AWARD NUMBER: W81XWH-19-1-0264

TITLE: Role of Lipid Dyshomeostasis in Cognitive Dysfunction of Parkinson's Disease

PRINCIPAL INVESTIGATOR: Vidyadhara D J

CONTRACTING ORGANIZATION: Yale University, New Haven**,** CT

REPORT DATE: August 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE:	**2. REPORT TYPE:**	**3. DATES COVERED:**
August 2021	Annual	15Jul2020-14Jul2021

4. TITLE AND SUBTITLE:
Role of Lipid Dyshomeostasis in Cognitive Dysfunction of Parkinson's Disease

5a. CONTRACT NUMBER:
W81XWH-19-1-0264

5b. GRANT NUMBER:
PD180058

5c. PROGRAM ELEMENT NUMBER:

6. AUTHOR(S): Vidyadhara D J

E-Mail:vidyadhara.dj@yale.edu

5d. PROJECT NUMBER:

5e. TASK NUMBER:

5f. WORK UNIT NUMBER:

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Yale University
Rebecca Balentin
105 Wall St
New Haven Ct 06511-8917

8. PERFORMING ORGANIZATION REPORT NUMBER:
4B992

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRAA

11. SPONSOR/MONITOR'S NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:
Lipid dyshomeostasis plays a prominent role in many cognitive disorders envisaging its contribution to cognitive dysfunction of Parkinson's disease (PD). Mutations in *GBA* that encodes glucocerebrocidase 1 (Gcase1) results in accumulation of glycosphingolipids (GSLs) leading to Gaucher disease (GD) and also the most common genetic risk factor for PD. Patients with homozygous *GBA* mutations exhibit PD accompanied by severe cognitive deterioration compared to sporadic cases. Even the heterozygous *GBA* carriers (who do not have GD) are at intermediate risk for developing cognitive dysfunction. A recent study in our lab revealed that the accumulation of glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph) promotes alpha-synuclein aggregation in vitro. Additionally, a mouse line obtained by crossbreeding novel long-lived mouse model of GD (*Gba* L444P KO) with alpha-synuclein transgenic PD mice (*SNCA* tg) featured accelerated PD progression including motor abnormalities (*Gba/SNCA* or GD/PD mice) alongside accumulated GSLs. In this study, we are evaluating if the similar accumulation of GSLs and subsequent cellular and circuit level disturbances in the hippocampus and cortical areas drive cognitive dysfunction in *GBA*-associated PD. To achieve this, We are using our well-established mouse models of GD (*Gba* L444P KO), PD (*SNCA* tg) and GD/PD (*Gba*/*SNCA*) mice along with wildtype controls. Considerable amount of time was spent on establishing these mice colonies in first year. We performed both motor and cognitive behavior assays longitudinally at 3, 6 and 9 months of age on these mice. Motor behavior analysis indicate GD/PD mice to have a severe progressive motor deficit compared GD mice. Results of cognitive behavior assay are being analysed. We standardized immunohistochemistry, confocal microscopy and image analysis methods as well as collected and stored the mice brain specimens at appropriate age for upcoming experiments. Along with publishing a review article, PI was also involved in various professional development and training activities. During the next reporting period, we will perform lipidomics and histopathological studies to correlate with our behavior experiments and identify time course of pathological events with respect to the onset of cognitive dysfunction. We will also perform functional analysis of hippocampus through electrophysiology and conduct sufficiency experiments to evaluate our proposed mechanism of GlcSph and GlcCer mediating cognitive dysfunction in PD. Thus, our study will provide novel insights into the role of GSLs in deciphering cortico-hippocampal dysfunction and associated cognitive decline in PD.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	18	**19b. TELEPHONE NUMBER** (include area code)
Unclassified	Unclassified	Unclassified			

TABLE OF CONTENTS

1. INTRODUCTION:

More than 80% of people with Parkinson's disease (PD) suffer from cognitive dysfunction, which is considered as one of the major unmet needs by the PD patients and their caregivers. Still, little is known regarding the causative mechanisms from circuit to cellular level. Lipid dyshomeostasis is implicated in a growing number of cognitive disorders, envisaging its contribution to cognitive dysfunction associated with PD. Mutations in *GBA* gene that encodes glucocerebrosidase 1 leading to accumulation of glycosphingolipids (GSLs), is the most common genetic risk factor for PD. Cognitive dysfunction is more prevalent and severe in PD patients with *GBA* mutations. Here, we propose to use our well-established mice models of *Gba* mutations with PD phenotype to decipher the role of lipid dyshomeostasis and its mechanisms leading to cognitive dysfunction of PD.

2. KEYWORDS:

Parkinson's disease, cognitive dysfunctions, *GBA*, Lipid dyshomeostasis, glycosphingolipids, glucocerebrosidase 1

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: *To characterize the cortical and hippocampal lipid profile in the brains of Gba KO, SNCA tg and Gba/SNCA mice as a function of age and correlate it with α-synuclein pathology. (n=5/mice line/time point/experiment)*
Major Task 1 : Lipidomics for glycosphingolipids and phospholipids in *Gba* L444P KO, *SNCA* tg and *Gba*/*SNCA* mice at 3, 6, 9 and 12 months of age (1-18 months of project period)
Major Task 2: Immunohistochemistry in *Gba* L444P KO, *SNCA* tg and *Gba*/*SNCA* mice of 3, 6, 9 and 12 months of age (1-24 months)
Major Task 3: Western blotting (1-14 months)

Milestones achieved: Lipidomics for symptomatic age of 12 months is completed (50%). 3, 9 and 12 months mice brain required for immunohistochemistry are collected (60%). 12 months old mice whole brain and cortex samples are collected for Western blotting (50%). Cryosectioning (sagittal) of mice brain, immunostaining to assess neuroinflammation, and confocal/fluorescence microscopy experiments were standardized. Mice required for lipidomics and immunohistochemistry at three months of age are being bred and aged.

Specific Aim 2: *To assess the cognitive capabilities of Gba KO and SNCA tg mice and to evaluate if it worsens in Gba/SNCA mice (n=12/mice line/time point/experiment) (3-15 months)*

Milestones achieved: Completed longitudinal behavior assay for WT (wild type), *SNCA* tg mice (PD mice), *Gba* L444P KO (n=12) and *Gba*/*SNCA* mice (n=13) (GD and GD/PD mice, respectively) at 3, 6, 9 & 12 months of age, except for WT and PD mice at 12 months of age.

Specific Aim 3: *Evaluation of LTP in Schaffer collateral synapses of hippocampal sections and to investigate if AAV-shRNA mediated inhibition of Asah1and Ugcg ameliorate hippocampal pathology and cognitive deficits in Gba KO, SNCA tg and Gba/SNCA mice (16-24 months)*

Milestones achieved: Stereotaxic experiments are standardized. shRNA plasmids for Asah1 and Ugcg are being tested for their transfection efficacy in N2a cells (mouse neuroblastoma cell lines). Mice are being bred for electrophysiology experiments.

What was accomplished under these goals?

A large part of the experiments and usage of funding was delayed due to covid-19 restriction which was in place for 75-80% of the total duration of this annual report (Aug. 2020 – Aug. 2021). PI has applied for a no-cost extension (NCE). PI used the extra time available out of bench to participate in various virtual career and professional development activities as in the "Researcher Development Plan". PI is also writing a review article related to the topic of this grant, which would prove to be valuable in furthering our understanding on cognitive dysfunctions in Parkinson's disease. We could still complete 90% of the behavior experiments, 50% of the immunohistochemistry and lipidomics experiment and 25% of AAV injection experiments. Details are described below. We are continuing to breed the mice to make up for those colonies we had to cut down during covid-19 lockdown. We are on track to complete all the experiments during the period of NCE.

Specific Aim 1: *To characterize the cortical and hippocampal lipid profile in the brains of Gba KO, SNCA tg and Gba/SNCA mice as a function of age and correlate it with α-synuclein pathology. (n=5/mice line/time point/experiment)*

Milestones achieved:

Immunohistochemistry: We collected mice brain and spinal cord specimens for 3, 9 and 12 months time point by intracardial perfusion using paraformaldehyde fixative (n=6/genotype, sex balanced) for all the genotypes. Cryosectioning (sagittal) of mice brain, immunostaining to assess neuroinflammation, and confocal/fluorescence microscopy experiments were standardized.

Lipidomics: We collected fresh brain, liver, and spleen samples at 12 months of age for lipidomics (n=5/genotype) and quickly froze them at liquid nitrogen and stored them at -80°C before subjecting to lipidomics. We have the results for half of these samples for the levels of (GlcCer) and glucosylsphingosine (GlcSph) in the liver and the brain. No difference in the liver GlcCer levels (figure 1, A. average, B. total) was seen across the mice strains. Interestingly, liver GlcSph level was upregulated in GD (Gba KO) and GD/PD (Gba/SNCA) mice (Figure 1C). These results suggest conversion of accumulated GlcCer in GD and GD/PD mice (which lack GCase1, an enzyme that breaks down GlcCer) to its toxic form GlcSph in the liver. In the brain too, GlcCer levels did not change (figure 1 D & E). Though brain GlcSph level showed a trend towards

upregulation in GD and GD/PD mice (figure 1F), it was not significant. We can better interpret the results after performing lipidomics at an earlier age (3 months).

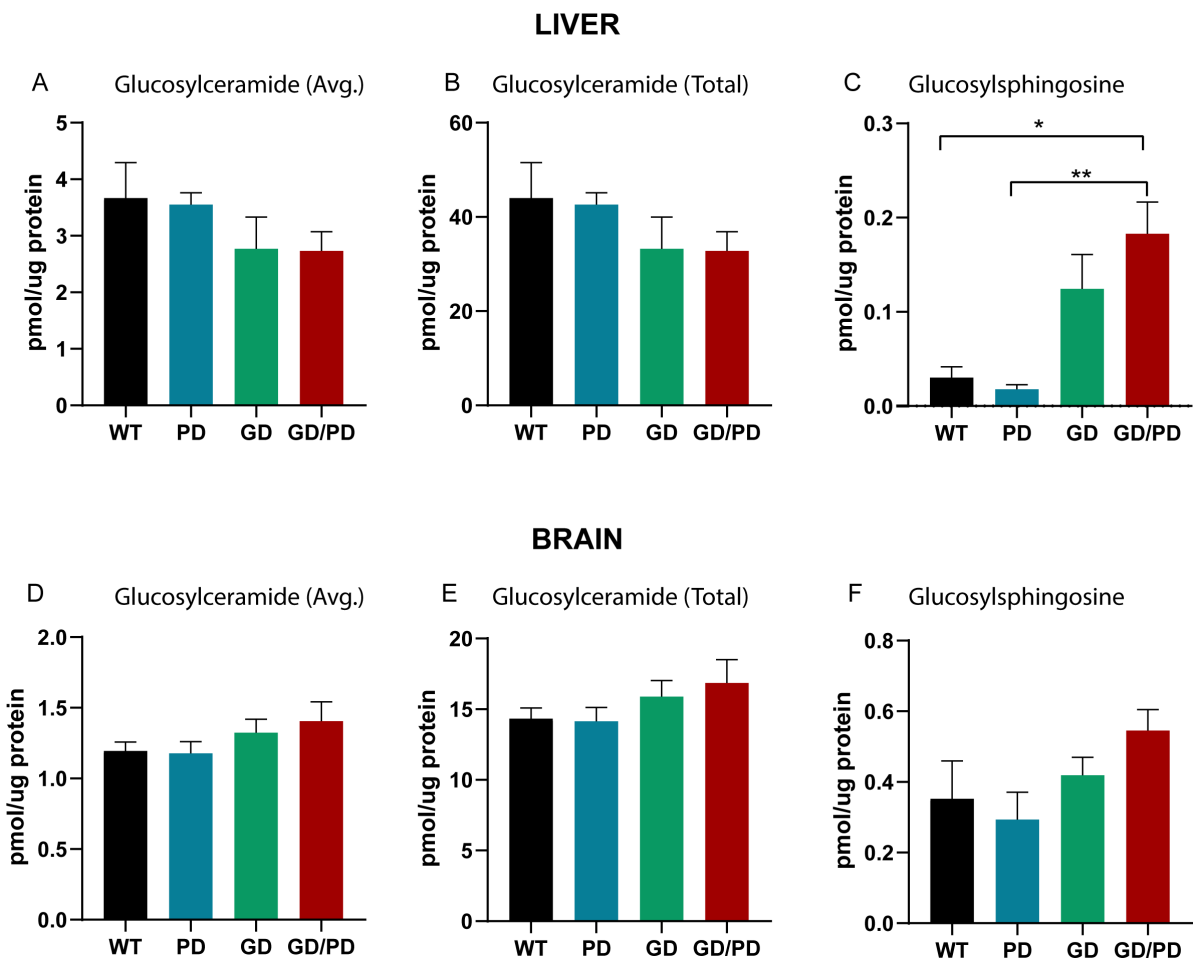

Figure 1: Lipidomics at postsymptomatic age of 12 months. A. Average levels of glucosylceramide in the liver. B. Sum of different glucosylceramide species in liver. C. Glucosylsphingosine level in the liver. D & E. Average and sum of different glucosylceramide species in the brain, respectively. F. Glucosylsphingosine levels in the brain.

Western blotting: We have collected 12 months fresh brain, liver and spleen homogenates, snap frozen in liquid nitrogen and stored at -80 for Western blotting experiment.

Specific Aim 2: *To assess the cognitive capabilities of Gba KO (GD) and SNCA tg mice (PD) and to evaluate if it worsens in Gba/SNCA mice (GD/PD) (n=12/mice line/time point) (3-15 months)*

During the first year of the project, behavior evaluation was completed only on GD and GD/PD mice at 3, 6, and 9 months of age. During second year, along with completing GD and GD/PD mice behavior for 12 months, we also performed behavior experiments on WT and PD mice at 3, 6 and 9 months of age (9 months behavior is yet to be analyzed). Motor behavior evaluation such as balance beam, grip strength and hind limb clasping was performed to assess age at which motor

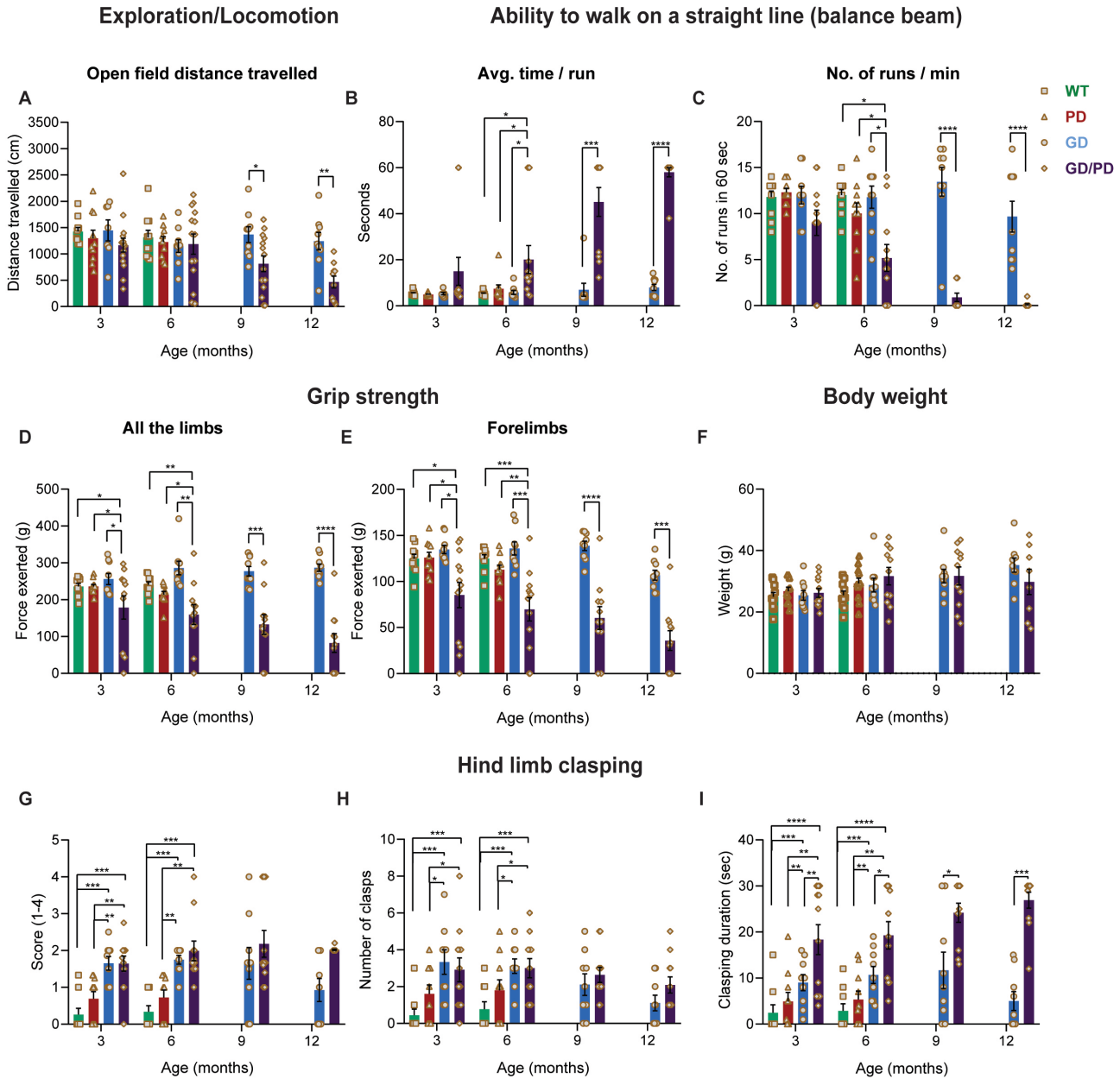

Figure 2: Behavior evaluation in WT, PD, GD and GD/PD mice evaluated longitudinally at 3, 6, 9, and 12 months of age. A. Distance travelled in an open field depicting exploratory behavior. B. Time taken on a balance beam to reach a safety box depicting motor abilities. C. Number of runs mice can perform on a balance beam. D. All the limb grip strength. E. Forelimb grip strength. F. Body weight. G. Scores for hind limb clasping, where higher scores mean increased motor disability. H. Number of clasps, higher they are, higher the disability. I. Clasping duration, higher it is, higher the disability.

behavior deficits that might arise in comparison to cognitive deficits. Cognitive behavior was assessed by open field (exploratory) and novel object recognition test (short- and long-term memory).

Open field behavior: Mice has an innate interest towards exploring a new environment, which is used in open field behavior assay. Distance travelled exploring an open box (Figure 2A) was comparable at 3 and 6 months among all the groups. At 9 months, a significant loss in exploratory/locomotory behavior was noted in GD/PD mice when compared to GD mice, which worsened further at 12 months (Figure 1A). Open field behavior for WT and PD mice at 9 and 12 months is yet to be evaluated.

Balance beam: Balance beam test is used to assess the ability to walk straight on a narrow beam from brightly lit end towards a dark and safe platform/box (Figure 2, B and C). At 3 months of age, all the balance beam parameters such as average time per run (Figure 2B) as well as number of runs per minute (Figure 2C) in PD and GD mice were comparable to WT mice. GD/PD mice showed a trend towards difficulty in performing balance beam task (not significant) compared to all other strains. At 6 months of age, average time per run (Figure 2B) as well as number of runs per minute (Figure 2C) were significantly impaired in GD/PD mice compared to other genotypes. WT and PD mice at 9 and 12 months is yet to be evaluated.

Grip strength: Grip strength (Figure 2, D & E) is a measure of force exerted by the animal in grasping specially designed pull bar assembly of a grip strength meter. A significant loss of grip strength was seen in GD/PD mice when compared to WT, PD & GD mice at 3 and 6 months of age (Figure 2, D. all the limbs, E. Forelimbs only).

Hind limb clasping: Mice, when picked up by the tail and made to descend towards the ground, extend their limbs reflexly in anticipation of contact. Mice with certain neurological dysfunction display hind limb clasping instead of extension. We made mice to perform this maneuver for 30 seconds and scored the hindlimb clasps (0: no clasp; 1: One hind limb clasp; 2: both the hind limbs clasp; 3: Hind limbs and trunk clasp; 4: Hind limbs and forelimbs clasp). Both GD and GD/PD mice revealed severe hind limb clasping when compared to WT and PD mice at 3 and 6 months of age. GD & GD/PD mice revealed a higher score (Figure 2G), a trend towards an increased number of clasps (Figure 2H) and a highly significant increase in clasping duration (Figure 2I). All the genotypes are yet to be analyzed and compared at 9 and 12 months.

Body weight: No significant difference was seen in body weight (Figure 2F). However, with age, there is significant intra-group variability in the body weight in GD/PD mice (Figure 2F).

Specific Aim 3: *Evaluation of LTP in Schaffer collateral synapses of hippocampal sections and to investigate if AAV-shRNA mediated inhibition of Asah1and Ugcg ameliorate hippocampal pathology and cognitive deficits in Gba KO, SNCA tg and Gba/SNCA mice (16-24 months)*

Milestones achieved:
We prepared the shRNA plasmids for ASAH1(Figure 3, green) and UGCG, and tested them for transfection efficacy in N2a cells (mouse neuroblastoma cell lines) (Figure 3, blue). We then tested them for their knockdown of Asah1 and Ugcg protein using Western blotting. For ASAH1, 4 different shRNA plasmids were tested for their transfection efficiency (Figure 3, ASAH 051, ASAH 657, ASAH 1045, and ASAH 1232). ASAH 1045 showed the best transfection efficiency among 4. It also showed good knockdown efficiency for asah1 protein as seen in Western blotting (Figure 3, Western blots, 1045). We are now preparing AAV from ASAH 1045 plasmid. Similar experiments are undergoing to decide best shRNA for UGCG.

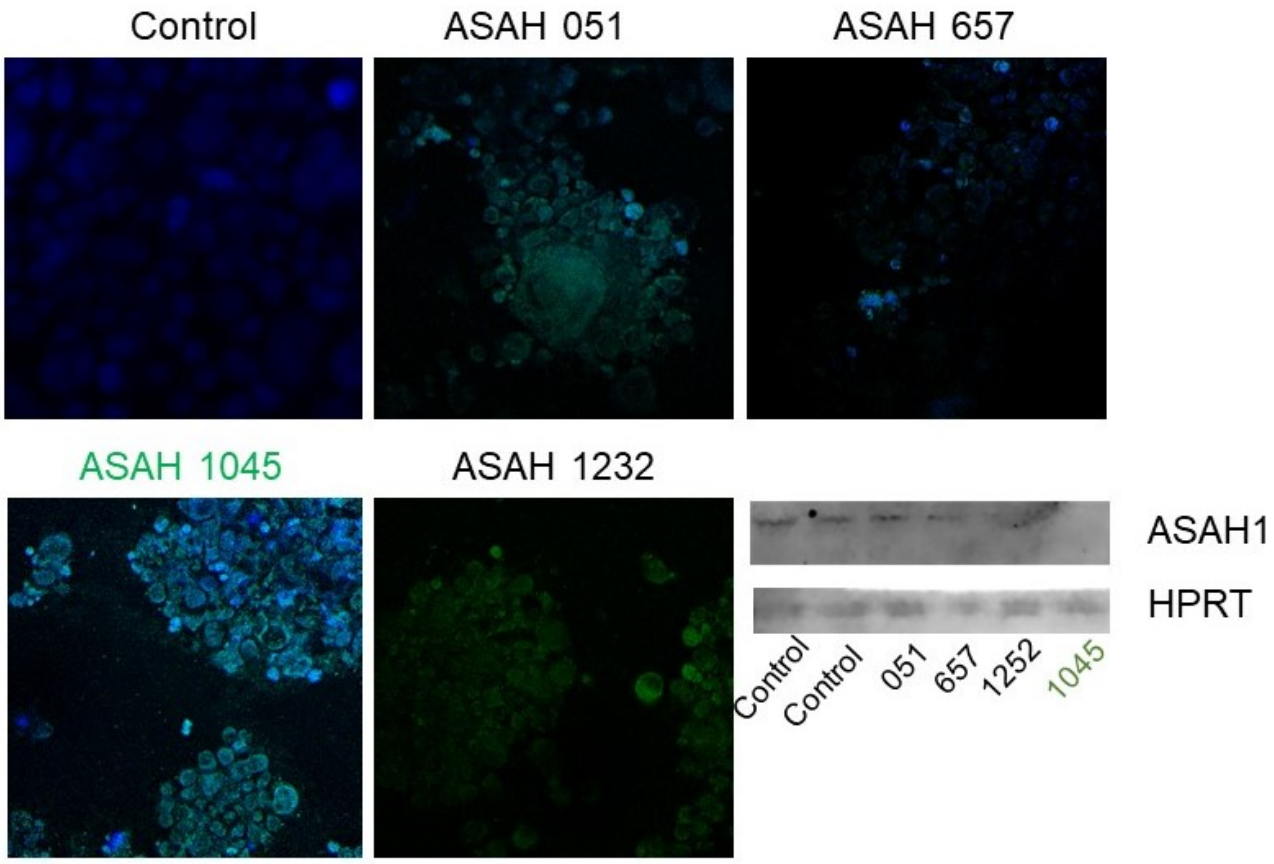

Figure 3: shRNA plasmids of UGCG (green)were tested for transfection efficiency to N2a cells (blue). Note highest transfection efficiency in ASAH 1045, and its efficiency to knockdown asah1 protein seen as no asah1 expression on Western blot.

Stereotaxy experiments for AAV-shRNA injections are standardized.

What opportunities for training and professional development has the project provided?

Training: In this reporting year, PI was trained in newer techniques such as *ex vivo* imaging of mice brain sections for dopamine transporters, AAV-stereotaxic injections, sample collection for lipidomics, proteomics data analysis and image analysis of electron microscopy samples. PI established successful collaboration with Dr. David Sulzer lab at Columbia University to study endolysosomal dysfunction in PD using cell culture models. PI also regularly interacts with Dr. Pramod Mistry who is a leader in *GBA*-related studies and his lab members through joint lab meetings. This is helping him develop a clinical perspective on *GBA*-associated PD. Dr. Sreeganga Chandra, who is the mentor in this project interacts with PI one-on-one every week and helps him with the resources and provide suggestions on the techniques and concepts. Overall administrative process to get all the regulatory approvals, renewing the animal protocol, utilizing the grant money, and applying for no-cost extensions was also a learning experience for the PI, especially in these difficult times of Covid-19.

Professional development: PI had a detailed discussion with his mentor Dr. Chandra on his career interests and professional development plan in the beginning of calendar year 2021. Below are few of his accomplishments for this report year.

a. Mentored three Yale undergrads for their "Senior Thesis".
b. Completed 80% of Certificate of College Teaching Preparation (CCTP).
c. Guest Editor, Methods Collection on Neurodegenerative Disorders, Journal of Visualized Experiments (JoVE).
d. Reviewed articles for Experimental Neurology, Frontiers in Pharmacology, Translational Stroke Research, BMC Complementary Medicine and Therapies, The FEBS Journal, & MDPI Nutrients.
e. Adjunct faculty at Dept. of Physiology, Kasturba Medical College, Manipal Academy of Higher Education, India.
f. Attended Introduction to Biomedical Data Science and Health Informatics course.
g. Attended CSHL Neurodegenerative Diseases: Biology & Therapeutics conference.
h. Attended Yale innovation summit, 2021.
i. Member of Yale Neuroscience Postdoc Committee.
j. Founding member Yale Neuroscience Committee for Diversity, Equity & Inclusion activities.
k. Organizer & Presenter at Yale Neuroscience Flipped Science Fair outreach program.
l. Judge at Yale-Hunter Conference, USA.
m. Judge at Annual Biomedical Research Conference for Minority Students.
n. Judge at Yale Undergrad Research Symposium.
o. Mentor at Yale Biological & Biomed. Sciences Diversity & Inclusion Collective.
p. Participating in Yale Postdoc Associations's Peer Mentoring Program, Postdoc-Faculty Mentoring Program and other career development activities.
r. Became member of International Parkinson and Movement Disorder Society, Aligning Science Across Parkinson's (ASAP), National Center for Faculty Development & Diversity and National Council for Behavioral Health.
s. Gave a talk in the Oxford College of Science, Bangalore University, India
t. Gave a talk in Indian Academy of Neuroscience - Bangalore Chapter Symposium
u. Gave a talk in Cold Spring Harbor Laboratory meeting on Protein Homeostasis in Health and Disease
v. Gave a talk in International Congress of Parkinson's Disease and Movement Disorders, 2020

How were the results disseminated to communities of interest?

PI discussed the developments of the project with Dr. Pramod Mistry and his lab members, who are working extensively on Gaucher Disease and other *GBA*-related disorders. PI presented the work in few meetings, as well as writing a review article related to the topic of this grant, which would prove to be valuable in furthering our understanding on cognitive dysfunctions in Parkinson's disease.

What do you plan to do during the next reporting period to accomplish the goals?

We couldn't complete the project in the intended timeline due to Covid-19 related restricted access to the laboratory as described in the appropriate section. However, we will be able to meet all the goals during the period of no-cost extension.

1. We will perform lipidomics at presymptomatic time-point of 3 months for all the genotypes. We are halfway through for validating lipidomics results by immunohistochemistry and Western blotting. We will use these techniques to also assess how perturbances in lipid levels influence the α-synuclein pathology and neuronal, glial, and synaptic health in the cognitive brain areas. This will cover the objectives of specific aim 1.
2. As a part of specific aim 2, we will analyze the accumulated data of Novel Object Recognition for WT, PD, GD and GD/PD mice. We will complete last time point of 12 months behavior for WT and PD mice, Correlating this with lipidomics and histopathological studies will allow us to identify the time course of pathological events and the onset of cognitive dysfunction.
3. We will evaluate these mice at 6 months of age for hippocampal Long Term Potentiation (LTP). This is widely used to assess hippocampal functions that can be directly correlated to biological and behavioral phenotypes. We will perform this experiment in collaboration with Dr. Pablo E. Castillo, Albert Einstein College of Medicine, New York. Also, sufficiency experiments for the proposed mechanism of GlcSph and GlcCer mediating cognitive dysfunction in PD by AAV-shRNA-mediated silencing of *Asah1* and *Ugcg* are under progress. We will assess whether this silencing successfully restores hippocampal health and cognitive function. These experiments will complete the goals of our specific aim 3.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

PD is diagnosed through motor abnormalities, and within several years, 80% of patients develop cognitive problems. Knowing this statistic provides a window for preventive measures but understanding the mechanisms through which cognitive problems arise is crucial. This work will provide major breakthroughs in this area and identify lipids as important modifiers of cognitive dysfunction in PD. Future therapies may be developed targeting lipid dyshomeostasis to prevent cognitive dysfunction in PD patients, which is considered one of the main unmet needs. There are already several drugs commercially available that blocks accumulation of glycosphingolipids (GSLs) such as GlcSph in cancer. Drugs targeting GlcCer are under clinical trials for GD. These two GSLs are the key targets of our study. If we are able to successfully establish the role of these in cognitive dysfunction of PD, those drugs can be repurposed to be used in PD, which would help patients in the near future. This study will also provide evidence to support if *Ugcg* and *Asah1* which produce GSLs can be the potential targets to treat cognitive decline in PD.

What was the impact on other disciplines?

GBA mutations are also known to increase risk for Dementia with Lewy Bodies (DLB), which is closely related to PD. Thus, our work will broaden the therapeutic landscape for treatment of cognitive dysfunction also in other synucleinopathies such as DLB, along with unravelling the causative mechanisms.

What was the impact on technology transfer?

We have streamlined a collaborative effort to perform lipidomics, which could be used in other projects too. The complicated breeding experiments involved in this study allowed us to standardize various genotyping protocols which could be useful for other studies too. The stereotaxic apparatus set-up and the standardization of AAV injection methodology could also be used in other studies. Expertise in breeding methods enabled us to help others in the lab to generate mice for large-scale omics studies.

What was the impact on society beyond science and technology?

Even after more than 200 years of its first description, PD remains predominantly a movement disorder even in the public domain. It is important to sensitize the caregivers and the community about the immense cognitive and psychiatric disturbances the patients go through. We will communicate the intent and the results of our study to the public in lay words in all the available digital and print platforms.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Effects of Covid-19: Restricted/limited access to the laboratory facilities was in place for most part of this reporting period, which has delayed our experiments and using allotted budget. PI also couldn't use the budget allotted for travel and attending conferences due to travel restrictions. We have requested for a no-cost extension to complete the project. PI used the extra time available out of bench to participate in various virtual career and professional development activities as in the "Researcher Development Plan". PI is also writing a review article related to the topic of this grant, which would prove to be valuable in furthering our understanding on cognitive dysfunctions in Parkinson's disease.

There was an inadvertent error in breeding and genotyping to obtain GD/PD mice, which delayed the experiments. This has been corrected now.

Changes that had a significant impact on expenditures

Shutdown or restricted entry to our laboratory due to Covid-19 pandemic for most of this reporting period along with limited access to the common facilities have significantly delayed our experiments. Because of this, we could not use 35-40% of the allotted grant for the budget period 1. This also includes grant that was allotted for travel and attending meetings. We plan to utilize the remaining budget in the period of no-cost extension.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: *Vidyadhara D J, PhD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0003-0974-0307
Nearest person month worked: 12
Contribution to Project: Dr. Vidyadhara has obtained Yale IACUC and ACURO approvals, procured mice and set-up breeding colonies, all the experiments, data analysis, purchase of consumables and prepared necessary progress reports.

Name: *Sreeganga S. Chandra, PhD*
Project Role: *Mentor*
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0001-9035-1733
Nearest person month worked: 12
Contribution to Project: Dr. Chandra has obtained IACUC approval for her proposal, number 2018-11117 which is in congruent with this proposal. Dr. Chandra has also mentored PI at all the stages of experiments towards successful execution of the grant.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable

QUAD CHARTS:

Submitted.

9. APPENDICES:

Not applicable