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

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CONTRACTING ORGANIZATION: California Institute of Technology, Pasadena, CA

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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.

15. SUBJECT TERMS

Parkinson's disease, human subjects, intestinal microbiome, stool specimens, gut-brain axis

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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 12 new human subjects (92% of targeted enrollment) were successfully recruited at the RUMC site to result in a total of 45 human subjects recruited in the first 4 years which partially meets our proposed goal. We consider 92% a very successful outcome considering the COVID-19 pandemic, and this level of recruitment has not prevented any proposed studies. 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 outside of the pandemic. Overall, we continue to make outstanding progress on all aspects of this project.

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

1. ACCOMPLISHMENTS:

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

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; 48 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?

Research. *Trainees meet weekly with the PI, both separately and together, to discuss their latest results, technical problems, collaborations, reagent needs and so forth.*

Group Meeting & Scientific discussions. *The Gradinaru laboratory holds formal 2 meetings every week, one for research updates from investigators and to discuss literature and the other to troubleshoot experiments. Each week a group member presents their work. Often, we have PIs, students and fellows from other laboratories join in our weekly meetings. Each trainee presents their work approximately every 8 weeks to the entire group. These lab meetings cover a range of topics, from, neuroscience, behavior to virology to animal models of disease. We frequently discuss relevant papers in the field and how they impact the research in our laboratory. Furthermore, each trainee participates in Caltech's vigorous seminar program in which outside scientists come to Caltech to present their research. They also have the opportunity to participate in the weekly "BioLunch", which features two half-hour presentations every week by a student and/or postdoc, thereby providing excellent exposure to ongoing projects in the Biology Division. Further, a student and/or postdoc will present their work once a year in a campus-wide seminar series called "Micro Mornings", where members of the microbiology community at Caltech discuss their work in front of an audience of peers that include not only biologists, but chemists and engineers as well. The diverse feedback from this worthwhile helps students and fellows craft dynamic research programs. In addition, the students and fellows in the laboratory organize their own weekly journal club, practice talks and brainstorming sessions, often without the PI.*

Mentoring*. The PI mentors each trainee on science, their careers, ethics, scientific strategy, interpersonal relationships, oral and written communication, graphics, and so forth. I realize that each young scientist has different talent sets, and thus try to help each individual*

improve all their skills. For example, we discuss appropriate and effective ways to network, how to turn potential competitors into collaborators, how to compete (if necessary) in a collegial way, etc. We also engage in open discussions about alternative career choices in addition to preparation for obtaining and succeeding in an academic career. I view my role as a mentor to primarily be a resource for the scholarly, academic and personal advancement of the careers of my trainees.

***Writing**. In general, the PI does not write the research papers from her laboratory, but discusses content, organization and figures as the papers are planned and being written, edits to enhance the personal style of each author, and rewrites key parts if necessary. My goal is to train superb writers. Other laboratory members continually critique each other's manuscripts, grant proposals, research statements, posters, etc.*

***Scientific meetings and conferences.** Trainees attend and present their data at 2 or 3 scientific meetings each year, locally, nationally or internationally. All trainees have presented their findings from this project at 3 scientific meetings in the past year. This provides not only the opportunity to receive feedback and critique on the project, but to network with researchers*

How were the results disseminated to communities of interest?

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

1) In the Year 4 of the Project, Dr. Keshavarzian's team at RUMC will continue vigorous patient and subject recruitment and sample collection. So far we have succeeded in hitting 92% of our 4 year enrollment target goal for human subjects recruitment (45/49). 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:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **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 (12/16; total 45/49 for 3 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

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

Nothing to report

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

Nothing to report

3. **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**

Nothing to report

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**

Nothing to report

- **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

4. **PRODUCTS:** *"Nothing to Report."*

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

  Report only the major publication(s) resulting from the work under this award.

  - **Journal publications.**

https://pubmed.ncbi.nlm.nih.gov/32066981/

https://pubmed.ncbi.nlm.nih.gov/32043464/

https://pubmed.ncbi.nlm.nih.gov/32071263/

https://pubmed.ncbi.nlm.nih.gov/33093662/

https://pubmed.ncbi.nlm.nih.gov/33067567/

https://pubmed.ncbi.nlm.nih.gov/34182773/

  - **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)

sarkis.caltech.edu

Technologies or techniques

Nothing to report

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

Nothing to report

- **Other Products**

Nothing to report

5. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

NAME:	**Viviana Gradinaru, PhD**
PROJECT ROLE:	PI, and Caltech Site PI
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	1.20 Calendar Months
CONTRIBUTION TO PROJECT:	Prof. Gradinaru is supervising the tissue imaging by CLARITY, electrophysiology, and the AAV aspects of the project.
FUNDING SUPPORT (If Applicable):	

NAME:	**Gerard Coughlin**
PROJECT ROLE:	Graduate Assistant, Investigator
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	1.00 Calendar Months
CONTRIBUTION TO PROJECT:	Gerard Coughlin is contributing to imaging CLARITY tissue by custom light-sheet microscopy (software and hardware) and to data analysis methods.
FUNDING SUPPORT (If Applicable):	

NAME:	**Elisha Mackey**
PROJECT ROLE:	Research Technician/Lab Manager
RESEARCHER IDENTIFIER:	
NEAREST PERSON MONTH WORKED:	1.00 Calendar Months
CONTRIBUTION TO PROJECT:	XXX
FUNDING SUPPORT (If Applicable):	

- **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

6. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *N/A*
   - **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*
7. **APPENDICES:** *N/A*