Targeting *GBA1* for Parkinson's disease research

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Background & Rationale

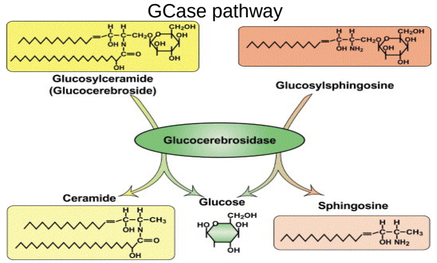
Mutations in the *GBA1* gene, which encodes for lysosomal glucocerebrosidase (GCase), have been identified as causative for Gaucher disease (GD), a rare lysosomal storage disorder and represent the most common genetic risk factor for Parkinson's disease (PD) (Sidransky et al., 2009). The proportion of PD patients that carry *GBA*1 mutations is estimated to be between 5 and 10%. The penetrance and lifetime risk of developing PD for *GBA1* mutation carriers is estimated up to 20% at 70 years (Schapira 2015).

Decreased GCase activity has been reported in both PD patients with *GBA1* mutations and without *GBA1* mutations (Murphy et al., 2014). Emerging experimental evidence in cell-free systems, cells, animal models and patient samples suggests a correlation between this decreased activity and accumulation of alpha-synuclein (aSyn) (Fishbein et al., 2014; Gegg et al., 2012; Mazulli et al., 2011; Sardi et al., 2011).

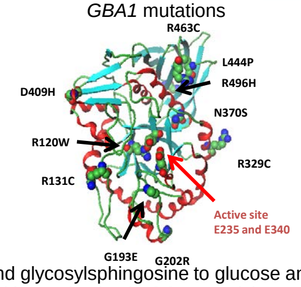
These strong genetic and pathological links make GCase an attractive target for PD drug development. As such, The Michael J. Fox Foundation (MJFF) has made robust investments to address key questions to effectively translate GCase therapeutically for PD patients. The current poster details MJFF activities which address critical gaps in our knowledge of role of *GBA1* in PD and tackle key challenges facing GCase drug development.

References: Sidransky et al., N. Engl. J. Med. (2009) 361: 1651-1661; Schapira, Mol Cell Neurosci. (2015) 66: 37-42; Murphy et al., Brain. (2014) 137: 834-848; Fishbein et al., Brain (2014) 137: 3235-3247; Gegg et al., Ann. Neurol. (2012) 72: 455-463; Mazulli et al., Cell. (2011) 146: 37-52; Sardi et al., Proc. Natl. Acad. Sci. (2011) 108: 12101-6.

GCase pathway



GBA1 mutations



GCase enzyme metabolizes glucocerebrosidase to glucose and ceramide and glycosylsphingosine to glucose and sphingosine. The presence of a *GBA1* mutation is invariably associated with a reduction in GCase activity and substrate accumulation. Over 300 different mutations of the *GBA1* gene have been described, but the N370S and L444P account for the majority found in both GD and PD.

Key Questions in Developing GCase Targeting Therapies

Genetics

- Incomplete penetrance suggests presence of modifiers. How do we identify such modifiers to garner insights into potential therapeutic targets and patient enrichment/stratification strategies?
- Is *GBA1* mutation-associated PD phenotypically similar to idiopathic PD?
- Do human or mouse genetic studies provide insight into minimum GCase activity needed for therapeutic efficacy?

Biomarkers

- What are the target engagement and pharmacodynamic markers to inform clinical dose selection and to track drug efficacy?
- What are the most optimal, standardized and validated assays to measure GCase ? To measure ceramide pathway analytes?
- Could alpha-synuclein serve as a good biomarker in GCase targeting trials?

Therapeutics

- What is the most optimal therapeutic modality for targeting GCase?
- How much of an increase (both amount and duration of increase) in GCase is needed to see efficacy?
- What cellular models, animal models and end-points are optimal for drug development?
- What is the potential liability of increasing GCase activity?

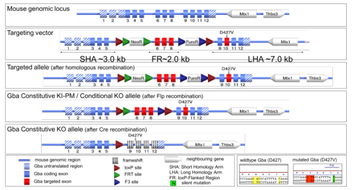
Clinical Cohorts

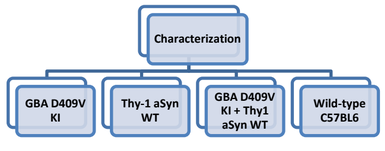
- What is the optimal patient population for Phase 1 and Phase 2/PoC trials?
- How can you enrich for a population most likely to respond to GCase activation?
- Would GCase targeted therapies work in idiopathic PD?
- What clinical outcome measures should be used for Phase 2/PoC trials?
- What should be the duration of the PoC trial?

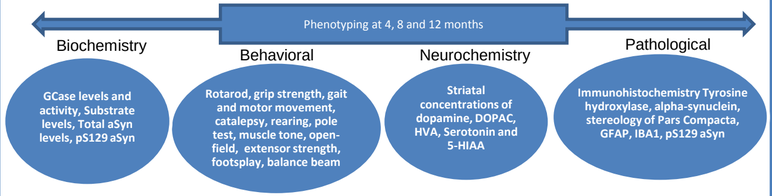
MJFF is Making Robust Investments to Address Research & Therapeutic Challenges

Generating and characterizing preclinical models

Homozygous *GBA1* D409V Knock-in Mice (available at The Jackson Laboratory Stock # 019106)

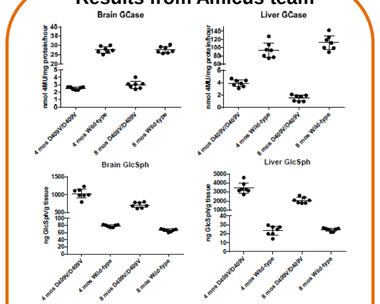






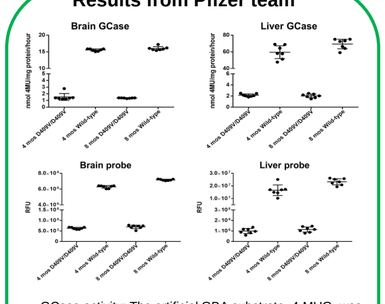
Biochemical analyses: The homozygous *GBA1* D409V KI mouse model was generated in collaboration with MJFF's industry tools consortium. In the study below, *GBA1* D409V KI and WT mice (n=7/group) were anesthetized with sodium pentobarbital and perfused using 0.9% buffered saline until blood was completely cleared. Brains (dissected into 2 equal hemispheres) and liver lobes (left and right) were collected and flash frozen in liquid nitrogen. Brain hemispheres and liver lobes were sent blinded to Amicus and Pfizer teams for determining GCase activity/levels, substrate levels, and aSyn levels.

Results from Amicus team



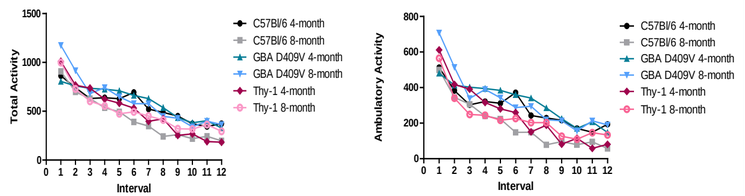
GCase activity: Lysosomal GCase activity was determined as the CBE-inhibitable release of 4-MU from 4-MUG in MI buffer pH 5.2 containing 0.1% TX-100, 0.25% Na-taurocholate, and the GBA2 inhibitor NB-DGJ.
GlcCer and GlcSph measurements: Sphingolipid quantities were determined by LC-MS/MS with appropriate internal standards and a chromatography method that achieves baseline separation of glucosyl and galactosyl epimers.

Results from Pfizer team

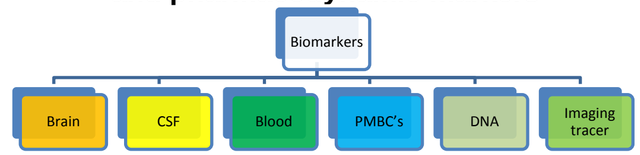


GCase activity: The artificial GBA substrate, 4-MUG, was used to assess GBA activity in the brain homogenates using an established enzymatic assay with a fluorescent product 4-MU. GBA1 specificity was defined by CBE.
Lysosomal GBA levels : MDW941, is an irreversible inhibitor of GBA, 8-deoxy-8-azidocyclophellitol (KY170), bound to a fluorescent molecule (BODIPY). This cell-permeable probe binds with a high degree of selectivity to active GBA molecules in the lysosomal compartment of cells *in vitro* and in tissue homogenates.

Behavioral phenotyping: Locomotor activity was monitored using Kinder Scientific Monitor System in 4 or 8 month old wild-type, homozygous *GBA1* D409V KI or hemizygous Thy-1 aSyn WT mice (n = 22/group) in a sound-attenuated room with white noise set to operate at 70 ± 10 db. Total and ambulatory counts were obtained at 5-min intervals for a total of 60 minute test session in the open-field. Data are shown as average counts.



Developing and validating target engagement and pharmacodynamic markers

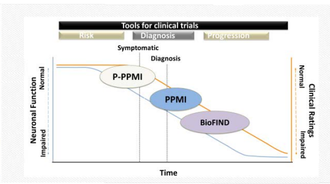


MJFF is funding studies to measure GCase activity/levels reliably and quantifiably in brain tissue, CSF, blood and PBMC's. MJFF is also funding genotyping studies of the *GBA1* locus in clinical studies as well as development of a PET ligand.

Defining clinical outcomes measures and biomarkers in *GBA1* cohorts

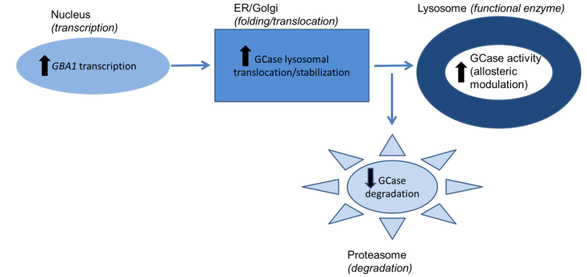
Parkinson's Progression Marker Initiative (PPMI) – Study Details

Study population	400 *de novo* PD subjects (newly diagnosed and unmedicated) 200 age- and gender-matched healthy controls ~70 SWEDD ~70 Prodromal - Olfactory+RBD ~250 LRRK2+ PD manifest and non-manifesting family members ~250 GBA+ PD manifest and non-manifesting family members ~100 SNCA+ PD manifest and non-manifesting family members Subjects will be followed for 3 to 5 years
Assessments/ Clinical data collection	Motor assessments Neurobehavioral/cognitive testing Autonomic, Olfaction, Sleep DaTSCAN, AV133, DTI/RS MRI, Amyloid
Biologic collection/	DNA collected at screening Serum and plasma collected at each visit; urine collected annually CSF collected at baseline, 6mo 12 mo and then annually Samples aliquotted and stored in central biorepository



MJFF initiated recruitment of 250 *GBA1* (125 affected and 125 unaffected) N370S mutation carriers to the Parkinson's Progression Markers Initiative to identify *GBA1*-related biomarkers and inform our understanding of the natural history of PD.

Funding diverse therapeutic programs targeting GCase



MJFF has funded small molecule drug-development programs which increase stabilization and lysosomal translocation of GCase (e.g. Amicus Therapeutics) or direct activation of GCase (e.g. Genzyme), which would increase the levels of functional enzyme in lysosomes.

Summary

MJFF's vision is to apply a holistic strategy to address research and therapeutic challenges to enable accelerated development of *GBA1*-targeting therapeutics and optimally informed clinical trials.

Homozygous *GBA1* D409V knock-in mouse shows significant reductions in GCase activity and GCase probe signal, and significant increase in GlcSph in both brain and liver. Studies are underway for *GBA1* D409V KI mouse cross-bred with aSyn transgenic mouse to determine if loss of GCase function affects aSyn induced pathology and related phenotypes.