Award Number: W81XWH-18-1-0233

TITLE: Harnessing Neuroplasticity Genes to Combat Synucleinopathy-Mediated Axonopathy

PRINCIPAL INVESTIGATOR: Joseph Patterson PhD

CONTRACTING ORGANIZATION: Michigan State University

REPORT DATE: JUNE 2020

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 *(From - To)*
JUNE 2020	Annual	1 Jun 2019 - 31 May 2020

4. TITLE AND SUBTITLE

Harnessing Neuroplasticity Genes to Combat Synucleinopathy-Mediated Axonopathy

5a. CONTRACT NUMBER
W81XWH-18-1-0233

5b. GRANT NUMBER
GRANT12471958

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Joseph Patterson, and Caryl Sortwell

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Translational Science and Molecular Medicine
Michigan State University
Grand Rapids Research Center
400 NW Monroe Ave.
Grand Rapids MI, 49503

8. PERFORMING ORGANIZATION REPORT NUMBER

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)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The earliest stages of synucleinopathy have been difficult to study due to the fact that most animal models of Parkinson's disease (PD) fail to recapitulate the progression of synucleinopathy to neurodegeneration. The alpha-synuclein (α-syn) preformed fibril (PFF) synucleinopathy model exhibits a distinct stage of accumulation of α-syn inclusions in tyrosine hydroxylase immunoreactive (THir) neurons in the substantia nigra pars compacta (SNpc) months prior to the ultimate degeneration of the nigrostriatal system. In the context of the early phases of synucleinopathy in the α-syn PFF model, laser capture microdissection was used to collect phosphorylated α-syn (pSyn) immunoreactive SNpc neurons in PFF-injected rats and SNpc THir neurons in control-injected rats. RNA was isolated and RNASeq used to identify gene expression changes between SNpc neurons with and without pSyn inclusions. Results from male rats have identified 102 candidate genes. Of these candidate genes, a subset associated with neuroplasticity or synaptic plasticity were validated in male and female rats with digital droplet PCR. Based on the validation results, we have selected SYN1 and CPLX2 as genes we will design adeno-associated viruses to overexpress in the next phase with the goal to mitigate neurodegeneration.

15. SUBJECT TERMS
Synuclein, Synucleinopathy, neurodegeneration, RNASeq

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	7	19b. TELEPHONE NUMBER *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)

Table of Contents

Page

Introduction……..4

Body……..5

Key Research Accomplishments……..6

Reportable Outcomes……6

Conclusion……6

References……7

Appendices……7

Introduction

Parkinson's Disease (PD) is the second most common neurodegenerative disease. Hallmarks of PD include motor impairment, progressive degeneration of the nigrostriatial dopamine (DA) neurons, and the accumulation of Lewy Bodies (LBs), or cellular inclusions containing alpha-synuclein (a-syn). The first genetic links to familial PD were mutations and copy number variants in the a-syn gene (*SNCA*) [1-4]. Though heredity is a factor in ~5-10% of total cases, the overall the etiology of sporadic PD is unknown. In regards to neurodegeneration, the loss of axons or axonopathy has been shown in animal models, as well as patients, to occur prior to the loss of cell bodies of the nigrostriatal neurons [5]. Furthermore, there is abundant evidence to support the role in early PD of impaired axonal guidance and transport, degeneration of synapses, as well as dysregulation of genes encoding proteins known to interact with a-syn in the synapse [6]. Ultimately, the pathophysiological mechanisms in PD appear to culminate in overt axonopathy, with near complete denervation of the caudate putamen within four years of PD diagnosis, preceding degeneration of nigral soma [7].

In the a-syn PFF-seeded synucleinopathy model, exogenous a-syn PFFs injected into the striatum are taken up into nigrostriatal terminals, leading to the templating, hyper-phosphorylation, accumulation, and subsequent formation of endogenous a-syn oligomers and fibrils (Figure 1A). In the both the terminals and cell bodies of the substantia nigra pars compacta (SNpc) there is accumulation of inclusions that are positive for phosphorylated a-syn (Figure 1B), Thioflavin-T and ubiquitin as well as proteinase-K resistant. In addition to the SNpc, inclusions are also present in the cortex, thalamus, and amygdala; regions with neurons that also innervate the striatum [8]. An overview of the time course of events in the a-syn PFF-induced synucleinopathy cascade in the rat nigrostriatal system is illustrated in Figure 1C. Specifically, a-syn hyperphosphorylated inclusions (gray line) form in the SNpc as early as one month, and peak around 1-2 months post-injection. At this same early time point a significant increase in tyrosine hydroxylase (TH) immunoreactivity is observed in the striatum (red line, Figure 1C, [9]), suggesting compensatory changes in the axon terminals in response to accumulating inclusions. This response may be linked to differential gene expression of neuroplasticity genes. At two months, nigral terminals and cell bodies begin to lose their dopaminergic phenotype as evidenced by loss of TH (green line, Figure 1C). Neuronal loss in the SNpc (cell death rather than earlier loss of phenotype)

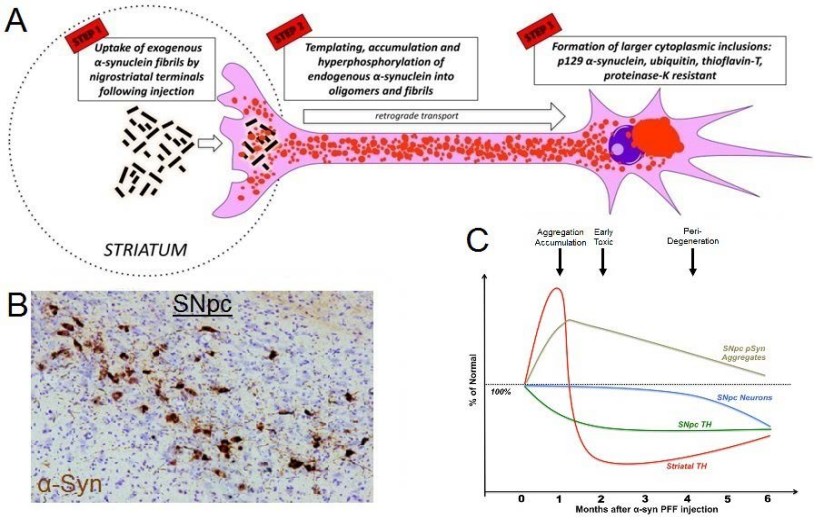

Figure 1. The a-syn PFF-seeded synucleinopathy model. ***A.*** *Diagram showing the uptake a-syn PFFs in the striatum and subsequent formation of inclusions in the SNpc.* ***B.*** *Representative image of inclusion containing SNpc neurons at one month post-injection.* ***C.*** *Time-course of inclusion formation, TH expression and neurodegeneration.*

however, does not occur until months 4-6 post-injection (blue line) associated with a >50% reduction in striatal DA tissue content. Using our current optimized parameters we can achieve 50% SNpc degeneration at this time point [9]. Further, loss of SNpc neurons occurs bilaterally despite the fact a-syn that inclusions are observed only within the ipsilateral SNpc [9]. Thus, the nigral synucleinopathy induced by a-syn PFF injection reveals at least three distinct stages:

1) *Month 1: Aggregation and Accumulation Phase*:
   Inclusions are newly formed/forming, neurons and terminals attempt to compensate.
2) *Month 2: Early Toxic Phase:* Decrease in TH protein in both the soma and terminals.
3) *Month 4: Peri-Degeneration Phase:* Nigral neuron death initiates and continues.

The a-syn PFF model presents a unique opportunity to examine gene expression changes that occur throughout the progression of the dynamic phases of synucleinopathy, specifically during aggregate accumulation and early toxic phases. In the PD brain, pathology of nigrostriatal terminals is likely to be the earliest consequence of synucleinopathy in the SNpc [7].

The project seeks to use laser capture microdissection (LCM) to specifically isolate DA neurons in the SNpc, followed by RNA sequencing (RNASeq) to identify genes associated with neuroplasticity altered in early phases of synucleinopathy in male and female rats. Candidate genes from the RNASeq results can then be selected and manipulated with adeno-associated viruses (AAVs) designed to increase or knockdown expression of the candidate gene. Manipulation of these candidate genes will then be tested in the context of the PFF model to determine if synucleinopathy has been mitigated.

Body

Throughout these two years, the project has been met with many issues that are out of our control, along with some difficulties associated with optimization of the technique use, laser capture microdissection. In terms of issues that were out of our control, we initially had a delayed start due to the financial processing associated with the university, an issue with a cohort of rats that were of a mixed genotype due to breeding issues at Charles Rivers Laboratories (set us back months), and most recently, we have been delayed due to COVID-19. The COVID-19 pandemic has led to supply/reagent shortages, closure of the sequencing facility we use, a 3-4 month time period where we were restricted from the lab, and additional weeks of quarantining for myself based on contact tracing. Based on all of these issues/setbacks, I requested and was granted a no cost extension (NCE) until June 2021 on the project.

During the last year, laser capture microdissection was further optimized and remaining female samples prepared for RNASeq. Sending these samples for RNASeq has been delayed due to multiple closures due to COVID-19, however, remaining RNA has been used to validate gene changes observed previously in the male RNASeq results. From the RNASeq results from the males, a number of genes involved in neuroplasticity and synaptic plasticity were examined with remaining RNA in order to validate the changes with droplet digital PCR. Genes changes selected for validation that were consistent with the decrease observed via RNASeq were SNCA, SYN1, SYN3, MAP2, CPLX1, CPLX2, SYT2, SYT3, UBE3A, RPTOR, SLC6A3, SLC18A2, and RIMS1; where From

these, all showed significant decreases in transcript except for SNAP25, FOXP1, BDNF, SYN2, STX3, DNAJC5 did not significantly change in the validation study. Based on the magnitude of decrease in males, the decreased expression of some of these genes were examined in the female cohort. SNCA, SYN1, CPLX2, MAP2, SLC6A3, SLC18A2, and UBE3A are all also downregulated in the female cohort. Based on this criteria, two genes related to synaptic plasticity that also appear to be critical components in neurotransmission were selected for the final portion of the project, the AAV-overexpression of these genes in order to determine if axonopathy could be mitigated. At the time of our COVID-19 shutdown, we had selected SYN1 and CPLX2 as our genes to overexpress. We are currently gearing up to work on cloning these genes in to the viral backbone for overexpression in neurons.

Key Research Accomplishments

- Lobbied Congress with other researchers in conjunction with the Michael J Fox Foundation for the increase and continuation DOD CDMRP and NIH funding (Researchers on the Hill Day 2019).

Reportable Outcomes

- Poster presentation at the 2019 World Parkinson's Congress (Kyoto, Japan)
- Selected for the poster tour at the 2019 World Parkinson's Congress (Kyoto, Japan)
- Poster presentation at 2019 Society for Neuroscience meeting (Chicago, Il)
- Research talk at 5th Annual Udall Center for Parkinson's Disease Research Symposium (Ann Arbor, Michigan)
- Selected for virtual poster at 2020 Movement Disorder Society Congress
- A hit from RNASeq not related to the neuroplasticity aim of this project indicated phosphoglycerate kinase 1 (PGK-1) is downregulated in early synucleinopathy. This result was used as supporting data for a Michael J Fox Foundation grant, "Assessment of the Neuroprotective Potential of Terazosin via PGK-1 Enhanced Glycolysis and Mitochondrial Function", that will be funded starting in September.

Conclusions

The project has led to the identification of 102 potential candidate genes in male rats, a subset of which is related to neuroplasticity and synaptic plasticity and have since been validated in male and female rats. Whether modulation of gene expression of these genes can mitigate axonopathy or subsequent neurodegeneration is still unknown, but is in the progress of being answered.

References

1. Polymeropoulos, M. H. Autosomal dominant Parkinson's disease and alpha-synuclein. *Annals of neurology* 44, S63-64 (1998).
2. Polymeropoulos, M. H. *et al.* Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276, 2045-2047 (1997).
3. Singleton, A. B. *et al.* alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302, 841, doi:10.1126/science.1090278 (2003).
4. Spillantini, M. G. *et al.* Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neuroscience letters* 251, 205-208 (1998).
5. Dauer, W. & Przedborski, S. Parkinson's disease: mechanisms and models. *Neuron* **39**, 889-909 (2003).
6. Dijkstra, A. A. *et al.* Evidence for Immune Response, Axonal Dysfunction and Reduced Endocytosis in the Substantia Nigra in Early Stage Parkinson's Disease. *PloS one* **10**, e0128651, doi:10.1371/journal.pone.0128651 (2015).
7. Kordower, J. H. *et al.* Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain : a journal of neurology* **136**, 2419-2431, doi:10.1093/brain/awt192 (2013).
8. Wall, N. R., De La Parra, M., Callaway, E. M. & Kreitzer, A. C. Differential innervation of direct- and indirect-pathway striatal projection neurons. *Neuron* 79, 347-360, doi:10.1016/j.neuron.2013.05.014 (2013).
9. Paumier, K. L. *et al.* Intrastriatal injection of pre-formed mouse alpha-synuclein fibrils into rats triggers alpha-synuclein pathology and bilateral nigrostriatal degeneration. *Neurobiology of disease* 82, 185-199, doi:10.1016/j.nbd.2015.06.003 (2015).

Appendices

None