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TITLE: Apolipoprotein E Regulation of Alpha-Synuclein Spreading and Functional Connectivity in Parkinson's Disease Dementia

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CONTRACTING ORGANIZATION: WASHINGTON UNIVERSITY

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14. ABSTRACT

Parkinson disease (PD) is characterized by accumulation of protein aggregates composed of the protein alpha-synuclein (aSyn) in structures termed Lewy bodies (LB) and Lewy neurites (LN) in multiple brain regions. Although motor symptoms are often the most visible features of the illness and are thought to relate to abnormalities in nigro-striatal dopaminergic signaling, PD also causes dementia with prominent cognitive and psychiatric symptoms in many patients. The molecular and circuitry mechanisms that lead to PD dementia are not clear, and there are no effective treatments to prevent or slow progression of dementia in PD. Multiple studies indicate that pathological aSyn aggregation plays a key role in PD, and a growing number of reports now demonstrate that aSyn spreading can be modeled in vivo by stereotaxic injection of aSyn preformed fibrils (PFFs) into the striatum where it then spreads in a time-dependent manner to multiple connected regions including the substantia nigra as well as the cortex and amygdala. Genetic association studies implicate the apolipoprotein E (APOE) genotype as a strong risk factor for dementia in PD. Our preliminary data indicates that apoE isoforms directly regulate aSyn spreading within the nigro-striatal circuit, but whether APOE genotype affects aSyn spreading in neocortical and limbic regions, and whether this contributes to deficits in cognitive behavior and disruption of function connectivity in brain networks is unknown. Hypothesis: APOE4 potentiates aSyn spreading to cortical and limbic regions, disrupting functional

15. SUBJECT TERMS

NONE LISTED

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1. INTRODUCTION:

Parkinson disease (PD) is characterized by accumulation of protein aggregates composed of the protein alpha-synuclein (aSyn) in structures termed Lewy bodies (LB) and Lewy neurites (LN) in multiple brain regions. Although motor symptoms are often the most visible features of the illness and are thought to relate to abnormalities in nigro-striatal dopaminergic signaling, PD also causes dementia with prominent cognitive and psychiatric symptoms in many patients. The molecular and circuitry mechanisms that lead to PD dementia are not clear, and there are no effective treatments to prevent or slow progression of dementia in PD. Multiple studies indicate that pathological aSyn aggregation plays a key role in PD, and a growing number of reports now demonstrate that aSyn spreading can be modeled *in vivo* by stereotaxic injection of aSyn pre-formed fibrils (PFFs) into the striatum where it then spreads in a time-dependent manner to multiple connected regions including the substantia nigra as well as the cortex and amygdala. Genetic association studies implicate the apolipoprotein E (*APOE*) genotype as a strong risk factor for dementia in PD. We recently published that apoE isoforms directly regulate aSyn spreading within the nigro-striatal circuit, but whether *APOE* genotype affects aSyn spreading in neocortical and limbic regions, and whether this contributes to deficits in cognitive behavior and disruption of functional connectivity in brain networks is unknown. The purpose of this project is therefore to test the hypothesis that *APOE* genotype regulates spreading of aSyn pathology to cortical and limbic regions, negatively affecting functional connectivity and behavior related to these regions.

2. KEYWORDS:

Parkinson disease, Parkinson disease dementia, Lewy body dementia, alpha-synuclein, apolipoprotein E, pre-formed fibril, cortex, amygdala, striatum, cognition, functional connectivity

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**

Specific Aim 1	Timeline	% Complete
Major Task 1: Generate mice and perform aSyn pre-formed fibril (PFF) Injections	Months	
Validate efficacy of aSyn PFFs in wildtype mice, including controls with aSyn monomer in wildtype mice and aSyn PFFs in aSyn knockout mice	1-3	100
Breed Apoe KO and APOE2, APOE3, and APOE4 mice	1-6	100
Inject cohorts of mice with aSyn PFFs	6-9	60
Local IACUC Approval (amendment needed for collaboration with Dr. Bauer for fcOIS)	1	100
Major Task 2: Assess pathology in neocortical and limbic regions		
Perfuse mice, section brains, and perform immunohistochemistry for pSyn, GFAP, CD68	10-14	60
Quantify and map brain pathology	12-16	20
Perform statistical analysis to relate APOE genotype to brain pathology	12-16	0
Specific Aim 2		
Major Task 3: Cognitive Testing in mice injected with aSyn PFFs		
Perform cognitive test battery in Apoe KO and APOE2, APOE3, and APOE4 mice injected with aSyn PFFs	12	80
Data analysis and correlation with brain pathology	12-14	0
Specific Aim 3		
Major Task 4: fcOIS imaging in mice injected with aSyn PFFs		
Validation of functional connectivity phenotype in aSyn PFF injected mice vs aSyn monomer control	1-6	100
fcOIS imaging in Apoe KO and APOE2, APOE3, and APOE4 mice injected with aSyn PFFs	7-13	80
Data analysis and correlation with brain pathology	9-15	0

   - **What was accomplished under these goals?**

1) **Major activities** during this project period included: 1) injection of cohorts of APOE knockin mice with aSyn PFFs, 2) interim behavior and fcOIS analysis at 3 months post-injection .

2) **Specific objectives** of this project include: 1) Investigate the effect of *APOE* genotype on aSyn spreading in cortical and limbic regions, 2) Determine the effect of *APOE* genotype on cognitive behavior in the context of pathological aSyn aggregation, and 3) Determine the impact of aSyn spreading on brain functional connectivity.

3) **Significant results** obtained during this project period include the following:

a. **Optimization of measurement of aSyn PFF-induced disruption of functional connectivity in living mice**. As described in our application, we have partnered with Dr. Adam Bauer in the Department of Radiology at Washington University who has expertise in measuring functional connectivity in living mice. This is performed using optical measurement of oxy- and deoxy-hemoglobin with a technique called functional connectivity – optic intrinsic signaling (fcOIS). Over the last project period we have continued to optimize measurement of functional connectivity using fcOIS in mice injected with aSyn PFFs, including assessment of anesthesia to improve signal to noise ratio. We have collected two fcOIS datasets for APOE knocking mice injected with aSyn PFFs and are currently analyzing that data.
b. **Behavioral analysis in APOE knockin mice injected with aSyn PFFs.** We did not observe any statistically significant differences in cognitive behavior across APOE genotypes at an interim timepoint after aSyn PFF injection. Since then we continued to age the mouse cohorts and have repeated the behavioral tests. These data are currently being analyzed.

c. **Stated goals not met**:
    i. We have not completed analysis of behavior, functional connectivity, and immunohistochemistry phenotypes in the cohorts of APOE knockin mice injected with aSyn PFFs.

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

This project has provided Dr. Davis with multiple opportunities for training and professional development, as outlined below:

1) Expand knowledge of basic cellular and molecular mechanisms of neurodegenerative disorders:
    a. Dr. Davis attended weekly journal club and seminar series through the Washington University Department of Neurology, Movement Disorders Section, Division of Biological and Biomedical Sciences, and Hope Center for Neurological Disorders.
    b. Dr. Davis developed curriculum content and delivered lectures to Washington University medical students and graduate students.
    c. Dr. Davis gave two platform presentations at the 2020 American Neurological Association annual meeting on topics separate from but related to this project.

   d. Dr. Davis gave a presentation at Neurology Grand Rounds at Vanderbilt University.
2) Gain expertise in basic scientific methods:
   a. Dr. Davis adapted methods for recombinant protein expression and purification and refined stereotaxic surgical techniques for induction of aSyn pathology in mice.
   b. Dr. Davis attended regular meetings and discussions with mentors and collaborators related to basic science laboratory work.
3) Obtain formal instruction in lipoprotein biology: Dr. Davis is participating in the formation of a new lipid research interest group in the Hope Center for Neurological Disorders at Washington University.
4) Continue clinical practice and gain exposure to clinical/translational research:
   a. Dr. Davis sees patients weekly in the outpatient Movement Disorders Center and attends periodically on the inpatient neurology consult service at Barnes-Jewish Hospital.
   b. Dr. Davis performed lumbar punctures and intrathecal infusions for a clinical trial for Huntington disease as well as lumbar punctures for multiple biomarker studies of neurologic disease.
   c. Dr. Davis continued to contribute to the leadership team of a multi-institutional consortium to study REM sleep behavior disorder as a prodromal condition of synucleinopathies, the North American Prodromal Synucleinopathy (NAPS) consortium. Dr. Davis is co-director of the Biofluid Core for NAPS and assisted with preparation of an NIH U19 application that was funded.
5) Navigate the process of transition to independent investigator: with mentoring support from committee members, Dr. Davis submitted an NIH R01 proposal which was reviewed and scored but ultimately not funded. Dr. Davis was awarded a Michael J. Fox Foundation Target Advanced Program award as Principal Investigator, and is currently preparing an R01 application.

- **How were the results disseminated to communities of interest?**

  Dr. Davis served as a panelist for a career development workshop for Washington University undergraduate students to increase awareness of basic and clinical research in neuroscience and neurology.

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

During the next reporting period, we plan to do the following:

1) Analyze behavior, functional connectivity, and immunohistochemistry data from APOE knockin mice injected with aSyn PFFs vs monomer.
2) Test the correlation between immunohistochemical measurements of aSyn pathology with behavioral phenotypes and functional connectivity.
3) Determine if any additional cohorts are needed to adequately assess potential differences between groups.

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**

     We have changed our Standard Operating Procedure for preparation and purification of aSyn recombinant protein based on knowledge gained during this project and anticipate that this will result in more reliable assessment of pathology specifically induced by aSyn aggregation rather than non-specific inflammation due to endotoxin contamination.

   - **What was the impact on other disciplines?**

     Nothing to report.

   - **What was the impact on technology transfer?**

     Nothing to report.

   - **What was the impact on society beyond science and technology?**

     Nothing to report.

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**

     We do not anticipate major changes to the approach or activities for this project. We encountered delays during the previous project period which have now been resolved as previously described.

   - **Changes that had a significant impact on expenditures**

Nothing to report.

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

  Nothing to report.

- **Significant changes in use or care of human subjects**

  Not applicable – no human subjects.

- **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:	**Albert Davis**
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-2042-8445
Nearest person month worked:	
Contribution to Project:	Dr. Davis planned and performed experiments including protein purification and animal surgeries, and analyzed data.
Funding Support:	N/A
Name:	**Adam Bauer**
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-8364-3209
Nearest person month worked:	
Contribution to Project:	Dr. Bauer planned fcOIS experiments and analyzed data.
Funding Support:	N/A
Name:	**Annie Bice**
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	
Contribution to Project:	Ms. Bice performed fcOIS experiments and analyzed data.
Funding Support:	N/A
Name:	**Jessica Patterson**
Project Role:	Senior Research Technician
Researcher Identifier (e.g. ORCID ID):	0000-0003-2560-6546
Nearest person month worked:	
Contribution to Project:	Ms. Patterson supervised mouse colonies, performed protein purification, and analyzed histology data.
Funding Support:	N/A
Name:	**Salman Kaleelurrahuman**
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	0000-0002-3338-0172
Nearest person month worked:	
Contribution to Project:	Mr. Kaleelurrahuman assisted with protein purification, managed mouse colonies, and performed histology.
Funding Support:	N/A

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

  Dr. Davis: 1K08NS101118-01 closed on 6/30/22

- **What other organizations were involved as partners?**

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.