations of lysosomal function are expected to affect α-synuclein levels. On the other hand, pathological forms of α-synuclein and DA modified α-synuclein have been shown to inhibit CMA (Cuervo et al., 2004; Martinez-Vicente et al., 2008). GD patients with parkinsonism and PD subjects carrying heterozygous *GBA* mutations present α-synuclein positive ubiquitinated inclusions (Neumann et al., 2009; Wong et al., 2004). The link between *GBA* mutations and LBD further supports a role for the relationship between *GBA* and α-synuclein in disease

Table 1
iPSC-based studies of *GBA* related mechanisms.

Genotype	iPSC-model	Key findings	References
GD (N370S/84GG); WT/WT	Midbrain DA neuronal cultures	• Reduced GCase activity and levels • Decreased degradation of long-lived proteins • Higher abundance of α-synuclein	(Mazzulli et al., 2011)
GD (N370S/N370S; L444P/L444P; L444P/RecNcil), WT/WT	Macrophages; DA neuronal cultures	• Reduced GCase activity and levels, and substrate accumulation in macrophages and neurons • Delayed clearance of phagocytosed RBCs • Inverse correlation between the rate of RBC clearance and the severity of the mutation • No difference in erythrophagocytosis • GD2 macrophages have increased pro-inflammatory (TNF-α) mRNA levels upon LPS stimulation • Phenotypic rescue by treatment with recombinant GCase and to a lesser extent by Isofagomine • In DA neuronal cultures reduced GCase activity and substrate accumulation	(Panicker et al., 2012)
PD (A53T and isogenic gene-corrected controls, SNCA trp)	Cortical neurons	• Increase of GCase in the ER and decrease in the post-ER–to–ER ratio • Syvn1 reduces the levels of immature GCase; Nedd4 and NAB2 increase post-ER GCase	(Chung et al., 2013)
GD (N370S/N370S; L444P/L444P; W184R/D409H; L444P/RecNcil), WT/WT	Macrophages	• Increased production of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and ChT1 • Phenotypic rescue with recombinant GCase, Ambroxol and Isofagomine	(Panicker et al., 2014)
GBA-PD (L444P/WT; N370S/WT; RecNcil/WT); GD (N370S/N370S; L444P/L444P); isogenic gene-corrected controls; WT/WT	Midbrain DA neuronal cultures	• Reduced GCase activity and levels • Increased α-synuclein levels • Substrate accumulation • Enlargement of the autophagosome and lysosomal compartment • Reduction of autophagic flux • Deficits in autophagosome-lysosome fusion • Increased calcium levels at basal conditions • Increased calcium release from the ER • Increased susceptibility against rotenone and A23187	(Schöndorf et al., 2014)
GBA-PD (N370S/WT; twins discordant for PD); WT/WT (healthy and sporadic PD)	Midbrain DA neuronal cultures	• Reduced GCase activity and levels • Increased α-synuclein levels • Reduced DA levels • Increased MAO-B levels in affected twin neurons	(Woodard et al., 2014)
GD (L444P/RecNcil; W184R/D409H; L444P/L444P; N370S/N370S)	Neuronal cultures	• Reduction of TFEB levels and lysosomal depletion • Block in clearance of autophagosomes • Increased neuronal cell death following autophagy induction	(Awad et al., 2015)
GD (N370S/N370S siblings, one diagnosed with PD; N370S/N370S; N370S/c0.84dupG; IVS2 + 1G > T/L444P); WT/WT	Macrophages, NPCs and midbrain DA neurons	• Treatment with NCGC607, a small-molecule non-inhibitory chaperone, restores GCase activity and protein levels, reduces substrate accumulation, and α-synuclein levels in DA neurons	(Aflaki et al., 2016a, 2016b)
GBA-PD (N370S/WT); WT/WT	Midbrain DA neurons	• Reduced GCase maturation • Aberrant composition of GlcCer species • ER stress • Defects in autophagic clearance and enlargement of lysosomal compartment • Increased α-synuclein secretion	(Fernandes et al., 2016)
PD (SNCA trp); GD (N370S/c.84dupG); WT/WT (healthy and idiopathic PD)	Midbrain DA neurons	• α-synuclein knockdown restores GCase, sulfatase, hexosaminidase and β-galactosidase, trafficking and activity • Overexpression of Rab1a restores Golgi structure, improves hydrolase trafficking and activity, and reduces pathological α-synuclein and neuronal viability	(Mazzulli et al., 2016a)
PD (SNCA trp; A53T SNCA mutant and isogenic gene-corrected controls; PARK9); GD (N370S/c.84dupG); WT/WT (idiopathic PD; healthy)	Midbrain DA neurons	• PARK9 neurons have decreased GCase activity and α-synuclein accumulation • Treatment with a small molecule GCase activator rescues α-synuclein phenotypes and toxicity	(Mazzulli et al., 2016b)
PD (DJ-1 c.192G > C/DJ-1 c.192G > C, parkin); DJ-1 c.192G > C/WT; DJ1-KO; idiopathic PD; WT/WT	Midbrain DA neurons	• Mitochondrial oxidative stress leads to oxidized dopamine accumulation that impairs GCase activity, lysosomal dysfunction, and α-synuclein accumulation	(Burbulla et al., 2017)
GBA-PD (N370S/WT); WT/WT	Midbrain DA neurons	• Decrease of α-synuclein tetramers and related multimers • Treatment with Miglustat reduces GSL accumulation, restores α-synuclein tetramers and related multimers, and protects against α-synuclein toxicity	(Kim et al., 2018)
GBA-PD (L444P/WT; N370S/WT; RecNcil/WT); isogenic (gene-corrected) controls; GBA-KO	Midbrain DA neurons	• Mitochondrial dysfunction in *GBA*-PD and *GBA*-KO neurons • Absence of gene-dosage effect • ER stress in *GBA*-PD not observed in *GBA*-KO • Reduced NAD/NADH redox state and NMNAT2 mRNA levels • Therapeutic rescue (mitochondrial and autophagic)	(Schondorf et al., 2018)

(*continued on next page*)

Table 1 (*continued*)

Genotype	iPSC-model	Key findings	References
GD (N370S/c.84dupG; L444P/L444P); PD (SNCA trp; A53T); WT/WT	Midbrain DA neurons	• GluCer and α-synuclein co-localize in iPSC-neurons • GluCer directly induces α-synuclein aggregation in human iPS neurons • α-synuclein potentiates GSL-induced neurotoxicity • Pathological events can be partially rescued by treatment with GlcCer synthase inhibitor or small molecule 758 (GCase activator)	(Zunke et al., 2018)

Abbreviations: ChT1 = chitotriosidase; DA = dopamine; ER = endoplasmic reticulum; *GBA*-KO = *GBA* knockout; GCase = glucocerebrosidase; GD = Gaucher disease; GLS = glycosphingolipids; GluCer = glucosylceramide; IL-1β = interleukin-1 beta; IL-6 = interleukin-6; MAO-B = monoamine oxidase B; midbrain DA neurons = midbrain dopaminergic neurons; NAB2 = N-arylbenzimidazole 2; NAD = nicotinamide adenine dinucleotide; NADH = nicotinamide adenine dinucleotide hydrogen; NMNAT2 = Nicotinamide mononucleotide adenylyltransferase 2; NPC = neural precursor cell; PD = Parkinson's disease; RBC = red blood cell; Synv1 = Synoviolin-1; TFEB = transcription factor EB; TNF-α = tumor necrosis factor-alpha; WT = wild-type.

pathogenesis. The initial hint towards an interaction between GCase and α-synuclein came from the evidence that the pharmacological inhibition of GCase with conduritol-β-epoxide (CBE) leads to increased α-synuclein levels in neuroblastoma cells and mice (Manning-Bog et al., 2009). Subsequent studies conducted in primary neuronal cell lines have shown that GCase inhibition is not sufficient to influence α-synuclein levels in neuronal cell cultures (Dermentzaki et al., 2013; Papadopoulos et al., 2018). Such discrepancy could be due to the different durations of CBE treatment as well as specific assay conditions. Several independent studies have then reported α-synuclein accumulation in the brains of GD mouse models (Cullen et al., 2011; Fishbein et al., 2014; Sardi et al., 2011; Xu et al., 2011). Stem cell models have proven valuable in the analysis of *GBA*-mediated mechanisms of α-synuclein pathology. iPSC-derived neurons from GD and *GBA*-PD patients recapitulate reduced GCase activity, protein level and maturation (Aflaki et al., 2016a; Mazzulli et al., 2011; Schöndorf et al., 2014; Woodard et al., 2014). The aforementioned decline in the proteolysis of long-lived proteins due to mutant GCase leads to an increase in α-synuclein protein levels in GD iPSC neurons (Mazzulli et al., 2011). Higher levels of α-synuclein in iPSC-derived neurons were also observed after inhibition of GCase, via CBE treatment, as well as in *GBA*-PD iPSC neurons compared to isogenic controls (Schöndorf et al., 2014; Woodard et al., 2014). Latter suggests that heterozygous *GBA* mutations may predispose to PD via altered α-synuclein degradation. On the other hand, high α-synuclein levels due to SNCA triplication or α-synuclein overexpression in iPSC-derived neurons result in soluble oligomers and insoluble amyloidogenic aggregates within cell bodies and neurites (Mazzulli et al., 2016a). Higher α-synuclein levels also lead to increased lysosomal mass, disturbed trafficking and impairment of lysosomal hydrolases, including GCase, which can be partially rescued by knockdown of α-synuclein (Mazzulli et al., 2016a). Research conducted in mouse and cell models have shown that *GBA* mutations may contribute to increased levels of oligomeric α-synuclein species not only via lysosomal dysfunction but also through altered lipid properties of membranes. According to the *loss-of-function* hypothesis, the defective GCase activity causes an accumulation of SPL that alter the lipid composition of cell membranes and disrupt the membrane binding of α-synuclein (Jo et al., 2000). GlcCer stabilizes α-synuclein soluble oligomers that, in turn, inhibit intracellular trafficking of GCase to the lysosomes, suggesting a positive feedback loop between decreased lysosomal GCase function and α-synuclein propagation, which eventually leads to pathogenic development and progression of neurodegenerative disease (Mazzulli et al., 2011). Thus, ALP dysfunction can serve as both a cause and a consequence of α-synuclein pathology in *GBA*-related PD pathogenesis. For a long time it has been assumed that α-synuclein exists as a natively unfolded monomer that can assemble into multimers with α-helical structure in vitro and monomeric α-synuclein species are in equilibrium with membrane-bound multimers under physiological conditions (Dettmer et al., 2016). However, this view has been challenged by Bartels et al. showing that α-synuclein occurs physiologically as a helical folded aggregation resistant tetramer (Bartels et al., 2011). Missense mutations in α-synuclein leading to familial PD significantly decrease tetramer:monomer ratio in neural cells, including human iPSC-derived neurons with the A53T mutation (Dettmer et al., 2015a). Lipids are thought to play an important role in the formation of α-synuclein tetramers (Dettmer et al., 2015b). Thus, *GBA* defects may affect the formation of α-synuclein tetramers. Recently, this hypothesis has been investigated using a variety of cell models including *GBA* knockout SH-SY5Y neuroblastoma cells, primary neurons from heterozygous L444P *GBA* mice, and N370S *GBA*-PD iPSC-derived human DA neurons (Kim et al., 2018). The analysis of N370S *GBA* mutant neurons showed that GlcCer accumulation disrupts α-synuclein tetramers and multimers, which results in an accumulation of monomeric α-synuclein (Kim et al., 2018). The role of GlcCer in destabilizing α-synuclein tetramers and multimers was further supported by the evidence that the reduction of the substrate with the glucosylceramide synthase inhibitor Miglustat, or enhanced enzymatic activity by genetic means, recovers the levels of α-synuclein tetramers and related multimers and thereby protects against neuronal toxicity induced by preformed fibrils in human DA neurons (Kim et al., 2018). Thus, the authors concluded that lipid homeostasis is required to sustain α-synuclein tetramers and related multimers in human DA neurons (Kim et al., 2018). Using CBE-treated iPSC-neurons and GD patient neurons, Zunke et al. have shown the existence of equilibrium between 35 Å-sized monomeric species and 100 Å HMW conformers (Zunke et al., 2018). While the structure of such HMW species needs further characterization, the authors have then shown that glycosphingolipids convert mainly the HMW species into a stable, assembly-competent form that can serve as seeds for recombinant monomeric α-synuclein. In this study, the importance of α-synuclein in neurotoxicity was further supported by the increased viability of α-synuclein knockout neurons after CBE treatment (Zunke et al., 2018). Taken together, α-synuclein accumulation due to loss of GCase function impairs hydrolase trafficking and lysosomal function leading to a further decrease in GCase activity as well as substrate accumulation in a vicious cycle (Mazzulli et al., 2016a). Furthermore, substrate accumulation due to the loss of the enzymatic activity may directly contribute to changes in lysosomal pH and decreased lysosomal function as well as autophagy-mediated breakdown (Bourdenx et al., 2016) and further aggravates α-synuclein pathology (Kim et al., 2018; Zunke et al., 2018). Due to the tight interconnections between the ER and endolysosomal structures, mutant *GBA* might contribute to ALP impairment via ER mediated mechanisms (Allison et al., 2017; Phillips and Voeltz, 2016; Wu et al., 2017). Future studies are required to address the role of gain-of-function mechanisms involving ER stress responses and UPR activation (Ron and Horowitz, 2005; Sawkar et al., 2005) in *GBA*-related ALP defects.

3.2. Endoplasmic reticulum stress

The ER is essential for protein processing, calcium homeostasis and

lipid synthesis. ER stress has been detected in various PD models including *GBA*-PD (Colla et al., 2012; Hoozemans et al., 2007). According to the *gain-of-function* hypothesis, mutant GCase is not correctly trafficked to the lysosomal compartment and undergoes ER associated degradation (ERAD) (Ron et al., 2010). Accumulation of misfolded GCase triggers ER stress and leads to dysfunction of the ubiquitin–-proteasome degradation (Ron et al., 2010). Supporting this evidence, defects in GCase folding and trafficking, ERAD and ER stress have been described in iPSC-derived DA neurons from PD patients carrying *GBA* mutations (Fernandes et al., 2016; Schondorf et al., 2018). Specifically, IRE1 and PERK related branches of ER stress are activated in *GBA*-PD iPSC-derived neurons (Schondorf et al., 2018). Thus, stem cell models represent a valuable system to test therapeutic approaches targeting neuronal ER stress mechanisms.

3.3. Mitochondrial dysfunction

Using *GBA* knockout mice as a model of neuronopathic GD, Osellame et al. have provided the first evidence of a link between *GBA* and mitochondria. This study showed that loss of GCase activity leads to mitochondrial fragmentation, respiratory chain defects, and defective mitophagy (Osellame et al., 2013). *GBA* mutations could affect mitochondrial function and dynamics by different mechanisms: i) by increasing α-synuclein levels and aggregation; ii) via ALP impairment and defective mitochondrial turnover; iii) via ER stress and altered inter-organelle communication. Thus, both gain- and loss-of-function mechanisms are potentially linked to mitochondrial demise in *GBA*-PD. In line with this hypothesis, the inhibition of GCase activity with CBE leads to altered mitochondrial morphology and function in neuronal cultures (Cleeter et al., 2013; Xu et al., 2014). A recent investigation conducted in *GBA* L444P/WT knockin mice has shown that heterozygous *GBA* mutations are also linked to mitochondrial dysfunction mainly via defects in mitophagy (Li et al., 2018). Patient specific stem cell models have helped unravel mechanisms of mitochondrial demise in *GBA*-PD. Neurons from *GBA*-PD patient iPSCs show defects in mitochondrial function and energy metabolism, characterized by morphological changes, reduced respiration, and increased oxidative stress. Interestingly, different *GBA*-PD mutations (N370S, L444P, RecNcil) have similar effects on mitochondrial function and no gene dosage effect was detected when comparing heterozygous *GBA*-PD with isogenic *GBA* knockout and wild-type neurons (Schondorf et al., 2018). Thus, different mechanisms likely contribute to mitochondrial dysfunction in *GBA* mutant and *GBA* knockout models. One such mechanism could be changes in mitochondria SPL composition that was observed in *GBA* KO, but not *GBA*-PD, cells. On the other hand, despite significant substrate accumulation, the complete loss of GCase enzymatic function in *GBA* KO neurons was not sufficient to trigger ER stress that was instead observed in heterozygous *GBA*-PD neurons. Due to the close apposition of ER and mitochondria, these findings suggest a role of gain-of-function mechanisms in ER stress responses in mitochondrial dysfunction and interorganelle communication in *GBA*-PD (Schondorf et al., 2018). Furthermore, *GBA*-PD neurons showed significant changes in the NAD + metabolism, and treatment with the NAD+ precursor nicotinamide riboside increased NAD+ levels in *GBA* DA neurons and rescued mitochondrial respiration and dynamics, supporting a link between decreased NAD+ and mitochondrial dysfunction in *GBA*-PD (Schondorf et al., 2018).

3.4. Calcium dyshomeostasis

GBA mutations affect the proper function of all the cellular calcium stores (lysosomes, mitochondria and ER). Different mechanisms may lead to impaired calcium homeostasis in *GBA* mutants, including changes in SPL on ER membranes, mitochondrial dysfunction and impairment of ER-mitochondrial communication. The first link between *GBA* and calcium originated from the work of Korkotian et al. showing the hypersensitization of ER ryanodine receptors in a pharmacological neuronal cell model of GD (Korkotian et al., 1999). ER calcium plays a key role in protein folding and manipulating the intracellular calcium levels partially restores the homeostasis of mutant lysosomal enzyme in LSD (Mu et al., 2008). Increasing calcium levels in ER via pharmacological or genetic means enhances the folding of mutant GCase (Ong et al., 2010). *GBA*-PD neurons show increased concentrations of calcium at basal conditions and enhanced calcium release from the ER stores (Schöndorf et al., 2014). More recently, Kilpatrick et al. identified age-dependent reciprocal changes in ER and lysosomal Ca2+ homeostasis in fibroblasts from GD and *GBA*-PD patients (Kilpatrick et al., 2016). Further studies in patient neuronal models are warranted to assess the therapeutic effect of calcium regulation in *GBA*-PD.

3.5. Dopamine metabolism

PD-linked mutations have a different impact on DA homeostasis: *Parkin* deletions lead to enhanced DA release (Jiang et al., 2012), whereas *LRRK2* G2019S is associated with reduced DA release (Nguyen et al., 2011). Studies using PET scan have shown similar patterns of DA loss in patients with GD, *GBA*-PD, and sporadic PD (Goker-Alpan et al., 2012; Saunders-Pullman et al., 2010; Sunwoo et al., 2011). Intracellular DA content and metabolism have also been investigated in iPSC-derived DA neurons from GD and *GBA*-PD patients. An initial study was performed in iPSCs from *GBA* N370S monozygotic twins clinically discordant for PD. DA neurons from both individuals showed a reduced capacity to synthesize and release DA (Woodard et al., 2014). Interestingly, neurons from the affected twin showed a decrease in DA level, an increase in monoamine oxidase B (MAO-B) expression, and impaired intrinsic network activity compared to unaffected ones. This suggests that non-genetic and environmental factors in addition to *GBA* mutations could perturb DA metabolism (Woodard et al., 2014). A subsequent study compared iPSC lines derived from subjects with GD with and without PD, including one set of N370S/N370S siblings discordant for PD (Aflaki et al., 2016a). The authors found that DA neurons from patients with type 2 and type 1 GD with parkinsonism have reduced vesicular DA levels and DA uptake. In addition, they observed reduced DA transporter and VMAT2 expression levels in GD1 PD neurons, which likely contribute to reduced DA uptake in these cells (Aflaki et al., 2016a). How *GBA* mutations perturb DA homeostasis is still unclear. One potential mechanism could be the increased levels of α-synuclein occurring in *GBA*-PD that may lead to defects in synaptic vesicle regulation. As *GBA* mutations are linked to defects in different forms of autophagy, disruption of protein homeostasis at synapses may contribute to defects in DA metabolism in *GBA*-PD. Further studies should investigate whether α-synuclein accumulation precedes DA dysfunction.

3.6. Sphingolipid dyshomeostasis

SPL are structural components of cell membranes and key signaling molecules, playing a role in regulating membrane structure and cellular processes such as migration, protein trafficking, and synaptic transmission. Lysosomal storage disorders accompanied by SPL accumulation may lead to a variety of neurological complications (Fuller and Futerman, 2018). Even in the absence of overt lipid accumulation, subtle changes in the lipid composition of cellular membranes may also affect neuronal function through poorly understood mechanisms (Fuller and Futerman, 2018). Due to their phagocytic activity and the increased burden of GlcCer, macrophages are the cells that mainly show substrate accumulation in GD patients (Cox and Schofield, 1997). Due to limitations of the periodic acid-Schiff (PAS) stain that is commonly used to identify Gaucher cells and the lack of reliable antibodies for GlcCer detection, the investigation of neuronal SPL pathology has faced challenges. Neuronal storage of GlcCer has been described in brains of GD patients and mouse models, is some cases with the pseudotubular

structures that are characteristic of GlcCer deposits in Gaucher cells (Farfel-Becker et al., 2013; Lloyd et al., 1956). In a recent study, Guedes et al. have identified increased serum levels of monohexosylceramide, ceramide and sphingomyelin in *GBA* mutation carriers; on the contrary, levels of phosphatidic acid (PA), phosphatidylethanolamine (PE), plasmalogen phosphatidylethanolamine (PEp) and acyl phosphatidylglycerol (AcylPG) were decreased (Guedes et al., 2017). Even though some of the aforementioned changes are also found in other neurodegenerative diseases (Wood et al., 2015; Wood et al., 2010), the elucidation of SPL changes in *GBA*-mediated PD could help identify pathways leading to PD in *GBA* mutants as well as novel biomarkers for disease risk and progression.

However, the exact role of substrate accumulation and SPL metabolism in *GBA*-related neurodegeneration is still unknown. Relevant cellular or animal models could help elucidate such mechanisms. Fibroblasts do not show substrate accumulation at basal conditions (Sillence et al., 2002) and the SPL pattern of neuroblastoma cell lines differs from the lipid profile of differentiated neurons (van Echten-Deckert and Herget, 2006). Importantly, neuronal differentiation of iPSC recapitulates the SPL changes observed during human brain development and iPSC-derived neurons show the SPL content of adult human brain (Schöndorf et al., 2014). These findings suggest that iPSC neuronal models are a suitable system to study SPL pathology. The inhibition of GCase with CBE as well as CRISPR-Cas9 mediated *GBA* knockout in human iPSCs lead to substrate accumulation in iPSC-derived neurons (Schondorf et al., 2018; Zunke et al., 2018), which has as well been described in GD and *GBA*-PD iPSC-derived neurons (Fernandes et al., 2016; Schöndorf et al., 2014). Interestingly, Fernandes et al. have shown that only certain GlcCer species accumulate in *GBA*-PD neurons, with an increase in C16:0 and C24:0 species, and a reduction for C20:0 GlcCer (Fernandes et al., 2016). Whether GlcCer species have a cell-specific role in the disease still needs further investigation. Another unsolved issue is the exact location of substrate accumulation (Elleder, 2006). While the main compartment of lipid storage is the lysosome (Takahashi et al., 1978; Willemsen et al., 1995), it is still unclear whether this also occurs in other cellular organelles. Evidence suggests that GlcCer accumulates at the ER membrane (Conradi et al., 1984; Korkotian et al., 1999; Lloyd-Evans et al., 2003). Interestingly, GlcCer and glucosylsphingosine (GlcSph) accumulation has been detected in enriched mitochondrial preparations from *GBA*-KO iPSC neurons (Schondorf et al., 2018). Increased levels of GlcCer and decreased levels of ceramide have also been reported in iPSC-neurons from a PD patient with α-synuclein triplication (Mazzulli et al., 2016a). Furthermore, a link between glycosphingolipids and α-synuclein conformation and aggregation has been demonstrated (Kim et al., 2018; Zunke et al., 2018). It is important to underline that even a slight increase in GlcCer and GlcSph, below those observed in symptomatic GD cases, is sufficient to induce alterations in glycolipid trafficking (Sillence et al., 2002). Thus, it is likely that subtle changes in substrate levels in neurons may lead to functional abnormalities and changes in the lipid membrane composition and together they accelerate the neurodegenerative process. In this scenario, restoring SPL homeostasis could represent a promising therapeutic target for *GBA*-PD and other neurodegenerative conditions. Supporting this hypothesis, Miglustat, an inhibitor of GlcCer production, decreases the level of pathological α-synuclein and confers neuroprotection against α-synuclein toxicity (Kim et al., 2018).

3.7. Non-cell autonomous mechanisms: The immune system in GBA-PD

PD is a neurodegenerative disease that is conventionally considered to arise and exclusively affect vulnerable neurons. However, increasing evidence suggests that complex interactions between the brain and the immune system contribute to the disease (Deleidi and Gasser, 2013). Both peripheral and brain resident immune cells may contribute to neuroinflammatory reactions observed in PD brain (Brochard et al., 2009; Lecours et al., 2018; Sommer et al., 2018). *GBA* is highly expressed in cells of the myeloid lineage and the accumulation of its substrates, GlcCer and GlcSph, leads to chronic inflammation that correlates with disease severity in GD patients (Liu et al., 2012). Both the innate and the adaptive immune system are involved with the presence of activated macrophages engulfed with lipids, increased levels of proinflammatory cytokines and chemokines as well as immunoglobulins (Aflaki et al., 2016b; Liu et al., 2012). Such hyper-inflammation has an influence on immune cell maturation, recruitment, and even on blood-brain barrier (BBB) infiltration by macrophages (Liu et al., 2012). Macrophages are the main cell type showing substrate accumulation and largely account for the multiorgan complications in GD (Vitner et al., 2012). Whether immune responses are also a driving force in neurodegeneration or simply represent a response to neuronal death remains unclear. iPSCs serve as a unique model to track the effect of disease-related mutations on immune cells. The group of Feldman generated for the first time iPSC-derived Gaucher macrophages from GD patients that showed low GCase enzymatic activity, accumulation of SPL, and impairment of lysosomal functions (Panicker et al., 2012). Interestingly, GD iPSC-derived macrophages showed a delayed clearance of red blood cells (RBC) that correlated with the severity of the mutations (Panicker et al., 2012). Treatment of GD iPSC-derived macrophages with recombinant GCase completely rescued the delay in RBC clearance, whereas the chaperone Isofagomine only had a partial effect (Panicker et al., 2012). These findings are in line with the known efficacy of these GD therapies and support the concept that iPSCs are not only a valuable tool to study disease pathogenesis, but also an important model for therapeutic development. A subsequent study from the same group showed that GD iPSC-derived macrophages expressed higher levels of inflammatory cytokines, including tumor necrosis factor α, IL-6, and IL-1β than control cells, and this phenotype was exacerbated by treatment with lipopolysaccharide (Panicker et al., 2014). Recombinant GCase and pharmacological chaperones (Isofagomine and Ambroxol) were able to reverse these functional abnormalities to an extent that reflects their known clinical efficacies (Panicker et al., 2014). Interestingly, GD iPSC-macrophages retain efficient phagocytic activity but show reduced production of intracellular reactive oxygen species and impaired chemotaxis (Aflaki et al., 2014). GD iPSCs have also been utilized to examine the effects of GCase deficiency on the developmental potential of the hematopoietic lineage. Using iPSC generated from GD patients, Sgambato et al. showed that GCase deficiency leads to a skewing towards increased myeloid differentiation and decreased erythroid differentiation (Sgambato et al., 2015). Gaucher macrophages generated from GD patient monocytes also show an activated macrophage phenotype with increased activation of the NLRP3 inflammasome as consequences of lysosomal storage and impaired autophagy (Aflaki et al., 2016b). Several pathological mechanisms could link *GBA* mutations to immune dysfunction, including SPL accumulation (Nagata et al., 2017; Pandey et al., 2017), deficits of the autophagy-lysosomal system (Ma et al., 2013), as well as mitochondrial demise (Weinberg et al., 2015). However, the exact contribution of these mechanisms to brain inflammation is still unclear. In addition, as most of the research has been conducted in the context of GD, further studies are needed to address whether heterozygous *GBA* mutations also impact immune cell function in PD.

4. PD-linked genes and GCase function

PD genes other than SNCA may influence GCase function. Several mechanisms including α-synuclein aggregation, disturbances of autophagy, and defects in vesicle trafficking may affect GCase folding, transport and activity in other forms of PD. While further research is needed to assess the exact role of gene interactions in *GBA*-PD, initial experiments in patient blood cells and iPSC-models already suggest that PD-related genes may functionally affect GCase. With respect to iPSC-models, a recent study has shown that α-synuclein increases oxidized

DA, inhibits GCase trafficking, and impairs lysosomal function in iPSC-derived neurons from sporadic and familial PD (α-synuclein, PARK9, Parkin and DJ-1 mutants) (Burbulla et al., 2017). GCase enzymatic activity has been reported to be higher in *LRRK2* G2019S carriers, both asymptomatic and with PD (Alcalay et al., 2015). LRRK2 may affect GCase function by different mechanisms including the expansion of the lysosomal compartment that has been observed in LRRK2 G2019S models including patient iPSC-neurons (Orenstein et al., 2013). Alternatively, LRRK2 dependent defects in vesicle trafficking may be responsible for changes in GCase turnover (MacLeod et al., 2013). Recently, perturbations in vesicle trafficking and recycling have emerged as central mechanisms in the pathophysiology of PD (Abeliovich and Gitler, 2016). Thus, defects in intracellular transport pathways to lysosomes may result in reduced GCase activity. In line with this evidence, intracellular trafficking of GCase is regulated by the lysosomal integral membrane protein type 2 (LIMP-2), encoded by the gene *SCARB2*, that functions as a trafficking receptor by targeting and delivering GCase to the lysosome (Reczek et al., 2007; Rothaug et al., 2014). Notably, SCARB2 mutations have been linked by GWAS to the risk of developing PD (Do et al., 2011).

5. iPSC for drug discovery in *GBA*-PD

The challenge for the treatment of *GBA*-PD is the relatively poor understanding of the exact mechanisms involved in neurodegeneration in these patients and the difficulties in designing drugs that efficiently cross the BBB. The pathogenesis of the systemic manifestations of GD may be different from mechanisms that lead to PD in these patients. However, results from therapeutic approaches in GD have instructed the development of therapies for *GBA*-PD patients. Increasing GCase activity via enzyme replacement therapy (ERT), reducing the accumulation of the substrate via substrate reduction therapy (SRT), and improving the folding and intracellular trafficking of the enzyme to the lysosomes via pharmacological chaperones have been the main therapeutic strategies. ERT has been proven safe and able to improve the natural history of GD; *in vitro*, the recombinant enzyme partially corrects the cellular defects resulting from *GBA* deficiency, by inhibiting the production of inflammatory cytokines (Panicker et al., 2014) and by restoring the clearance of phagocytosed RBC (Panicker et al., 2012). However, the large molecular weight of the enzyme prevents it from crossing the BBB and limits its therapeutic application in the neuronopathic forms of GD and *GBA*-related parkinsonism. Alternatively, GCase activity could be enhanced via gene therapy approaches. Normalizing the level of lysosomal GCase via gene therapy has been proven to be protective in experimental models of neuronopathic GD and synucleinopathies (Massaro et al., 2018; Morabito et al., 2017). Both neonatal and fetal intracranial AAV-mediated gene delivery of *GBA* reduced visceral pathology as well as neuroinflammation and neurodegeneration (Massaro et al., 2018). However, normalization of GCase activity in the brain did not completely restore glycosphingolipid levels, nor prevent long-term microglial and astrocyte activation (Massaro et al., 2018). While these results have been attributed to differences in transduction efficiency, a contribution of non-cell-autonomous mechanisms and peripheral macrophages to brain pathology in this model should also be taken into consideration. Relevant to *GBA*-PD, recent work by Morabito showed that the intravenous injection of the AAV-PHP·B variant expressing *GBA* in adult A53T-SNCA mice leads to an increase of GCase activity by 1.5 fold and significantly reduces the accumulation of α-synuclein (Morabito et al., 2017). Importantly, excess GCase appeared to be secreted and taken up by neighboring cells (Morabito et al., 2017). Even though a supraphysiological activity compared to wild-type conditions has not been described in these models, further studies will assess the potential side effects of the long-term increase of GCase activity. A different strategy to ameliorate *GBA*-PD pathology is to halt the GCase/α-synuclein feedback loop by inhibiting glucosylceramide synthase. The iminosugar eliglustat is a potent and well-tolerated inhibitor that has been used in GD1 patients (Larsen et al., 2012; Lukina et al., 2010). However, being a substrate of the P-Glycoprotein (MDR1), it does not cross the BBB (Larsen et al., 2012). Treatment with Miglustat, another glucosylceramide synthase inhibitor, has been shown to reduce GSL accumulation, to reverse the destabilization of α-synuclein tetramers, and protect against α-synuclein preformed fibril-induced toxicity in human iPSC-DA neurons (Kim et al., 2018). However, Miglustat also inhibits the non-lysosomal glucosylceramidase (GBA2) (Nietupski et al., 2012). Furthermore, in a mouse model of Niemann-Pick disease type C, an increase in GlcCer was observed after treatment with Miglustat, which the authors attribute to the inhibition of GBA2 (Nietupski et al., 2012). In addition, Miglustat causes adverse effects including gastrointestinal disturbances, weight loss, tremor or worsening of an existing tremor and peripheral neuropathologies (Hollak et al., 2009; Pastores et al., 2005). Inhibitors with high specificity and good brain penetration have been developed, including the SRT molecule GZ/SAR402671 that is currently under evaluation in a clinical trial in *GBA*-PD patients (NCT02906020). An inhibitor with similar profile, GZ667161, has been shown to decrease GlcCer and GlcSph levels as well as to ameliorate α-synuclein, ubiquitin, and tau aggregates in the brain of *GbaD409V/D409V* mice. This was accompanied by improvement in memory deficits in this model (Sardi et al., 2017). Treatment with GZ667161 was also able to reduce membrane-associated α-synuclein and ameliorated cognitive deficits in A53T–SNCA mice (Sardi et al., 2017). A third therapeutic strategy to enhance GCase activity is based on the use of chaperones that promote the folding and the trafficking of the misfolded enzyme. Molecular chaperones are relatively small in size and cross the BBB (Santos and Tiscornia, 2017). Importantly, molecular chaperones could target a broader spectrum of intracellular and molecular changes such as the ER retention of the mutant GCase and subsequent ER stress, UPR and ERAD. While initial GCase were iminosugar-based inhibitors with low selectivity, non-iminosugar inhibitory chaperones (quinazoline analogues) with chaperone activity, high selectivity, and increased ER to lysosome translocation have been subsequently developed (Marugan et al., 2011). The balance between the inhibitory and chaperoning capacity represents a challenge for the clinical translation of such inhibitory chaperones. A phase II clinical trial with the inhibitory chaperone Isofagomine in adult type 1 GD patients has failed to meet efficacy expectations and has been discontinued. To face this challenge, new non-inhibitory GCase compounds with chaperone activity have been identified by high throughput screening of a small molecule library and medicinal chemistry structure optimization (Goldin et al., 2012; Patnaik et al., 2012). A non-inhibitory chaperone binds to a site that is different from the active site and promotes the folding of mutant enzyme in the ER and its translocation to lysosomes. The non-inhibitory chaperone can also directly induce the residual lysosomal activity of the mutant enzyme. Importantly, such small chemical chaperones can efficiently cross the BBB (Patnaik et al., 2012). Among small chemical chaperones, NCGC607 and NCGC758 have been further explored for therapeutic development (Aflaki et al., 2016a; Aflaki et al., 2014). One of the most studied small molecular chaperones is Ambroxol, a metabolite of bromhexine commonly used as a mucolytic (Balestrino and Schapira, 2018). The therapeutic effects of Ambroxol are currently investigated in two clinical trials for PD patients with *GBA* mutations (ClinicalTrials.gov identifiers NCT02941822 and NCT02914366). Macrophage and neuronal cell models have been crucial for the evaluation of chaperones. The iminosugar Isofagomine and Ambroxol, have been tested in iPSC-derived macrophages in the context of GD (Panicker et al., 2014; Panicker et al., 2012). Interestingly, in comparison to those treated with recombinant *GBA*, Isofagomine has relatively lower efficiency in reversing the phagocytosed RBC clearance defect in mutant *GBA* macrophages (Panicker et al., 2014). These results are consistent with the outcome of previous clinical studies with Isofagomine (Boyd et al., 2013). Treatment of primary macrophages and iPSCs-derived macrophages with the non-inhibitory chaperone

NCGC758 resulted in increased GCase translocation to lysosomes, improved chemotaxis, and reduced substrate storage (Aflaki et al., 2014). Studies on iPSC-derived human mDA neurons from patients with PD, GD and PD, and GD types 1 and 2 showed that treatment with NCGC758 and NCGC607 restores GCase activity and protein levels, reduces substrate accumulation, and enhances the clearance of pathological α-synuclein (Aflaki et al., 2016a; Mazzulli et al., 2016b). These effects have been confirmed in multiple iPSC neuronal lines derived from PD patients that harbor distinct mutations in SNCA (triplication or A53T), GBA1, or PARK9 genes, and idiopathic PD neurons, supporting the potential therapeutic role of GCase activation in PD patients without *GBA* mutations (Mazzulli et al., 2016b).

Finally, several cellular pathways of different organelles are involved in *GBA*-PD and can be a therapeutic target. Mitochondrial phenotypes correlated with *GBA*-PD can be rescued by treatment with NR in iPSC-derived neurons (Schondorf et al., 2018). The importance of mitochondrial dysfunction in *GBA*-PD is further supported by the evidence that treatment with the mitochondrial antioxidants mito-TEMPO or NAC reduces the levels of oxidized DA and insoluble α-synuclein, and increases GCase activity in sporadic and familial PD (Burbulla et al., 2017).

6. Considerations for human iPSC-based modeling: advantages, limitations, and future directions in iPSC disease modeling

Human iPSC disease modeling offers several advantages: it retains the patient genetic background allowing genotype-phenotype correlations without the need for overexpression systems; it is an unlimited source of patient specific cells that can be used for disease studies and drug screenings; it allows the generation of disease relevant cell types, including neurons, astrocytes, as well as macrophages and microglia. Patient-derived human iPSCs also facilitate the study of PD non-coding risk variants (Soldner et al., 2016), such as *SNCA* intron variants (Nalls et al., 2014), whose modeling would be challenging in primary cell cultures. Relevant to PD studies, a recent study has shown that human mDA neurons display an inherent vulnerability to degeneration compared to mouse neurons, mostly due to species-specific difference in DA metabolism, thus highlighting the importance of conducting studies in human neurons in parallel to animal model systems (Burbulla et al., 2017).

Despite fast and growing advances in the field, iPSC-based disease modeling still faces challenges. The interaction between the individual genetic background with environmental factors and ageing significantly influences the phenotype of a complex disorder such as PD. The comparison between patient and isogenic gene-corrected cell lines allows genotype-phenotype functional correlation studies (Reinhardt et al., 2013). Even though with some limitations, the effect of environmental factors could be modeled in vitro (i.e. toxins, inflammatory mediators, specific mitochondrial or ER stressors). However, the lack of the ageing component in iPSC models is still a challenge. Furthermore, many diseases, like PD, are complex disorders, in which epigenetic modifications may play a key role in the disease development and progression (Labbe et al., 2016). In this respect, one major drawback of iPSC modeling is that somatic cell reprogramming requires a global remodeling of the epigenetic landscape (Papp and Plath, 2013). Such iPSC rejuvenation reshapes several cellular processes including mitochondrial function, cellular senescence, and DNA damage. By using direct cell conversion into neurons, some of these limitations may be overcome (Mertens et al., 2018).

While current iPSC disease modeling studies and screenings use mainly neuronal cell types, such applications should be extended to non-neuronal cell types that are also involved in the disease process, namely immune and glial cells. This will improve our understanding of the role of cell-cell interactions in the pathogenesis of neurodegenerative diseases. In this respect, co-culture experiments are now feasible and provide the possibility of modeling the long-term interactions of neurons and microglia in tissue-like conditions (Haenseler et al., 2017; Muffat et al., 2016). Recently, brain organoids are emerging as an increasingly valuable tool for disease modeling, drug discovery and validation (Di Lullo and Kriegstein, 2017). The three-dimensionality (3D) of brain organoids may better recapitulate the pathogenesis and mechanisms of diseases on an organ-level instead of single cells. Unlike traditional cell culture or 2D systems, 3D organoid technology harnesses the self-organizing properties of PSCs to recreate complex multicellular tissues, which allows studying intra- and intercellular signal transduction in the aspects of cell-cell and cell-matrix interactions. Single-cell RNA sequencing reveals the similarities of cell composition of neural progenitor proliferation and differentiation comparing iPSC-derived human cerebral organoids to fetal neocortex (Camp et al., 2015). In addition, brain organoids resemble the multi-layer progenitor zone organization of human brain with the ventricular zone, the inner and outer subventricular zone (Camp et al., 2015; Lancaster et al., 2013). Several groups have generated a broad variety of 3D self-organizing organ-like structures from mouse and human PSC to study the occurrence and/or development of neurological disorders. Importantly, in brain organoids cells organize and interact with each other and their extracellular matrix (ECM). Human 3D stem cell–derived models of familial Alzheimer's disease improve neuronal maturation and promote tauopathy through the accumulation of β-amyloid aggregates in the ECM. These could be observed only in 3D cultures and did not occur in 2D culture or mice (Choi et al., 2014). Raja et al. recapitulated AD phenotypes including β-amyloid (Aβ) aggregation, hyperphosphorylated Tau (pTau), and endosome abnormalities in AD patient-derived 3D brain organoids (Raja et al., 2016). Interestingly, the cellular composition, maturation and functionality of brain organoids can be manipulated according to the growth factors and culturing period to differentiate into various human brain regions, including midbrain (Jo et al., 2016). Therefore, the recapitulation of some of the features and functions of specific brain regions by organoids provides a new platform for researchers to explore neurodegenerative diseases. Due to the fact that neurodegenerative diseases are complex in nature and often involve various cell types as well as affect different areas of the brain, organoids not only could serve as an important model for disease modeling but also for drug development. Using live cell imaging, Birey et al. detected an increase in saltation frequency of neurons derived from patients with Timothy syndrome, a severe neurodevelopmental disease, accompanied by reduced levels of saltation length and mobile speed during migration in fused human cortical and subpallium spheroids (Birey et al., 2017). However, these effects can be rescued by lowering the activity through inhibition of L-type calcium channels using a Cyclin-dependent kinase (CDK) inhibitor, Roscovitine (Birey et al., 2017). Although they recapitulate many key properties of stem cell niches and tissue development, most organoid models are still simplified tissue models mainly because of the lack of vascularization. The shortage of oxygen supply and nutrition not only prevents the further growth and differentiation of inner cells of the organoid but can also serve as a potential risk for necrosis and leads to the release of cytotoxic signals.

Finally, iPSCs facilitate biological and drug screens on patient-specific cells with a defined genetic background that allows the direct correlation of the observed cellular phenotype and response to defined drugs with the patient clinical and genetic data. On the other hand, the development of optimized stem cell-based platforms for drug and small molecules screenings have faced several challenges including the lack of robustness of most differentiation protocols, the high degree of variation, high costs and time-consuming procedures (Ebert and Svendsen, 2010).

7. Concluding remarks

GBA represents an interesting therapeutic target due to its link to the ageing processes, sporadic PD and other neurodegenerative

conditions. Further understanding the mechanisms linking *GBA* and neurodegeneration will therefore be a key factor for developing novel therapeutic approaches. Ongoing clinical trials will help understand the actual role of candidate mechanisms in *GBA*-PD pathogenesis. In the meantime, it will be fundamental to pursue further research aimed at elucidating *GBA* biology and identifying novel targets. The discovery of iPSCs and recent advances in disease modeling tools have provided an important step forward for studying human brain disease. With regard to *GBA*-PD, iPSCs offer advantages for disease modeling including the possibility of investigating SPL biology in relevant cells, the role of DA metabolism as well as non-cell autonomous mechanisms. In addition to the investigation of disease mechanisms, iPSC models are a valuable tool for the development of pharmacological compounds for therapy. Key to precision medicine approaches, iPSCs can also facilitate drug screenings and patient selection for clinical trials. Still, the key and most challenging question in *GBA*-PD remains why most of the *GBA* carriers never develop the disease. Functional genome-wide CRISPR/Cas9 screenings can be performed to investigate gene function and genetic regulators of *GBA*-PD disease pathways (Potting et al., 2018). Patient specific stem cell models would therefore represent the ideal set for the validation of candidate gene modifiers emerged from GWAS, linkage studies, and CRISPR screenings.

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