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TITLE: A Multidisciplinary Approach to Study the Role of the Gut Microbiome in Relapsing and Progressive MS

PRINCIPAL INVESTIGATOR: Rob Knight

CONTRACTING ORGANIZATION:
UNIVERSITY OF CALIFORNIA, SAN DIEGO
La Jolla, CA 92093

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Rob Knight

E-Mail: Rknight@ucsd.edu

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UNIVERSITY OF
CALIFORNIA, SAN DIEGO
9500 Gilman Dr, La
Jolla, CA 92093

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have completed recruitment of all subjects. We are currently processing samples for sequencing and genotyping in order to complete the project in September of 2019.
On the experimental side, a group of 24 germ-free mice was colonized with human microbiota and EAE was induced. Two additional experiments to replicate these initial findings are ongoing. The project is both scientifically and financially on track.

15. SUBJECT TERMS

microbiome, multiple sclerosis, progressive, relapsing

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1. INTRODUCTION

We hypothesize that specific human GI microbiota can alter the balance of inflammatory and regulatory immune cell populations thus leading to disease in genetically susceptible hosts. Furthermore, we hypothesize that gut microbiota from subjects with RMS and PPMS is fundamentally different and can elicit distinguishable effects when transferred into susceptible animal models of the disease.

2. KEYWORDS

Microbiome
Multiple sclerosis
Primary Progressive
Relapsing Remitting
Knockout mice
Bioinformatics
Immunology

3. ACCOMPLISHMENTS

3a. Major goals of the project: This project has two major goals or aims. In the SOW, each Aim was subdivided into Major Tasks and subtasks:

Aim#1: To compare the gut microbiome of subjects with RMS and PPMS.

- Major Task 1: To seek and obtain HRPO approval –**COMPLETE**
- Major Task 2: Identification and recruitment of research subjects -- **COMPLETE**
  - Subtask 1: Perform Chart reviews to identify eligible patients from MS clinic at UCSF and Mt Sinai. -- **COMPLETE**
  - Subtask 2: Clinical evaluation and invitation to participate in the study -- **COMPLETE**
- Major Task 3: Sample collection and initial processing -- **COMPLETE**
  - Subtask 1: Preparation of collection mailing kits -- **COMPLETE**
  - Subtask 2: Bacterial DNA extraction from stool material -- **ONGOING**
  - Subtask 3: Genotyping and HLA characterization of host DNA. – **TO DO**

Milestone #1: Recruitment and processing samples from 150 RMS, 150 PPMS and 150 healthy controls.

- Major Task 4: 16S ribosomal gene sequencing and initial bioinformatics analysis.
  - Subtask 1: sequencing of 16S ribosomal RNA gene in all DNA samples from MS patients and controls. – **TO DO**

Milestone #2: Sequencing of the MS microbiome.

- Major Task 5: Data integration and advanced bioinformatics analysis.
  - Subtask 1: Integration of microbiome and genomic data – **TO DO**

Aim#2: To test the effect of human MS microbiota in spontaneous and induced experimental models.

Major Task 1: microbiota transfer into germ-free mice and EAE induction -- **ONGOING**

Subtask 1: re-derivation of Tob1/2D2 mice into a GF line -- **ONGOING**

Subtask 2: Transfer of live microbiota from select patients into germ-free mice, EAE induction and follow-up -- **ONGOING**

Major Task 2: Immuno-pathological characterization of experimental mice

Subtask 1: tissue dissection, harvesting and pathological analysis – **TO DO**

Subtask 2: Flow cytometry – **TO DO**

Subtask 3: Immunohistochemistry – **TO DO**

Subtask 4: Molecular characterization – **TO DO**

*Milestone #3: Co-authored manuscript – **TO DO***

3b. Accomplishments to date:

We have completed recruitment at both Mt. Sinai and UCSF. At the end of our recruitment period (October 2018), Mt. Sinai recruited a total of 167 subjects. Of these, 81 are RRMS, 25 PPMS and 61 healthy controls. Blood samples were collected for all subjects and clinical visits stool and blood samples are completed.
UCSF recruited a total of 350 subjects: 115 with RRMS, 120 with PPMS and 115 healthy controls. Blood and stool samples were collected from all subjects and all subjects completed their clinic visits.
In total, between both sites, we have recruited 537 subjects (196 RRMS, 145 PPMS and 176 controls). While we ended up 5 PPMS subjects short of the goal (n=150), we consider this will not impact significantly in our analysis and in the interest of time, we will carry on with analysis. The few extra samples from RRMS and healthy controls will be evaluated for quality and the number reduced to n=150 accordingly.

With the recruitment goal accomplished, we also conclude the participation of Dr. Cree (clinical PI). Thus, this constitute his final report.
In the EWOF period we plan to complete sequencing, DNA genotyping and analysis of all samples to provide a final report by Oct 2019.

Additional Accomplishments:

- Bacterial DNA is being purified from stool samples. Quality control is being performed (Specific Aim 1. Major Task 3. Subtask 2)
- Tob1/2D2 mice are currently being derived germ-free (Specific Aim 2. Major Task 1. Subtask 1)
- Germ-free C57Bl/6 mice (7-8/group) have been colonized with whole microbiota from a RRMS, a PPMS and a healthy subject. After 5 weeks, EAE was induced. Experiment is ongoing at this time.

3c. Opportunities for training and professional development

Exchange of protocols and samples between experimental sites is ongoing. Caltech (Sub: Mazmanian) has received microbiota samples from UCSF (PI: Baranzini) to colonize germ-free mice. This experiment required careful coordination between the Caltech, UCSF and Mt Sinai groups to transfer samples for analysis.
The UCSF group (Baranzini) performed the first experiment on its germ-free facility. Know-how from the Caltech group (Mazmanian) was leveraged for this experiment.
Also, the UCSF group introduced a protocol to re-derive germ-free mice with guidance from Caltech.

UCSF and MT Sinai teams received specimens for analysis from the experiment performed at Caltech. Investigators at each of the 3 sites are in close communication. Now that recruitment has been completed, UCSF (PI: Baranzini) and ICSD (PI: Knight) investigators are permanent contact to process samples for bacterial DNA sequencing.

3d. Dissemination of results to communities of interest
Since most of the activities so far have been concentrated on recruitment, there are no results from this project to report yet.
A related article from our groups (UCSF, Caltech, UCSD and MT Sinai) was published in PNAS and received significant media attention for its design, and impact (Cekanaviciute et al. PNAS 2017). This study was funded by the National MS Society and philanthropic contributions.
Two more articles have been published from the UCSF group that are closely related to this project (Rojas et al. Cell 2019 and Cekanaviciute et al. mSystems 2018).

3e. Plans for accomplishing project goals during the next reporting period
This report is the final report for the clinical portion of this grant (PI: Cree). The other two PIs (Baranzini and Knight) have requested an EWOF to finalize the analysis of all samples obtained.
Work to isolate DNA from obtained samples is underway at UCSF. DNA will be sent to UCSD (Knight) for sequencing in the winter.
Additional experiments with microbiota transfer into GF mice are planned both at UCSF and Caltech.

4. IMPACT

4a. Impact on the development of the principal discipline(s) of the project

Nothing to report

4b. impact on other disciplines

Nothing to report

4c. impact on technology transfer

Caltech has transferred significant know-how and technical proficiency to UCSF throughout this project. Most notably, for experiments involving microbiota transfer into GF mice and for re-derivation of GF mice.

4d. impact on society beyond science and technology

Nothing to report ye.

5. CHANGES/PROBLEMS

5a. Changes in approach and reasons for change

Nothing to report

5b. Actual or anticipated problems or delays and actions or plans to resolve them

While we had a number of setbacks, we are glad to report we have been able to complete recruitment of all subjects as planned.
We are currently repeating experiments of microbiota transfer from humans (RRMS and PPMS) to germ free mice. While our first experiment was encouraging, we could not replicate these findings in a second set of experiments. In addition, the sterility of GF mice was compromised while trying to replicate the original results at UCSF. We plan to repeat these experiments with additional samples this year.

5c. Changes that had a significant impact on expenditures

Nothing to report

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. PRODUCTS

6a. Publications, conference papers, and presentations

A related article from our groups (UCSF, Caltech, UCSD and MT Sinai) was published in PNAS and received significant media attention for its design, and potential impact in MS (Cekanaviciute et al. PNAS 2017). Two more articles have been published from the UCSF group that are closely related to this project (Rojas et al. Cell 2019 and Cekanaviciute et al. mSystems 2018).
These study were funded by the National MS Society and philanthropic contributions.

6b. Website(s) or other Internet site(s)

Nothing to report

6c. Technologies or techniques

Nothing to report

6d. Inventions, patent applications, and/or licenses

Nothing to report

6e. Other Products (Reportable outcomes)

Aim 1 is in its analysis phase. No reportable outcome at this time.
Aim 2 is in progress. No reportable outcome at this time.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. Individuals working on the project

Personnel	Project PI	Role	Nearest person Month	Contribution
Sergio Baranzini	Baranzini	PI	1	Project overview
Bruce Cree	Cree	PI	1	Clinical PI
Sneha Singh	Cree	Coordinator	6	Clinical coordinator
Adam Santaniello	Cree	Database manager	1	Set up project database
Rob Knight	Knight	PI	0.5	Technical development PI
Stefan Maximilian Janssen	Knight	Post Doc	0.9	development of methods to analyze exRNA sequences to assess interkingdom communication
Tomasz Piotr Kosciolek	Knight	Post Doc	1.25	development of methods to identify ncRNAs in the genomic and metagenomic data
Jon Sanders	Knight	Post Doc	0.5	development of genome annotation pipeline and fast matching for separating human reads from microbial that will be used in the shotgun metagenomics.
Daniel McDonald	Knight	Bioinformatics Programmer	1	computational studies of sequenced genomes, extracting and integrating biological information and data.
Jeffrey E Dereus	Knight	Programmer	1.7	management of the software

	Analyst	team, setup of project tracking and LIMS for this project.

7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No

7c. What other organizations were involved as partners?

Partner (sub-contract) 1
Organization Name: California Institute of Technology
Location of Organization: Pasadena, CA
Partner's contribution to the project (identify one or more): Germ-free mouse experiments

Partner (sub-contract) 2
Organization Name: Icahn School of Medicine at Mount Sinai
Location of Organization: New York, NY
Partner's contribution to the project (identify one or more): Subject recruitment and experimental work (immunohistochemistry of brain sections, immunophenotyping and RNAseq of selected mouse tissues)

8. SPECIAL REPORTING REQUIREMENTS:

This is a Collaborative award (3 Principal Investigators).
As approved by the Program official assigned to this project (Amie Bunker) the same report is being submitted under each PI's account.