

● PERSPECTIVE

## Chaperoning glucocerebrosidase: a therapeutic strategy for both Gaucher disease and Parkinsonism

Gaucher disease (GD) is a lysosomal storage disorder (LSD) affecting approximately 1 in 50,000 individuals in the general population. Mutations in both alleles of the *GBA1* gene result in deficient glucocerebrosidase (GCase) activity, which in turn leads to the accumulation of glycolipid substrates and impaired lysosomal function. GD is a multisystem disorder with a vast spectrum of clinical phenotypes, including both non-neuronopathic type 1 Gaucher disease (GD1) and neuronopathic types 2 and 3 Gaucher disease (GD2 and GD3). In addition to its role in the rare disorder GD, mutations in *GBA1* are the most frequent known genetic risk factor for the common complex disorder Parkinson's disease (PD) (Sidransky et al., 2009). Depending on ethnicity, patients with Parkinson's disease are 2–31% more likely to carry a mutation in *GBA1* than matched controls (Sidransky and Lopez, 2012). There appears to be a reciprocal relationship between levels of GCase and levels of the aggregate-prone protein alpha-synuclein (Mazzulli et al., 2011; Murphy and Halliday, 2014). Thus therapies focused on increasing GCase, especially in the brain, have attracted increased attention. To enhance the development of new therapeutics targeting GCase, attention has turned toward induced human pluripotent stem cell (iPSC)-derived models of GD (Aflaki et al., 2014). These new patient-derived models can be used to test the efficacy of new therapeutic strategies. Very recently, two novel small-molecule non-inhibitory chaperones of GCase were evaluated in such models with promising results (Aflaki et al., 2014, 2016; Mazzulli et al., 2016). The findings may pave the way for pharmacological development relevant to both GD and Parkinson's disease

The starting point for the development of most treatments for GD resides in the restoration of GCase activity. Currently, enzyme replacement therapy (ERT) with recombinant GCase is the most common treatment for non-neuronopathic GD (Barton et al., 1991). While ERT has had a major impact on the disease course, its high cost, requirement for lifelong treatments, and ineffectiveness in neuronopathic GD have contributed to a substantial need for more effective and less expensive therapeutic strategies. The use of pharmacological chaperones to assist in the refolding of mutated enzyme has emerged as an exciting and novel concept in treating lysosomal storage disorders (LSDs) (Aflaki et al., 2014; Jung et al., 2016). Chemical chaperone therapy, in contrast to enzyme replacement therapy, provides an avenue for treating neuronopathic GD as many chaperones can pass through the blood-brain barrier thus restoring GCase activity in neurons (Aflaki et al., 2016). Many of the chaperones explored for GCase are inhibito-

ry chaperones that bind to the active site, most frequently iminosugar derivatives (Joosten et al., 2014). Non-inhibitory chaperones circumvent the issues of poor selectivity and reduced enzyme functionality observed in inhibitory compounds (Rogers et al., 2010). High throughput screens using mutant human GCase have led to the identification of several non-inhibitory series that are particularly promising (Goldin et al., 2012). Lead compounds NCGC758 and NCGC607, identified by high throughput screening of an extensive small molecule library, have been explored as potential new prototype molecules for therapeutic development (Aflaki et al., 2014, 2016).

A major impediment facing research into neuronopathic GD, PD and other neurodegenerative disorders is the lack of appropriate tissue and cellular models. To circumnavigate this issue, investigators have exploited the ability to generate iPSCs that can be induced to produce different cellular fates, a technique first introduced by the Yamanka lab in 2006. In the two recent studies, this technology was harnessed to generate stem cells and dopaminergic neurons from human fibroblast lines from patients with Gaucher disease and/or Parkinson disease.

The first study by Aflaki et al. (2016) studied cells from six patients with GD1, GD1-PD, GD2, and an adult control. These lines were differentiated into macrophages (iMacs) and dopaminergic neurons (iDA neurons). The ability of this compound to chaperone GCase was initially validated in iPSC-derived patient macrophages, where storage of the substrates is abundant. After treating iPSC macrophages from GD patients with NCGC607, increased GCase activity, improved translocation of GCase to the lysosome, and a stark reduction in glycolipid accumulation were observed. Similar findings had previously been reported with compound NCGC758 (Aflaki et al., 2014).

After observing the effect of NCGC607 in iMacs, attention was turned to dopaminergic neurons. Functionality of the induced dopaminergic (iDA) neurons was confirmed by evaluating dopamine storage and by measuring their action potential. Prior to treatment, GD iPSC-neurons showed low GCase activity, with GD1 lines having about 30% of control GCase activity, and the GD2 lines exhibiting approximately 2% of control GCase activity. iPSC-derived dopaminergic neurons from patients with from GD1-PD and GD2 had elevated levels of  $\alpha$ -synuclein. After 21 days of-treatment with NCGC607, multi-fold increases in GCase activity were observed in both GD1 and GD2 neurons. The compound appeared to also have further utility, as it was associated with a reduction in  $\alpha$ -synuclein levels in dopaminergic neurons derived from patients with GD1 who also had parkinsonism. Confocal microscopy revealed that GCase was successfully translocated to the lysosome, as determined by co-staining using antibodies to both Lamp2 and GCase. As a result of this enhanced translocation of GCase to the lysosome, a significant reduction in the substrate glucosylsphingosines observed in all five GD lines. These findings indicate that this compound has an effect on both non-neuronopathic GD and neuronopathic GD, and since NCGC607 led to a reduction

of  $\alpha$ -synuclein levels, derivatives of this lead molecule may have potential for Parkinson disease therapeutics. It was also important to note that NCGC607 successfully translocated GCase with mutations other than the common N370S seen in GD1. This ability of NCGC607 to affect different mutations is vital in producing unrestricted therapies to all forms of GD.

The study by Mazzulli et al. (2016) also used iPSC-derived dopaminergic neurons to evaluate a previously described non-inhibitory chaperone of GCase. However, they used neurons generated from patients with mutations in different PD-associated genes. This included patients with mutations in PARK9, an A53T mutation in  $\alpha$ -synuclein, an  $\alpha$ -synuclein triplication (SNCA trp), one patient with Gaucher disease, and one with idiopathic PD. Treating cells with small molecule NCGC758, a molecule shown to have reasonable brain distribution in the mouse (Patnaik et al., 2012), reduced substrate levels and resulted in the reduction of pathological  $\alpha$ -synuclein. This treatment was also reported to reverse downstream pathology. Accordingly, this second study also indicated that such chaperones may prove beneficial for the treatment of synucleinopathies.

Gaucher disease, like nearly all LSDs, is a relatively rare disorder, not well known in the general population. Fortunately, the recently elucidated connection between Gaucher and the more well-recognized Parkinson's disease has intensified the speed of development of new practical therapeutic strategies. These recent studies show that small-molecule chaperone therapies may play an increasing role in the treatment of LSDs. The research is particularly exciting because such small-molecule chaperones appear to have an effect on Parkinson's disease. Modulating the reciprocal relationship between GCase and  $\alpha$ -synuclein opens new therapeutic opportunities for both Gaucher disease and Parkinson's disease, with implications for many of the other synucleinopathies.

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