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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
Chicago, IL 60612

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
Ali Keshavarzian, MD

E-Mail: ali_keshavarzian@rush.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Rush University Medical Center
Jennifer Garcia
1653 Congress Parkway
Chicago, IL 60612-3833

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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 16 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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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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 16 new human subjects (100% of targeted enrollment) were successfully recruited at RUMC site. 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 reporting period 16 new human subjects were successfully recruited at this Rush University Medical Center performance site. This achieved the targeted goal of 16 subjects for this performance site for this time period.

- **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 16 human subjects with stool and tissue collection successfully recruited. 100% completed
Subtask 2- microbiome sequencing / metagenomics. 24 month timeline. 0% completed
Subtask 3- SCFA analysis for stool and serum. 12 month timeline. 20% completed
Major Task 2: Animal colonization and phenotyping
Subtask 1 – colonization of mice with human microbiota 36 month timeline. 50% completed
Subtask 2 – microbiome profiling. 36 month timeline. 0% completed
Subtask 2 – motor testing, neuroinflammation status. 36 month timeline. 75% completed
Subtask 3 – AAV cloning and injection. 6 month timeline. 90% completed
Subtask 4 – CLARITY analysis and electrophysiology. 36 month timeline. 0% 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. 0% completed

What was accomplished under these goals?

Accomplishments: Activities accomplished in this quarter include: 1) reached our 12 month goal for recruitment, with the target of 16 subject already 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, and 5 motor testing mice fed prebiotic fibers. We are excited to report that the first round of SCFA feeding to SPF animals showed an effect on motor symptoms. Namely, feeding designer prebiotic diets enriched in 20% butyrate or acetate each improved motor symptoms in mice, whereas the 20% propionate 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. There have been no setbacks or failure 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.

Figure legend: As shows by the sticker test, and in the other motor tests, butyrate and acetate improve motor performance while propionate has no effect.

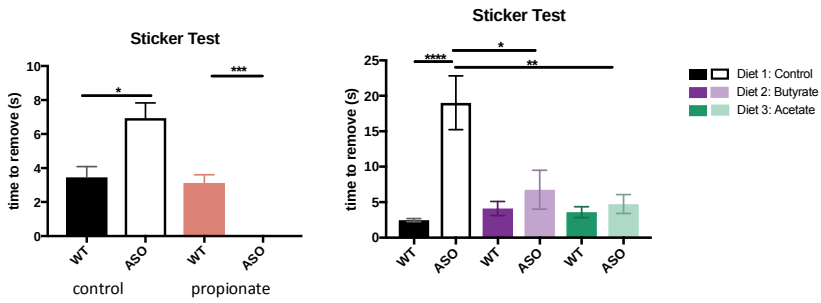
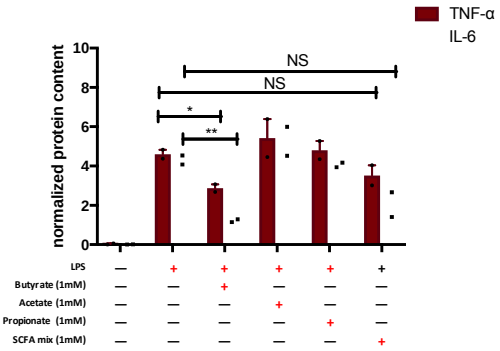


Figure legend: Primary mouse microglia, cultured in vitro with LPS for activation, shows reduced TNF and IL-6 production with butyrate treatment.



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?
Activities for next reporting period. 1) In the Year 2 of the Project, Dr. Keshavarzian's team at RUMC will continue vigorous patient and subject recruitment and sample collection (target for Year 2 for RUMC is 17 subjects). So far we have succeeded in hitting our enrollment target for human subjects (16/16). 2) Microbiome sequencing and SCFA analysis will be done in batches. 3) Dr. Mazmanian's group will analyze motor symptoms, neuroinflammation and pathophysiology in the "humanized" mouse models following prebiotic treatment. 4) We will evaluate short chain fatty acid (SFCA) levels in the prebiotic treated mice. 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 subject recruitment and enrollment (16/16) 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.*

- **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.** "Nothing to Report." For the Rush University Medical Center site.
- **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/12 months.
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: Gian Pal, MD
Project Role: RUMC Neurologist/PD Specialist, Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 0.3 pm/12 months.
Contribution to Project: Dr. Pal 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: Prachi Chakradeo, MS/PhD
Project Role: Clinical Coordinator

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3 pm/12 months.
Contribution to Project: Ms. Chakradeo 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. She meets with Dr. Pal each day and 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: see following page 9.

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

W81XWH-17-0589
PD160030 P2

PI: Ali Keshavarzian MD **Org:** Rush University Medical Center **Award Amount:** $601,400

Study/Product Aim(s)

- Specific aim 1. Profile the human stool microbiome, and serum and stool SCFA levels, in early PD patients.
- Specific aim 2. Determine how the microbiome regulates gut-brain interactions in a PD model.
- Specific aim 3. Develop and test "designer" fiber diets to modulate SCFA levels in PD mice.
- Specific aim 4. Test dietary and probiotic treatments in mice with PD-like symptoms.

Approach

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.

Insert a picture or graphic here, with a caption, that represents the proposed work

Activities accomplished: 1) on track with expected rate of patient / subject recruitment -16/16.

Timeline and Cost (Entire Grant)

Activities CY	17	18	19	20
Recruitment & Sequencing				
Animal Colonization				
Fiber Testing in Animals				
Estimated Budget ($K)	706,545	857,941	861,612	731,637

Updated: (September 27, 2018)

Goals/Milestones (Example)

CY17 Goal – Subject Recruitment and initial animal colonizations
- ☑ Subject recruitment and sample collection
- ☑ Colonization of mice with human microbiota
- ☑ Motor testing and neuroinflammatory status
- ☑ Treat PD mice with fibers, motor test

CY18 Goals – Fiber testing and animal studies

CY19 Goal – Fiber optimization and animal studies

CY20 Goal – Integrated mutli-omics analysis of human and mouse data

Comments/Challenges/Issues/Concerns
- No issues to report
- ***Rush University Budget Expenditure to Date*:**
- **Year 1 budget: $157,960**
- **Year 1 expenditures: $135,125 (*this is the adjusted balance after pending labor cost transfers post within the year 1 budget period)**