AWARD NUMBER: W81XWH-15-1-0652

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

PRINCIPAL INVESTIGATOR: Sergio E. Baranzini

CONTRACTING ORGANIZATION:
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
675 Nelson Rising Ln, San Francisco, CA 94158

REPORT DATE: Dec 29, 2019

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Dec 29, 2019	FINAL	30SEP2015 - 29SEP2019

4. TITLE AND SUBTITLE
A Multidisciplinary Approach to Study the Role of the Gut Microbiome in Relapsing and Progressive MS

5a. CONTRACT NUMBER
W81XWH-15-1-0652

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sergio E. Baranzini, Ph.D.

E-Mail: Sergio.Baranzini@ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of California San Francisco
675 Nelson Rising Ln
San Francisco, CA. 94158

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system (CNS) and a common cause of progressive neurological disability in young adults. The typical disease course (85% of patients) is relapsing or bout-onset MS (RMS) but 15% of patients do not have relapses and experience progression of disability from disease onset or primary progressive form of MS (PPMS). We hypothesize that the gut microbiota in RMS and PPMS patients is different, thus potentially influencing disease course. In this project we recruited 381 subjects (149 RMS patients, 84 PPMS and 148 healthy) and performed fecal (bacterial) DNA by 16S ribosomal RNA gene sequencing, and high-resolution HLA typing. In addition, we transferred microbiota from RMS (n=2) and PPMS (n=2) patients into germ free mice before inducing experimental allergic encephalomyelitis (EAE). Despite the heterogeneous and modest sample size, we observed increased frequency of Enterobacteriaceae in RRMS patients and a reduction of Prevotella Copri in PPMS subjects. Transplantation of fecal microbiota from RMS patients into germ free mice resulted in milder neurological disability (EAE) compared to mice receiving microbiota from PPMS patients. While results need to be replicated, this suggests that gut microbiota from PPMS is less

15. SUBJECT TERMS
microbiome, multiple sclerosis, progressive, relapsing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

1. INTRODUCTION: 4

2. KEYWORDS: 4

3. ACCOMPLISHMENTS: 4

WHAT WERE THE MAJOR GOALS OF THE PROJECT? 4
WHAT WAS ACCOMPLISHED UNDER THESE GOALS? 5
SPECIFIC AIM 1: TO COMPARE THE GUT MICROBIOME OF SUBJECTS WITH RMS AND PPMS. 5
MILESTONE #1: RECRUITMENT AND PROCESSING SAMPLES FROM 150 RMS, 150 PPMS AND 150 HEALTHY CONTROLS 6
MILESTONE #2: SEQUENCING OF THE MS MICROBIOME 10
SPECIFIC AIM 2: TO TEST THE EFFECT OF HUMAN MS MICROBIOTA IN A SPONTANEOUS AND INDUCED EAE MOUSE MODEL 10
MILESTONE #3: CO-AUTHORED MANUSCRIPT. 12

4. IMPACT: 14

5. CHANGES/PROBLEMS: 14

6. PRODUCTS: 15

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS 16

8. SPECIAL REPORTING REQUIREMENTS 17

9. APPENDICES: 17

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. To test these hypotheses, we will conduct a series of experiments including high-throughput bacterial DNA sequencing, immunological profiling of patients and controls, and live microbiota transfer from patients into mice followed by their immuno-molecular characterization.

2. KEYWORDS:

microbiome,
multiple sclerosis
progressive
relapsing

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To compare the gut microbiome of subjects with RMS and PPMS.	**Timeline**
Major Task 1: Identification and recruitment of research subjects	Months
Major Task 2: Sample collection and initial processing.	
Milestone #1: Recruitment and processing samples from 150 RMS, 150 PPMS and 150 healthy controls.	1-24
Major Task 3: 16S ribosomal gene sequencing and initial bioinformatics analysis.	
Milestone #2: Sequencing of the MS microbiome.	12-28
Major Task 4: Data integration and advanced bioinformatics analysis.	
Specific Aim 2: To test the effect of human MS microbiota in a spontaneous and induced EAE mouse model	
Major Task 1: microbiota transfer into germ-free mice and EAE induction	
Major Task 2: Immuno-pathological characterization of experimental mice	
Milestone #3: Co-authored manuscript.	28-36

What was accomplished under these goals?

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

Specific aim 1 was a complete success. Identification and recruitment of 381 subjects was accomplished (major task 1), and although it took longer than anticipated, the quality of data and biological samples obtained resulted in a valuable resource (Major task 2). Furthermore, sequencing of the bacterial DNA from stool and HLA typing from the host DNA was also successful (Major task 3).

Major Task 1: Identification and recruitment of research subjects

Subtask 1: Chart reviews to identify eligible patients from MS clinic at UCSF and Mt Sinai.

Chart reviews to identify potential study participants was the first tasks each of the two teams had and it was accomplished in a timely manner. Both teams (UCSF and Mount Sinai) were recruiting patients within the first 3 months of study initiation.

Subtask 2: Clinical evaluation and invitation to participate in the study

Clinical evaluation was a critical step in the recruitment process and strict inclusion and exclusion criteria needed to be verified before enrolling patients into the study. This process involved neurological evaluation of prospective subjects, subsequent invitation to participate in the study, signing of the informed consent and biological specimen collection (i.e. blood and stool).

Table 1. Participants' characteristics

	Control	MS	RRMS (4 CIS)	PPMS
Number	148	233	149	84
Site				
San Francisco	93	129	69	60
New York	55	104	80	24
Age (y)	51.3(42,62)	53.8(40,60)	45.5(36,56)	68.4(52,65)
Female (%)	64 (43.2%)	148(63.5%)	111(74.5%)	37(44%)
BMI	29.6(23.1,29.5)	25.7(22,28.9)	25.5(21.6,28.3)	26.1(22.9,29.6)
Disease duration(y)		12.4(5,18)	12.6(4.75,20)	12(5,16)
EDSS		2.8(1,4)	1.5(0,2.5)	5.0(3.5,6.4)
Treated(%)		133 (57.1%)	103(69.1%)	30(35.7%)
Treatment				
Tecfidera		33	29	4
Copaxone		29	28	1
Ocrevus/Rituxan		32	11	21
Gilenya		17	15	2
Interferon		11	10	1
Tysabri		11	10	1

Data present as mean (Interquartile range, IQR). y, year; EDSS, Expanded Disability Status Scale.

Overall, 149 relapsing-remitting MS patients, 84 primary progressive MS patients and 148 healthy participants were recruited from UCSF and Mount Sinai (Table 1, Excel File 1). Patients and controls were age matched, while as a consequence of the uneven sex distribution of MS, 63.5% of the MS participants were female, compared with 43.2% of controls. As more males are controls, a higher body mass index (BMI) was observed in the control group. More than half (57.1%) MS patients were treated, and specifically a higher proportion of RRMS patients (69.1%) were treated than PPMS patients (35.7%).

Major Task 2: Sample collection and initial processing.

Subtask 1: Preparation of collection mailing kits

In order to collect biological samples, a considerable logistical apparatus needed to be put in place. This included the creation of sample collection kits to facilitate stool collection from participants in the privacy of their homes. The stool collection kit contained all materials needed for a safe and hygienic sample collection, along with written instructions to ensure homogeneity of procedures across all subjects, thus minimizing systematic errors. Additional materials included videos and clinical coordinator availability in case questions about sample collection arose. Once participants collected their stool samples, they were instructed to ship it by overnight parcel to each of our labs (UCSF and Mount Sinai) for further storage and processing. All samples acquired at Mount Sinai were shipped in batch to UCSF at the end of the recruitment stage for final storage and processing.

Subtask 2: Bacterial DNA extraction from stool material.

Once samples arrived in the laboratories, trained personnel recorded them in a dedicated database, and stored each specimen at -80 C. Subsequently, bacterial DNA was purified by robotic assistance, followed by manual gel electrophoresis and spectrophotometric quantification to assess DNA quality

Subtask 3: Genotyping and HLA characterization of host DNA.

Host DNA was purified from the collected blood samples and DNA was subjected to High-resolution HLA typing by targeted gene sequencing.

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

Milestone # 1 was largely accomplished by the recruitment of nearly all RMS (n=149) and control (n=148) subjects proposed as shown in Table 1. We fell short in recruitment of PPMS subjects (n=84), largely in part due to unanticipated difficulty in identifying enough participants with this rare disease subtype. However, we deemed necessary to stop recruitment and start analysis in order to have results ready by the end of the grant period.

Major Task 3: 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

Sequencing of all DNA samples took place at UCSD (Knight). Amplicon sequencing variants (ASVs) were resolved from the 16S rRNA sequencing data and classified based on the Greengenes reference dataset (Excel File 2).

Subtask 2: bioinformatics analysis.

To understand how the microbial composition differs between patients and healthy controls, we compared the diversity of microbial composition characterized by ASVs within (α-diversity) and between individuals (β-diversity). As expected, no significant difference in α-diversity was observed between MS and healthy control groups as measured by Shannon and Chao1 index, neither among the disease course (Fig.1).

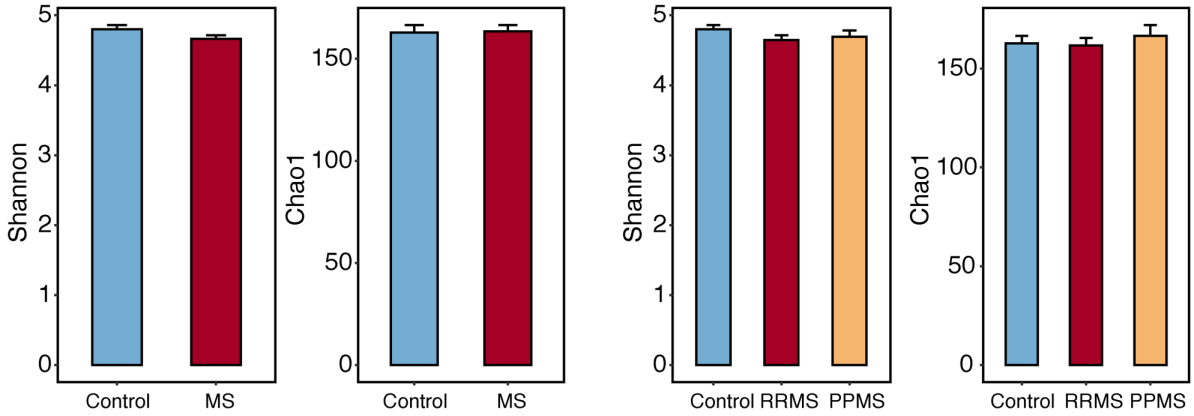

Figure 1. Microbiome diversity. Microbiome α-diversity measured by Shannon and Chao1 index was compared by disease status and disease course. Data are presented as mean ± SEM (ANOVA, not significant).

A significant difference of β-diversity among healthy control, RRMS and PPMS was observed even though the samples were not clearly clustered by disease status (Fig. 2A). Given the heterogeneity of human samples, we applied PERMANOVA to test how much microbiome differences are influenced by demography, medication use and physiology (i.e. confounders). Besides disease course, three other confounders were also identified to be significantly associated with microbiome diversity measured by inter-individual weighted uniFrac distance (Fig. 2B). Recruitment site accounted for the largest variation and a PCoA of the microbiome beta-diversity showed the significant difference of samples from San Francisco and New York (adonis R2=0.0465, Fig. 2C). We also observed the difference of microbiome diversity associated to sex (Fig. 2D), which has to be adjusted in the disease associated analyses as more MS patients are females while more controls are males (Fig .2E). The impact of these factors on shaping gut microbiome raised the possibility that the largest difference given by recruitment site was potentially co-contributed by a biased distribution of disease status or sex, we then quantified the sample size by disease course and sex in each site (Fig. 2E). No uneven distribution of disease or sex was observed in the two recruitment sites, hence the impact of geographic location had to be adjusted. Body mass index (BMI) also exerted significant effects (Fig. 2A), but the effect could be reduced by the sex adjustment as a consequence of the observed higher BMI of males compared to that of females (Fig. 2F).

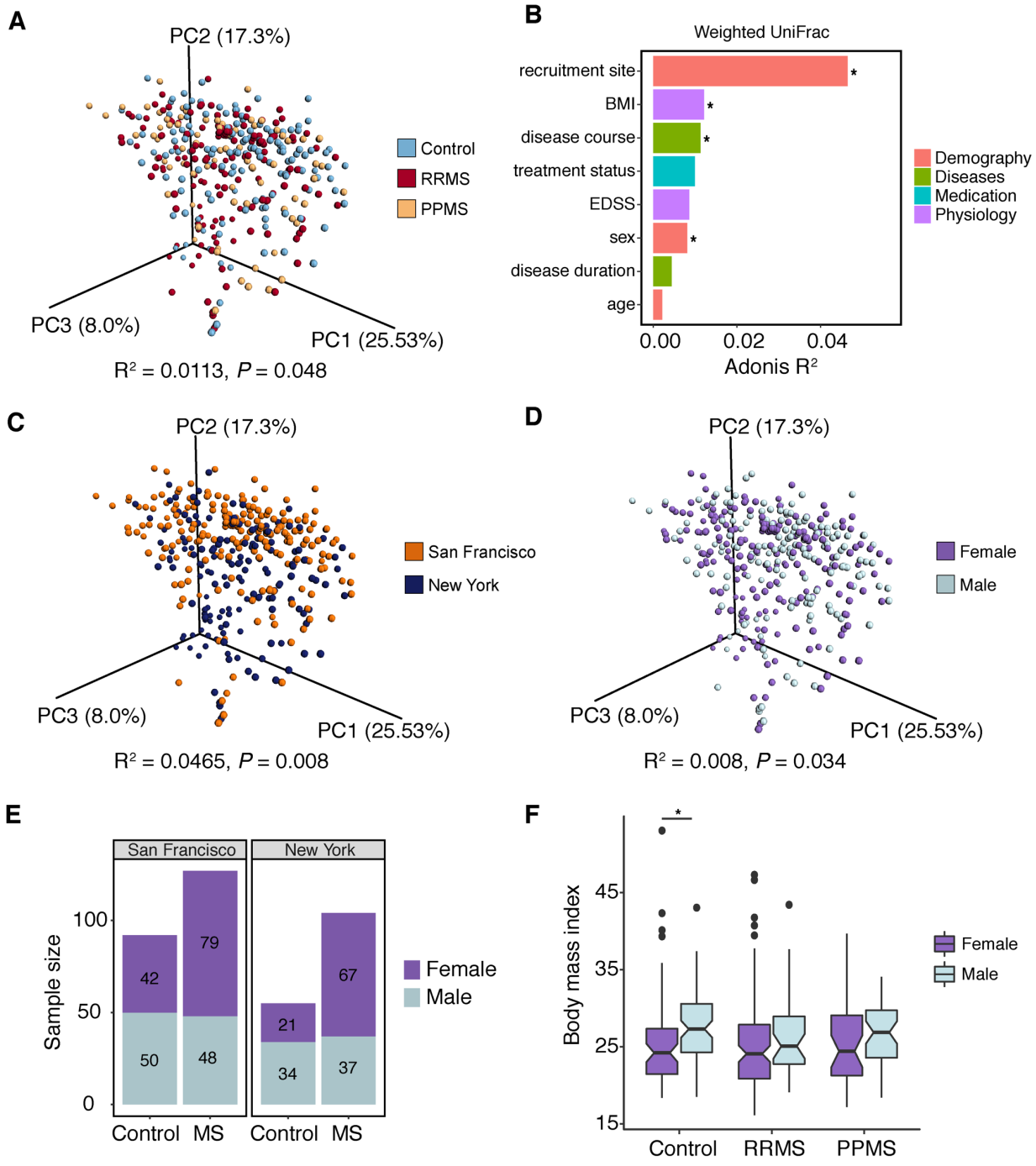

Figure 2. Effect size of confounders. (A) PCoA of weighted UniFrac community distance compared by disease status. (B) Bar plot showing the size effect (Adonis R^2) of confounders associated with gut microbial variations (weighted UniFrac distance). Confounders showing significant impact on gut microbiome were labeled (Multiple testing correction *P* value < 0.05). BMI, body mass index; EDSS, expanded disability status scale. (C) PCoA of weighted UniFrac community distance compared between recruitment sites. (D) PCoA of weighted UniFrac community distance by sex. (E) Sample size summarized by disease course, sex in two recruitment sites. (F) Body mass index compared between female and male by disease course. Statistical significance was determined by ANOVA (multiple testing corrected *P* value,*$P \leq 0.05$).

Individual microbes associated with MS

To identify the individual microbes associated with MS disease course, we performed differential analyses to compare the microbial community between MS patients and heathy controls by controlling the impact of recruitment site and sex as mentioned above. Only one microbe, belonging

to the Enterobacteriaceae family, was identified to be different between RRMS and controls subjects in this study (Fig. 3A). The abundance of Ruminococcus torques, Bacteroides caccae and Eubacterium dolichum were increased in PPMS patients compared to healthy controls while Provetella Copri was reduced in PPMS patients (Fig. 3B). Both R. torques and B. cacca are mucin degradation bacteria. Another mucin degradation bacterium, Akkermansia muciniphila, has also been reported in MS by our previous studies [1, 2]. R. torques was also enriched in patients with autism spectrum disorder and inflammatory bowel disease (IBD) [3]. A TonB-linked outer membrane protein, termed OmpW, was identified from B. caccae and shown to induce adaptive immune responses in pediatric IBD patients more frequently than in healthy controls [4]. These data suggest the possibility that these mucin degradation bacteria could induce proinflammatory responses in MS. Eubacterium dolichum was reported to associate with propionate production in human intestine, and predominant in patients with relapsing polychondritis [5] and individuals consuming a high-fat diet. There is no significant correlation between this bacterium and BMI in our study. Further studies are needed to identify the roles of these bacteria in the pathogenesis of MS. Patients with RRMS had more P. copri compared to PPMS as a result of the reduction in PPMS (Fig. 3C), but it was decreased in RRMS patients shown in a related study [6]. By stratifying the comparisons based on treated status, we still observed an increase of R. torques in both treated PPMS and untreated PPMS compared to healthy controls, and increase of B. caccae in treated PPMS (Fig. 3D). P. copri was only significantly reduced in untreated PPMS patients, which was also reported in another study [Intestinal microbiota in multiple sclerosis: influence of treatment with interferon β-1].

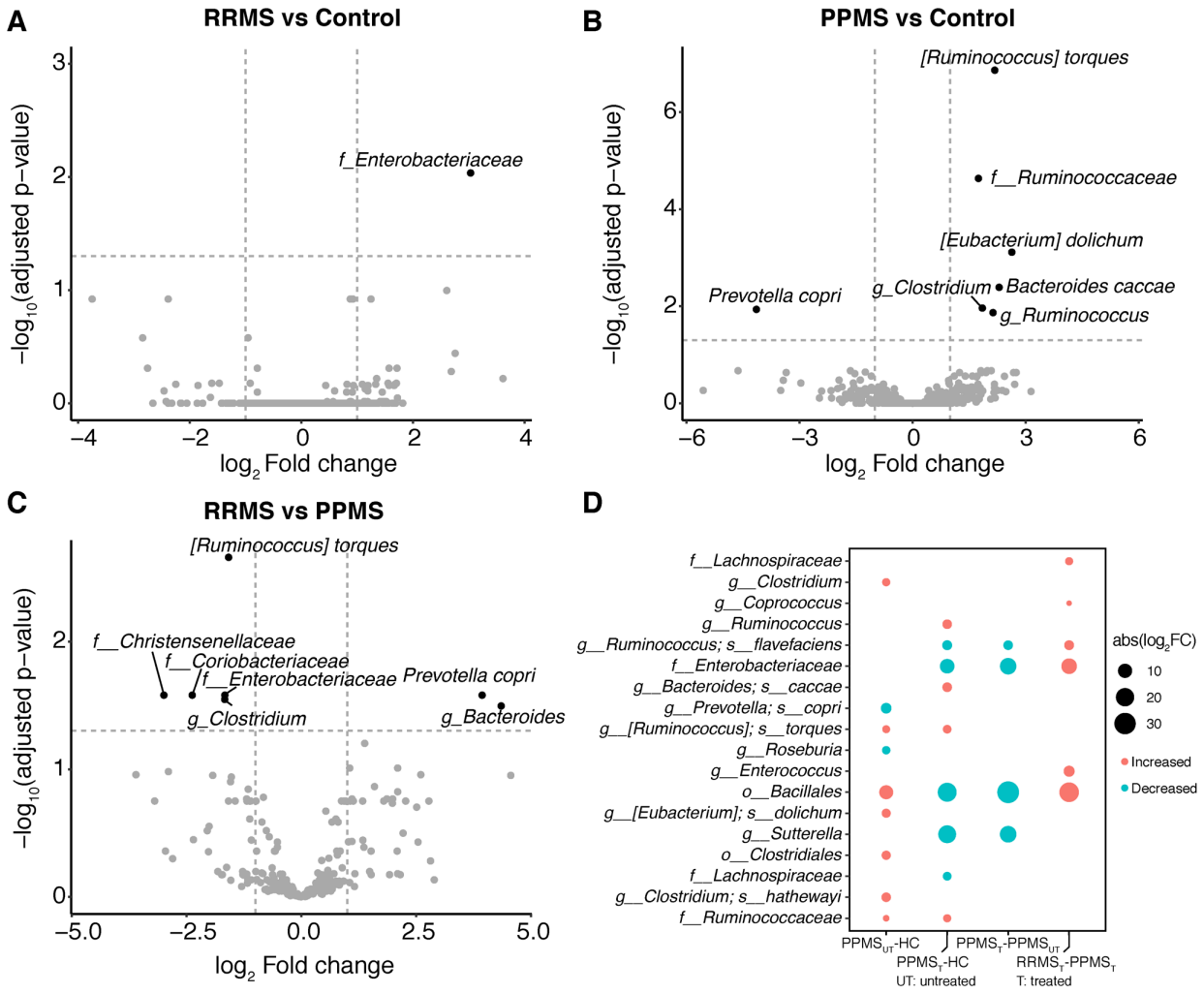

Figure 3. (A-C) Significantly differential ASVs associated with MS disease course by controlling the impact of recruitment site and sex (Fold change ≤ 2 and FDR ≤ 0.05). Only ASVs classified to genus/species level were labeled. (D) Significantly

differential ASVs compared between HC and MS stratified by treated status controlled by recruitment site and sex (Fold change ≤ 2 and FDR ≤ 0.05). Dot size is proportional to the absolute log fold change at base 2, red dots represent an increase of microbial abundance and yellow ones represent a decrease of the abundance.

Milestone #2: Sequencing of the MS microbiome.

This milestone was completed successfully.

Major Task 4: Data integration and advanced bioinformatics analysis.

Subtask 1: Integration of microbiome and genomic data

To understand how host genetics impact on the gut microbial composition, we applied the Chi-squared statistics to test whether there is an association between HLA genotype and the structure of a specific bacterium. The HLA genotyping reported 735 alleles in 22 HLA genes (Excel File 3), we first truncated the alleles from four sets of digits to two sets of digits covering only the difference in one or more nucleotide substitutions that change the amino acid sequence of the encoded protein, which identified 367 HLA alleles (Excel File 4). The presence/absence of each bacterium and the status (positive/negative) of each HLA allele were quantified among controls samples, MS patients and all samples, respectively, and then calculated for the association. We observed multiple significant associations between HLA alleles and gut microbes either characterized by 16S rRNA sequencing (Excel File 2) or shallow shotgun sequencing (Excel File 5) but only few or none remained significant after multiple test correction for all HLA alleles and bacteria (Excel File 6). We did not observe significantly associated bacteria with the MS-associated allele DRB1:15:01. Further effort is needed to stratify the samples into high MS risk or low risk groups based on the HLA alleles carried. A differential comparison of the microbes in these groups would identify the bacterial that associated with MS susceptibility rather than one allele.

Specific Aim 2: To test the effect of human MS microbiota in a spontaneous and induced EAE mouse model

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

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

Our proposal originally included experimental work in two mouse strains. The first one is C57Bl/6, which was already derived germ-free in the Mazmanian Lab and was ready to use. The second strain was a cross between Tob1-/- and 2D2, which results in a spontaneous form of EAE. Unfortunately, re-derivation of this strain was not possible due to technical impediments. Despite repeated attempts to derive this line germ-free, we were not successful and ultimately decided to carry out all experiments in C57Bl/6 mice.

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

We successfully performed a microbiota transplant from RRMS and PPMS patients into C57Bl/6 GF mice.
As Figure 4 below summarizes, EAE scores of mice receiving microbiota from the PPMS subject (red symbols) were significantly lower than those from mice receiving microbiota from a RRMS subject (green symbols). Colonization with microbiota from a control individual (orange symbols) resulted in EAE scores that were intermediate between the previous two groups (GF mice –black symbols-, as

expected developed minor signs of EAE).

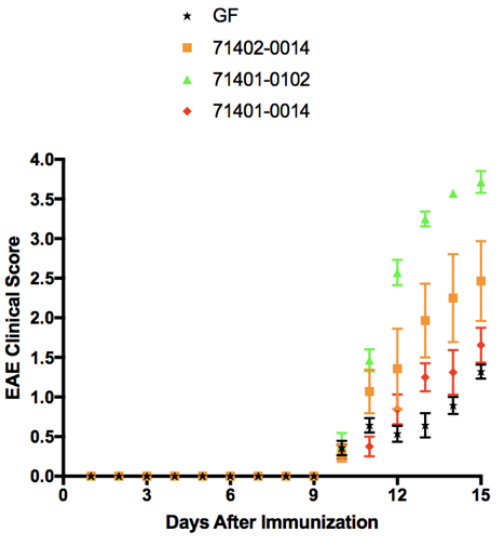

Figure 4. microbiota transfer from RMS and PPMS into GF mice.

We subsequently performed a second experiment, but unfortunately, some of the animals became contaminated and the experiment had to be terminated early. Yet another group of germ-free mice was bred to be able to repeat this experiment again but contamination was also detected causing the experiment to be terminated.
Noteworthy, each of these experiments take between 6-8 months to complete, and we run out of time in our award period to perform additional attempts. This being said, the infrastructure and the samples are available to continue these experiments with independent funds.

Major Task 2: Immuno-pathological characterization of experimental mice

Subtask 1: tissue dissection, harvesting and pathological analysis

In this set of experiments, lymph nodes, brains and spinal cords were harvested from the experimental mice shown in Figure 4, dissected and analyzed. Specifically, CNS tissues (brain and spinal cords) were stained with Hematoxylin & Eosin (H&E) and myelin specific stainings. Figure 5 depitcs a representative H&E stain showing increased lymphocytic infiltrates in mice receiving microbiota from RRMS (RR) and PPMS (PP) subjects, but not in those receiving microbiota from a healthy control (HC). Note that similar levels of infiltration are seen in the PP and RR groups. Minimal infiltration was observed in GF mice not colonized with any microbiota.

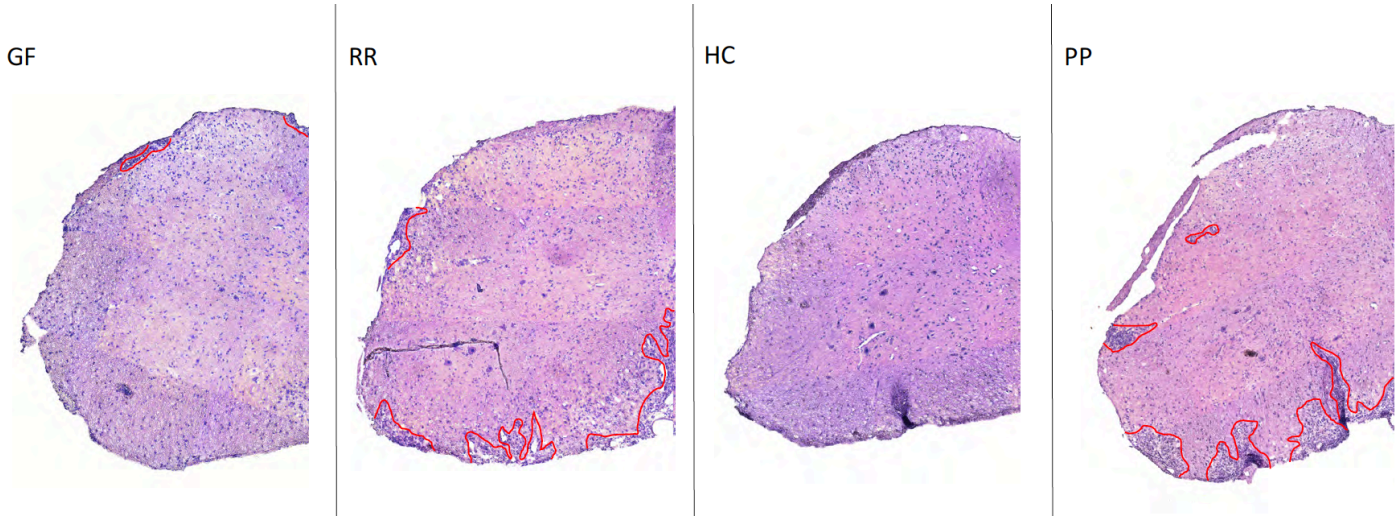

Figure 5. Histopathological staining of colonized GF mice.

Subtask 2: Flow cytometry

Flow cytometric analysis was not completed due to limited sample availability. Since we were not able to repeat the experiment due to contamination, additional blood samples were not available for this analysis.

Subtask 3: Immunohistochemistry

Immunohistochemistry was not completed due to limited sample availability. Since we were not able to repeat the experiment due to contamination, additional tissue blocks were not available for this analysis.

Subtask 4: Molecular characterization

Molecular characterization of tissues from these animals was not completed due to lack of available tissues.

Milestone #3: Co-authored manuscript.

Additional experiments to replicate our initial findings are underway with independent funds. We expect to finish these experiments and publish a manuscript in a timely manner.

References

1. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE *et al*: **Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice**. *Proceedings of the National Academy of Sciences of the United States of America* 2017, **114**(40):10719-10724.
2. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL *et al*: **Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models**. *Proceedings of the National Academy of Sciences of the United States of America* 2017, **114**(40):10713-10718.
3. Matsuoka K, Kanai T: **The gut microbiota and inflammatory bowel disease**. *Semin Immunopathol* 2015, **37**(1):47-55.
4. Wei B, Dalwadi H, Gordon LK, Landers C, Bruckner D, Targan SR, Braun J: **Molecular cloning of a Bacteroides caccae TonB-linked outer membrane protein identified by an inflammatory bowel disease marker antibody**. *Infection and immunity* 2001, **69**(10):6044-6054.
5. Shimizu J, Kubota T, Takada E, Takai K, Fujiwara N, Arimitsu N, Murayama MA, Ueda Y, Wakisaka S, Suzuki T *et al*: **Propionate-producing bacteria in the intestine may associate with skewed responses of IL10-producing regulatory T cells in patients with relapsing polychondritis**. *Plos One* 2018, **13**(9):e0203657.
6. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW *et al*: **Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters**. *Plos One* 2015, **10**(9):e0137429.

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

During this project, several opportunities for training and professional development were realized.

At the beginning of the project Keith Place (Baranzini Lab) visited the Mazmanian Lab in Caltech to learn from their senior rodent technician on sterile technique to keep mice germ-free within the gnotobiotic facility.

Elva Kogl (Baranzini lab) was trained in rederivation of germ-free mice by members of the Mazmanian (Caltech) and Turnbaugh (UCSF) Labs.

Xiaoyuan Zhou (Baranzini Lab) visited the Knight lab (UCSD) to learn about processing and analysis of 16S ribosomal RNA gene sequencing data.

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

Dissemination mostly occurred via scientific presentation in academic institutions. Since the analysis is still ongoing, most of the dissemination has been among the team members and closely related collaborators.

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

This is the final report.

4. IMPACT:

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

This project generated the largest microbiome dataset ever described in MS to date. In particular, this is the largest dataset of Primary progressive MS patients.

In addition, we have generated preliminary evidence suggesting that transfer of stool microbiota from PPMS patients into germ-free mice has lower impact than transferring stool microbiota from RRMS patients.

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

This project was focused in MS.

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

N/A

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

This project addressed a major question in the field. While it is difficult to measure societal impact, patients suffering from MS and their relatives have shown enthusiasm and curiosity to learn about our results.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

No changes to the original project were needed.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

The major challenge associated with this project was the time-consuming nature of performing experiments in germ-free conditions. While we were successful in setting up and maintaining our colonies for long periods, we were only able to conduct two successful microbiota transfer experiments. This in part was due to the long breeding period needed to bring germ-free mice to adulthood with control littermates. The slow breeding was compounded by the small size of our germ-free facility at UCSF, which only allowed us to house mice in the ISO-cage system (which offers higher flexibility but lower scale operations). A persistent challenge of any germ-free facility is bacterial contamination, which can occur at any cage change and jeopardize or invalidate an experiment. Unfortunately, 2 of our experiments needed to be terminated due to bacterial contamination.

In addition, due to time constraints, we were unable to rederive germ-free and experiment with the Tob1-/- mice.

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

None.

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

None.

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

None.

- **Significant changes in use or care of vertebrate animals.**

None.

- **Significant changes in use of biohazards and/or select agents**

None.

6. PRODUCTS:

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

- **Journal publications.**

Since analysis is still ongoing, no publications have yet resulted from this work.

- **Books or other non-periodical, one-time publications.**

None.

- **Other publications, conference papers, and presentations.**

None.

- **Website(s) or other Internet site(s)**

None.

- **Technologies or techniques**

None

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

None

- **Other Products**

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Personnel	Role	Person month
Sergio Baranzini	PI	10
Bruce Cree	PI	5
Rob Knight	PI	5
Ilana Katz-Sand	Patient evaluation and recruitment	5
Patrizia Casaccia	Molecular biology, immunology	2
Sneha Singh	Clinical Coordinator	15
Adam Santaniello	Database manager	5
Xiaoyuan Zhou	Post Doc	8
Tomasz Piotr Kosciolek	Post Doc	5
Jon Sanders	Post Doc	5
Stefan Janssen	Post Doc	5
Daniel McDonald	Bioinformatics Programmer, PhD	7
Jeffrey E Dereus	Lab Technician - Programmer	8

Sergio Baranzini: Submitting PI. Oversaw and coordinated all scientific and budgetary aspects of this proposal.

Bruce Cree: Clinical PI. Directed patient evaluation and recruitment efforts at UCSF.

Rob Knight: oversight and management of sequencing phase of project overall.

Ilana Katz-Sand: Directed patient evaluation and recruitment efforts at UCSF.

Patrizia Casaccia: Conducted molecular and immune profiling of mouse tissues derived from the fecal transfer experiments.

Adam Santaniello: Built and oversaw the sample and patients database resulting from this project.

Xiaoyuan Zhou: Analyzed all 16S and shotgun sequencing data from the Knight lab. Also conducted statistical analysis on metadata and HLA.

Amnon Amir: Developed fast algorithms for OTU picking that were used in the 16S analysis. Amnon left UC-SD in May 2017.

Stefan Maximilian Janssen: Developed methods to analyze exRNA sequences to assess interkingdom communication.

Tomasz Piotr Kosciolek: Developed methods to identify ncRNAs in the genomic and metagenomic data.

Daniel McDonald: Conducted computational studies of sequenced genomes, extracting and integrating biological information and data. Daniel joined the project in May 2017.

Jon Sanders: Developed a genome annotation pipeline and fast matching for separating human reads from microbial that will be used in the shotgun metagenomics.

Jeffrey Dereus: Managed the software team, setup of project tracking and LIMS for this project.

- **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 change from last reporting period.

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

- **Organization Name: N/A**
- **Location of Organization:**
- **Partner's contribution to the projec**t:
- **Financial support:** Additional support has been obtained from the Valhalla Charitable Foundation and the National MS society**.** This additional support will enable continuation of this project.
- **In-kind support:**
- **Facilities**:
- **Collaboration**:
- **Personnel exchanges:**
- **Other:**

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:**

9. APPENDICES: