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TITLE: Targeting Diet-Microbiome Interactions in the Pathogenesis of Parkinson's Disease

PRINCIPAL INVESTIGATOR: Ali Keshavarzian, MD

CONTRACTING ORGANIZATION: Rush University Medical Center

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6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

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

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1653 Congress Parkway
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms. During this reporting period 17 new human subjects were successfully recruited at this performance site.

15. SUBJECT TERMS
Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis

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Table of Contents

Page

Targeting Diet-Microbiome Interactions in the Pathogenesis of Parkinson's Disease

INTRODUCTION: The current project will analyze the gut microbiome and metabolites from PD patients and controls, and employ clinically relevant mouse models to determine how metabolites produced by the microbiome from dietary substrates affect motor symptoms. We propose to test whether directly regulating microbial metabolite profiles using "designer" dietary fibers and probiotics offers new avenues for ameliorating PD-like symptoms. During this reporting period 7 new human subjects (92.8% of targeted enrollment) were successfully recruited at the RUMC site to result in a total of 52 human subjects recruited (92.8% of targeted enrollment) in the first 4 years which partially meets our proposed goal. We have also made remarkable progress on the animal studies, defining specific diets that impact motor deficits in a mouse model of PD, and initiating mechanism of action studies. We have advanced the objectives of the project either on time, or in some cases ahead of schedule. The project has, to date, not experienced any major setbacks.

1. **KEYWORDS:** Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis, intestinal bacteria, dietary fiber, short chain fatty acids

- **ACCOMPLISHMENTS:** During this Y4 reporting period 7 new human subjects were successfully recruited at this Rush University Medical Center performance site. This achieved 92.8% of the targeted goal total of 52/56 human subjects proposed for the first 4 years.

- **What were the major goals of the project?**

- **Major goals of the project as stated in the approved SOW:**

Major Task 1: Recruitment and Microbiome Sequencing

Subtask 1- subject recruitment and sample collection.	12 month target of 12 human subjects with stool and tissue collection successfully recruited.	92% completed
Subtask 2- microbiome sequencing / metagenomics.	24 month timeline.	100% completed
Subtask 3- SCFA analysis for stool and serum.	12 month timeline.	70% completed

Major Task 2: Animal colonization and phenotyping

Subtask 1 – colonization of mice with human microbiota	36 month timeline.	100% completed
Subtask 2 – microbiome profiling.	36 month timeline.	100% completed
Subtask 2 – motor testing, neuroinflammation status.	36 month timeline.	100% completed
Subtask 3 – AAV cloning and injection.	6 month timeline.	100% completed
Subtask 4 – CLARITY analysis and electrophysiology.	36 month timeline.	70% completed

Major Task 3: Fiber testing and treatment of animals

Subtask 1 – treat PD mice with fibers and motor tests. 12 month timeline. 100% completed
Subtask 2 – treat PD mice with "optimized" fibers & test 36 month timeline. 75% completed

What was accomplished under these goals?

Activities accomplished in this quarter include: 1) partially reached our 48 month goal for recruitment, with the target of 56 subjects; 52 subjects have now been recruited; 2) colonization of germ-free WT and ASO mice with human microbiota; 3) SCFA treatment of SPF mice followed by motor testing; 4) feeding of SCFAs to SPF mice and analysis of neuroinflammation; 5) production and treatment of animals with prebiotic fibers, 6) motor testing mice fed prebiotic fibers; 7) microglia analysis by RNAseq of SCFA fed mice. We are excited to report that acetate feeding to SPF animals showed an effect on motor symptoms. Namely, feeding designer prebiotic diets enriched in 20% butyrate or acetate promoting fibers each improved motor symptoms in mice, whereas the 20% propionate fiber diet did not have this effect, showing specificity for different SCFAs in our mouse model of PD. Further, we show that butyrate reduces activation of microglia in vitro, and thus may affect neuroinflammation in vivo. Finally, we have profiled the transcriptome of microglia from brain regions of mice fed SCFAs, and find preliminarily very interesting results that we will fully describe in the next Quarterly Progress Report. There have been no setbacks or failures to achieve a goal, and the project is progressing on the proposed timeline or in some cases such as the microglia studies, ahead of schedule. Finally, we have published 4 major papers in this reporting cycle, all supported by DoD funding. We are now preparing 2 more manuscripts for imminent submission, and believe these will be high quality / high impact publications.

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

- "Nothing to Report." For the Rush University Medical Center site.

How were the results disseminated to communities of interest?

- "Nothing to Report." For the Rush University Medical Center site.

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

<u>Activities for next reporting period.</u>

1) In the Year 4 of the Project, Dr. Keshavarzian's team at RUMC will continue vigorous patient and subject recruitment and sample collection (target for Year 5 for RUMC is 4 subjects). So far we have succeeded in hitting 92.8% of our 4 year enrollment target goal for human subjects recruitment (52/56). 2) Microbiome sequencing and SCFA analysis are completed, and we are finalizing the single cell RBAseq of microglia from mice fed the prebiotic diet. 3) Dr. Mazmanian's group will analyze motor symptoms, neuroinflammation and pathophysiology in the "humanized" mouse models following prebiotic treatment. 4) We will evaluate the requirement for microglia in the prebiotic treated mice via microglial depletions. 5) Dr. Gradinaru's group will image brain tissues from these mice. 6) Drs. Mazmanian and Hamaker will finish the "optimized" prebiotic diets.

2. **IMPACT:**

   - **What was the impact on the development of the principal discipline(s) of the project?**
   - *Rush University Medical Center site and Dr. Keshavarzian's team achieved the targeted new human subject recruitment and enrollment goal (total 52/56 for*

4 years) which is required for the success of the project. The animal studies at Caltech further corroborated the preliminary data for a role by SCFAs in motor symptoms in mice. The fecal samples from all subjects collected at Rush are currently being sequenced at UCSD and will be published shortly after bioinformatic analysis.

- **What was the impact on other disciplines?** "Nothing to Report." For the Rush University Medical Center site.
- **What was the impact on technology transfer?** "Nothing to Report." For the Rush University Medical Center site.
- **What was the impact on society beyond science and technology?** "Nothing to Report." For the Rush University Medical Center site.

3. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change** "Nothing to Report." For the Rush University Medical Center site.
- **Actual or anticipated problems or delays and actions or plans to resolve them** "Nothing to Report." For the Rush University Medical Center site.
- **Changes that had a significant impact on expenditures** "Nothing to Report." For the Rush University Medical Center site.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents** "Nothing to Report." For the Rush University Medical Center site.
- **Significant changes in use or care of human subjects** "Nothing to Report." For the Rush University Medical Center site.
- **Significant changes in use or care of vertebrate animals.** "Nothing to Report." For the Rush University Medical Center site.
- **Significant changes in use of biohazards and/or select agents.** "Nothing to Report." For the Rush University Medical Center site.

4. **PRODUCTS:**

- **Publications, conference papers, and presentations.** "Nothing to Report." For the Rush University Medical Center site.

- **Journal publications.** "
-
- https://pubmed.ncbi.nlm.nih.gov/34182773/

- **Books or other non-periodical, one-time publications.** "Nothing to Report." For the Rush University Medical Center site.

- **Other publications, conference papers, and presentations.** "Nothing to Report." For the Rush University Medical Center site.

- **Website(s) or other Internet site(s).** "Nothing to Report." For the Rush University Medical Center site.

- **Technologies or techniques.** "Nothing to Report." For the Rush University Medical Center site.

- **Inventions, patent applications, and/or licenses**. "Nothing to Report." For the Rush University Medical Center site.

- **Other Products.** "Nothing to Report." For the Rush University Medical Center site.

5. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

- **For the Rush University Medical Center site:**

Name: Ali Keshavarzian, MD
Project Role: RUMC Site PI
Researcher Identifier (e.g. ORCID ID): ORCID 0000-0002-7969-3369.
Nearest person month worked: 1.2 pm.
Contribution to Project: Dr. Keshavarzian has directed the project at RUMC and regularly consults with Dr. Mazmanian and his team by conference call and Skype. He meets with the Rush team weekly.

Name: Leonard Verhagen, MD
Project Role: RUMC Neurologist/PD Specialist, Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 0.26 pm
Contribution to Project: Dr. Pal has left RUMC employment, Dr. Verhagen a renowned Movement Disorder and PD specialist assists in recruiting PD subjects and performs the medical evaluation of them as well as healthy subjects and obtains informed consent. He meets with Dr. Keshavarzian weekly.

Name: Alexander Yerkan, BS
Project Role: Clinical Coordinator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1.8 pm
Contribution to Project: Mr. Yerkan assists in recruiting subjects and performs the initial instructions and chart evaluation of them including administering questionnaires and assists with informed consent and pays the subjects. He meets with Dr. Verhagen and with Dr. Keshavarzian weekly or more often.

- **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." For the Rush University Medical Center site.
- **What other organizations were involved as partners?** "Nothing to Report." For the Rush University Medical Center site.

6. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** N/A
- **QUAD CHART:** Quad Chart for Rush University Medical Center site.