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The Michael J. Fox Foundation's efforts to understand the relationship between GBA1 and alpha-synuclein through the development and characterization of preclinical models

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Introduction

Heterozygous mutations in the *GBA1* gene, which encodes lysosomal glucocerebrosidase (GCase), are the most common genetic risk factor for Parkinson's disease (PD). In addition, decreased GCase activity has been reported in both genetic and sporadic cases of PD. Experimental evidence suggests a correlation between decreased GCase activity and accumulation of alpha-synuclein (aSyn). Thus, understanding the potential synergistic effect of increased aSyn and decreased GCase activity is important for understanding how alterations in GCase activity may contribute to or exacerbate PD-related pathology. To enable a better understanding of the relationship between aSyn and GCase activity, The Michael J. Fox Foundation (MJFF) has developed and characterized two mouse models allowing investigation of aSyn pathology in the context of reduced GCase activity. The first model analyzes the neurodegeneration/pathology induced through constitutive overexpression of wild type human alpha-synuclein directed by the murine Thy-1 promoter (hemizygous transgenic) in the context of the GCase activity-reducing D409V mutant form of GBA (homozygous knock-in *GBA1*). The second model analyzes the level of nigrostriatal degeneration and synuclein pathology in the GBA D409V knock-in model versus wildtype mice following stereotaxic injection of aSyn preformed fibrils into the striatum. Here, we outline and discuss the preliminary results of these model characterization efforts. Together, these models provide important platforms for understanding the mechanisms underlying GCase and aSyn dynamics, and for evaluating therapeutics targeting this pathway/relationship.

Characterization of a GBA D409V KI x Thy-1 aSyn Overexpression Mouse Model

Table 1. Summary of the models included in the characterization summary with the abbreviated name, short description of the line, and line identification number at Jackson Laboratories.

Model	Description	JAX ID
C57Bl/6 WT	Wild type C57Bl/6NJ mice obtained from JAX.	5304
GBA D409V KI	Targeted KI of D409V mutation in mouse GBA gene on B6N. Homozygous.	19106
Thy-1 aSyn	Human wildtype aSyn driven by the murine Thy-1 promoter (line 15) on B6N. Hemizygous.	17682
GBA D409V KI x Thy-1 aSyn	Cross of above GBA D409V KI and Thy-1 aSyn lines. Hom GBA x Hemi Thy-1 aSyn.	29124

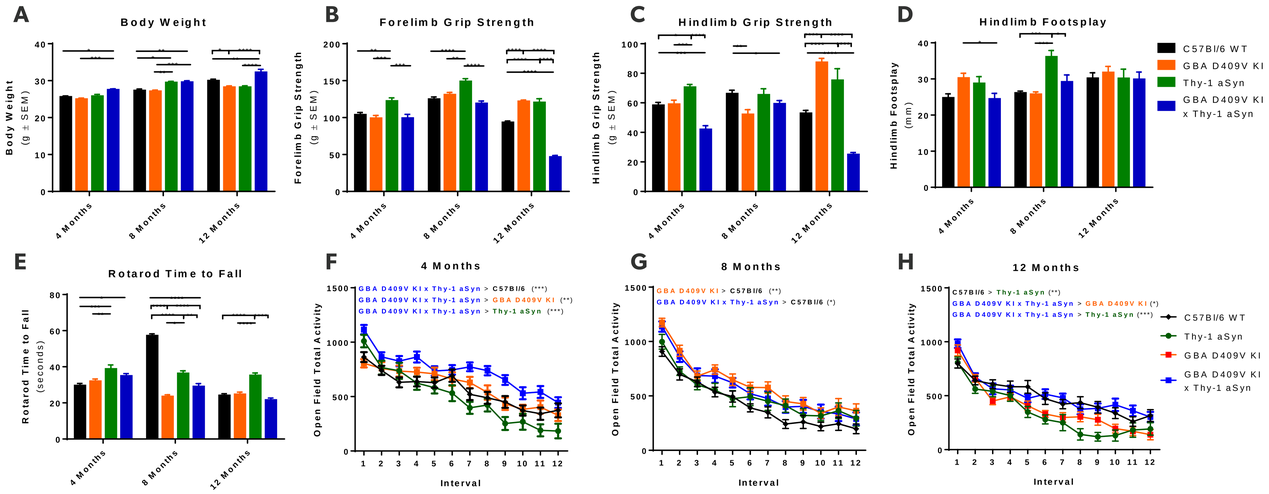

Figure 1. Assessments of motor phenotype demonstrate a potential decrease in grip strength of GBA D409V KI x Thy-1 aSyn mice at 12 months of age but otherwise no significant, sustained differences from C57Bl/6 WT mice. Twelve cohorts of mice were used (4 genotypes at 3 ages) for this characterization study. A) Body weight measures were taken at the designated ages, with animals weighed using a properly calibrated electronic scale. Forelimb (B) and hindlimb (C) grip strength were measured by allowing the mice to grip a bar and pulling back gently to measure the force to release by continued pulling (average of three valid measurements are reported). D) Hindlimb foots play was assessed by painting the heel pads of the hindfeet with nontoxic paint and dropping the mouse over a test sheet on a table while holding to measure the distance between the inner edges of the ink blots (average of three valid measurements are reported). E) Rotarod was performed on a 3cm rotating rod (rotation rate 12 rpm) for a maximum of 120 s intervals with the test session performed after a training session. F-G) Open field locomotor activity was obtained at 5-min intervals for a total of 60 minute test sessions in an open-field using Kinder Scientific Monitor System in a sound-attenuated room with white noise set to operate at 70 ± 10 db. Bars represent mean ± SEM (n= 22 males/group for C57Bl/6 WT, GBA D409V KI, and GBA D409V KI x Thy-1 aSyn; n=15 males/group for Thy-1 aSyn). Two-way ANOVAs were performed for graphs A-E with Bonferroni post hoc tests for significance within ages between genotypes. Two-way repeated measures ANOVAs were performed for graphs F-H with Bonferroni post hoc tests for significance between genotypes. * P<0.05, ** P<0.01, *** P<0.001 **** P<0.0001.

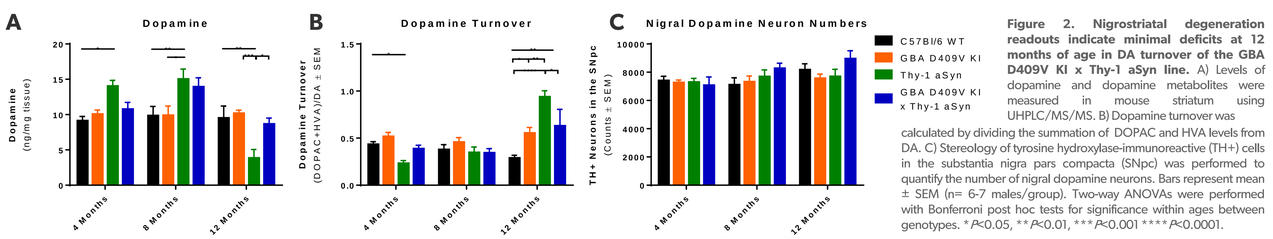

Figure 2. Nigrostriatal degeneration readouts indicate minimal deficits at 12 months of age in DA turnover of the GBA D409V KI x Thy-1 aSyn line. A) Levels of dopamine and dopamine metabolites were measured in mouse striatum using UHPLC/MS/MS. B) Dopamine turnover was calculated by dividing the summation of DOPAC and HVA levels from DA. C) Stereology of tyrosine hydroxylase-immunoreactive (TH+) cells in the substantia nigra pars compacta (SNpc) was performed to quantify the number of nigral dopamine neurons. Bars represent mean ± SEM (n= 6-7 males/group). Two-way ANOVAs were performed with Bonferroni post hoc tests for significance within ages between genotypes. * P<0.05, ** P<0.01, *** P<0.001 **** P<0.0001.

Characterization of the aSyn PFF Model in GBA D409V KI Mice

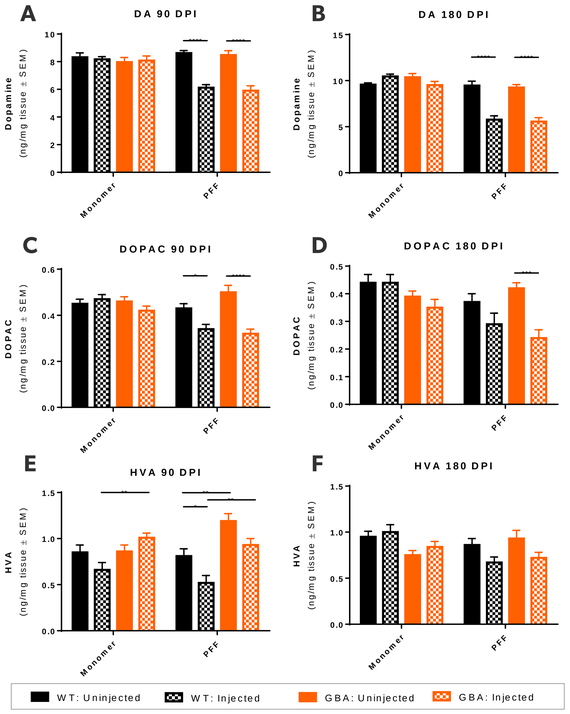

Figure 3. Striatal neurochemistry at 90 and 180 days post-injection of alpha-synuclein preformed fibrils (PFF) reveals decreases in dopamine and DOPAC levels agnostic of genotype, with less consistent changes in HVA between genotypes following PFF administration. Homozygous GBA D409V KI mice (GBA) and wildtype (WT) littermate controls were injected intrastriatally with alpha-synuclein (aSyn) monomers or aSyn PFFs and analyzed 90 (A, C, E) and 180 (B, D, F) days post-injection (DPI) for levels of dopamine (A-B) and the dopamine metabolites DOPAC (C-D) and HVA (E-F) via striatal HPLC. A-B) Dopamine levels were significantly decreased at 90 and 180 DPI following aSyn PFF, but not aSyn monomer, injection in WT and GBA D409V KI mice. No differences were observed between WT and GBA D409V KI mice at either time point. C-D) The dopamine metabolite DOPAC was significantly decreased at 90 and 180 DPI following aSyn PFF, but not aSyn monomer, injection in GBA D409V KI mice. In WT mice, aSyn PFF administration resulted in a significant reduction in DOPAC at 90, but not 180, DPI. No statistically significant differences were observed between WT and GBA D409V KI mice at either time point. E) At 90 DPI (5 months of age), HVA levels were statistically elevated in GBA D409V KI vs WT mice in the ipsilateral and contralateral hemisphere post-PFF injection and in the ipsilateral hemisphere post-monomer injection. HVA was only significantly decreased in WT mice post-PFF injection. F) At 180 DPI (8 months of age), all differences in HVA levels between genotypes and between hemispheres were absent. Two-way ANOVA with Tukey post hoc tests for differences between injected vs uninjected hemisphere and differences between genotypes were performed. N=11-12 mice/group. Bars represent mean ± standard error of the mean. * P<0.05, ** P<0.01, *** P<0.001 **** P<0.0001.

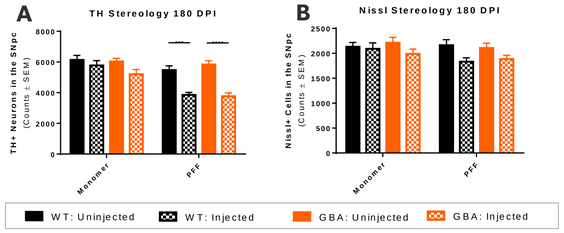

Figure 4. Numbers of tyrosine hydroxylase-positive neurons are significantly decreased in the ipsilateral hemisphere at 180 days post-aSyn PFF injection, regardless of genotype. Stereology was performed in the SNpc of mice using tyrosine hydroxylase (TH) immunostained and Nissl immunostained tissue. A) At 180 DPI, the number of TH-immunoreactive (TH+) neurons in the SNpc were significantly decreased in the ipsilateral hemisphere following aSyn PFF, but not aSyn monomer, injection. No differences were observed between genotypes. B) At 180 DPI, the number of Nissl-immunoreactive (Nissl+) cells in the SNpc that were not TH-immunoreactive were not significantly different between genotype, hemisphere, or injectate. Two-way ANOVA with Tukey post hoc tests for differences between injected vs uninjected hemisphere and differences between genotypes were performed. N=11-12 mice/group. Bars represent mean ± standard error of the mean. * P<0.05, ** P<0.01, *** P<0.001 **** P<0.0001.

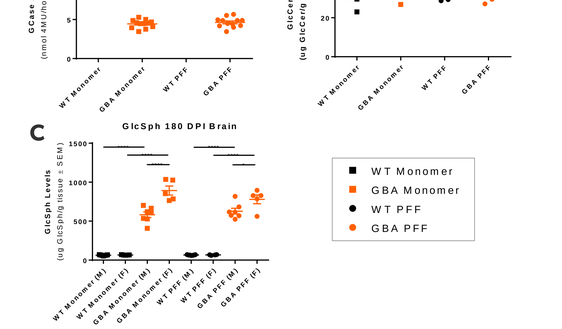

Figure 5. GCase activity and GlcSph levels in the are significantly altered in the GBA D409V KI mice, with no differences between aSyn monomer vs PFF injection. A) GCase activity was measured via the CBE 4MU method in ipsilateral hemisphere brain homogenate lacking striatum (used for HPLC). GBA D409V KI mice exhibit significantly reduced GCase activity as compared to WT mice. aSyn PFF injection had no effect on GCase activity at 180 DPI. B) Glucosylceramide (GlcCer) levels were measured by LC-MS/MS in ipsilateral hemisphere brain homogenate lacking striatum. No differences were observed between WT and GBA D409V KI mice or between aSyn monomer vs aSyn PFF injection. C) Glycosphingolipid (GlcSph) levels were measured by LC-MS/MS in ipsilateral hemisphere brain homogenate lacking striatum. GBA D409V KI mice exhibit significantly greater GlcSph levels as compared to WT mice, with female GBA D409V KI mice consistently displaying greater levels than males. aSyn PFF injection had no effect on GlcSph at 180 DPI. One-way ANOVA with Tukey post hoc tests for differences between injected vs uninjected hemisphere and differences between genotypes were performed. N=11-12 mice/group. Bars represent mean ± standard error of the mean. * P<0.05, **** P<0.0001.

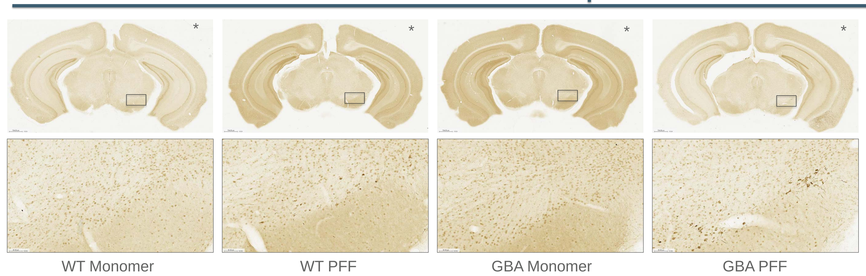

Figure 6. α-Syn striatal PFF injection induces midbrain synuclein aggregation in GBA D409V mice Homozygous GBA D409V KI mice (GBA) and wildtype (WT) littermate controls were injected intrastriatally with alpha-synuclein (aSyn) monomers or aSyn PFFs and analyzed 180 days post-injection. 40um sections of postfixed whole brain were mounted and stained for phospho-Serine129 (pS129). Asterisk (*) indicates treated hemisphere. Area shown magnified in inset is indicated against coronal section. Images shown are representative of overall results for the groups compared. IHC utilized anti-pSer129 aSyn IHC, Wako Cat#010-26481 at a primary dilution of 1:15,000.

MJFF is invested in providing the PD research community with high-quality tools and models to support rapid new discoveries and encourage reliable, reproducible data. Models and studies described in this poster are the result of recent collaborative efforts aimed at generating and characterizing animal models for PD research. More information can be found in the Research Tools Catalog at www.michaeljfox.org/toolscatalog (questions can be sent to tools@michaeljfox.org). In addition, MJFF also offers patient biosamples (eg blood, CSF, plasma, serum, urine, iPSCs) and clinical data to the research community. Information on available biosamples can be found at www.michaeljfox.org/biosamples and data can be found at www.michaeljfox.org/datasets. **Visit us at Booth #2030 to speak with us about these resources and our funding programs.**