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Role of the Gut Microbiome as Determinant of Depression in MS Subjects

PRINCIPAL INVESTIGATOR: Laura Piccio, MD, PhD

CONTRACTING ORGANIZATION: The Washington University

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this annual report, we detailed our activities in the dates since initial IRB approval (11/23/2021). Biosample collection supplies were ordered and received in sufficient quantity. We have begun to contact and schedule patients for study visits. Twenty-two patients have completed study visits, 12 patients have been scheduled for visits occurring in the coming 2 months. The REDCap database was developed and moved into production, participant data have been entered and are up to date for those that completed the study visits. Training was completed for the study team members involved in administering cognitive and functional assessments, entering data into the REDCap database, and processing the lab samples.

15. SUBJECT TERMS
Multiple Sclerosis, Gut microbiome, Depression, Metabolites, Immune cells

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a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
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1. INTRODUCTION:

We will study if specific gut microbiome or gut-derived metabolites are associated with depression in people with Multiple Sclerosis (pwMS). Mechanistically, we further hypothesize that depression in pwMS is related to decreased abundance of gut bacteria with GABA-producing activities and/or with anti-inflammatory properties. To determine if the presence of depression in pwMS is associated with specific gut microbiome, gut-derived metabolites or peripheral blood immune profiles. We will perform a cross-sectional study in clinically stable pwMS recruited at the John L. Trotter MS Center. We will evaluate the presence of depression using the Quality of Life in Neurological Disorders (Neuro-Qol) depression scale, one of the 13 scales in the Neuro-Qol recently developed by the NIH using modern psychometric techniques and validated in pwMS.

A total of 120 pwMS will be recruited: 60 with and 60 without depression based on the Neuro-Qol depression scale. At the study visit each participant will be asked to provide a stool sample for microbiome analyses and a blood sample for peripheral blood immunophenotyping. Potential confounders will be collected and treated as covariates in the analyses. These include: 1) degree of disability (EDSS); 2) treatment with anti-depressants and DMTs; 3) a 4-days food diary to evaluate diet composition; 4) weight and height to calculate the BMI; 5) fatigue; 6) level of physical activity; 7) sleep quality.

2. KEYWORDS:

Multiple Sclerosis, Gut microbiome, Depression, Metabolites, Immune cells

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of the project were as follows:

Major Task 1: Obtaining IRB and HRPO approvals, establish the work-flow for enrollment and study visits

a) Subtask 1. Obtain regulatory approvals from IRB and HRPO
b) Subtask 2. Setting up the forms in REDCap for all the variables collected during office visits and outcome measures (e.g. microbiome and flow cytometry data)
c) Subtask 3. Set up meetings with all personnel involved (study coordinator, dietician, technician, post-doc) to establish the work-flow for enrolling patients and study visits
d) Subtask 4. Ordering all the reagents and supplies needed for stool and blood sample collection and processing (eg. all reagents for flow cytometry)
e) Milestone: Getting everything ready to start patient's enrollment

Major Task 2: Enrollment of MS subjects in the study, sample collection and collection of clinical data

a) Subtask 1. Perform all study visits (~8 subjects/month; total of 150 subjects to enroll at least 120 subjects; 1 study visit/patient)
b) Subtask 2. Collect all stool and blood samples
c) Subtask 3. Collect all the clinical data: body mass index, EDSS, measures of fatigue, sleep, physical activity
d) Milestone: Enroll all patients, collect all biological specimens and clinical data

Major Task 3: Perform microbiome and metabolome analyses

a) Subtask 1. DNA extraction, 16S rRNA gene sequencing, metagenomic shotgun sequencing, LC-Mass Spectrometry.
b) Subtask 2. Microbiome and metabolome data analysis
c) Milestone(s) Achieved: Identify the microbiome and metabolites associations with depression

Major Task 4: Perform peripheral blood immune phenotyping analyses

a) Subtask 1. Perform flow cytometry staining on fresh blood samples
b) Subtask 2. Immuno-phenotyping and cytokine production analyses
c) Milestone(s) Achieved: Identify immune profiles associated with depression

Major Task 5: To quantify GABA production and determine microbiome-immune system interaction in vitro

a) Subtask 1. To quantify GABA levels in whole stool and blood from pwMS with or without depression
b) Subtask 2. Anaerobic culture of taxa of interests and test their GABA production in vitro
c) Milestone(s) Achieved: Determine correlations between GABA production in stool or specific bacteria with depression.

Major Task 6: Test microbiome-immune system interaction in vitro.

a) Subtask 1. Determine immune cell population and cytokine production in coculture of supernatant from whole stool and PBMCs.
b) Subtask 2. Determine immune cell population and cytokine production in coculture of supernatant from specific bacteria and PBMCs.
c) Milestone(s) Achieved: Determine anti- or pro- inflammatory effects of GABA producing bacteria and other bacteria strongly correlated with depression

Major Task 7: Final analyses and manuscript preparation
a) Subtask 1. Analyses will be completed and results compiled in one or more manuscripts
b) Milestone(s) Achieved: Manuscript preparation and submission

What was accomplished under these goals?

For the first Major Task, 100% completion of this goal was achieved April 2022. Under this goal initial materials, including the finalized consent form, were submitted to the Washington University IRB on 7/13/2021 and received approval on 8/20/2021. Data capture forms were developed for the project REDCAP database, which was moved from development into production on March 2022. Investigators and study coordinators were trained on the entry of study data into the REDCAP database. Supplies for biosample collection and processing were ordered and ready for patient enrollment by April 2022. We experienced a significant delay in the receipt of several vacutainer tubes for blood sample collection due to vendor backorder.

For the second major task, to date we have called >60 patients, screened and consented 49 patients, and completed 22 patient visits. Currently, there are 12 patient visits scheduled to occur in the next 2 months. We are meeting weekly to review recruitment efforts and are adjusting our efforts accordingly. We have observed ~50% drop-out rate (patient initially consented, but did not come to the study visit), for this reason we have adjusted out workflow for enrollment. We initially planned to mostly recruit patients who followed a three-month timeline (screening, sample/food diary collection, in-person visit completion), but are also now recruiting participants who will return in 4-6 months for a follow up visit in our clinic with the intention to complete their study visit on the same date. This has proved to be more convenient for participants who live far from our facility or who have trouble taking time off for work.

For the third and fourth major tasks, we have only completed processing blood samples to the point where we performed flow cytometry and prepare samples for further analyses.

For the fifth and sixth major tasks, the blood and stool samples are being stored for future shipment to the University of Connecticut for planned GABA and micriobiome-immune system analyses.

The seventh major task is dependent on the completion of the previous five tasks and is not yet started.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

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

In the next reporting period we plan to continue to contact participants and complete study visits. Collected data that has been organized for enrolled participants will be added into the study's redcap database. We will continue to process and store collected biospecimens as planned.

4. IMPACT:

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

Nothing to Report.

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.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

We initially planned to mostly recruit patients who followed a three month timeline (screening, sample/food diary collection, in-person visit completion), but are also now recruiting participants who will return in 4-6 months for a follow up visit in our clinic with the intention to complete their study visit on the same date. This has proved to be more convenient for participants who live far from our facility or who have trouble taking time off for work.

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

We have experienced delay in our ability to acquire some of the biosample collection materials, specifically vacutainer tubes for blood collection, due to a four-month backorder attributed to supply chain problems with the vendor.

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.

6. PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Journal publications

Nothing to Report.

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)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Laura Piccio
Project Role	Principal Investigator
Nearest person month worked	
Contribution to Project	Dr. Piccio has overseen the overall study, the build of the REDCap database, and performed study visits.
Funding Support	(effort supported by this grant)

Name	Amber Salter
Project Role	Co-Investigator
Nearest person month worked	
Contribution to Project	Dr. Salter has contributed to the build of the REDCap database.
Funding Support	(effort supported by this grant)

Name	Valeria Tosti
Project Role	Research Instructor
Nearest person month worked	
Contribution to Project	Dr. Tosti was involved in overall study supervision, patient enrollment and in all the procedure and assessment planned during the visits.
Funding Support	(effort supported by this grant)

Name	Courtney Dula
Project Role	Research Coordinator
Nearest person month worked	
Contribution to Project	The study coordinator will be involved in patient enrollment and in all the procedure and assessment planned during the visits including: drawing blood, coordinating for collection of the stool samples, clinical testing planned at the visits. She will maintain informed consent documents, and interact with the IRB for all the aspects related to this study.
Funding Support	

Name	Giovanni Pastori
Project Role	Research Coordinator
Nearest person month worked	
Contribution to Project	The study coordinator will be involved in patient enrollment and in all the procedure and

	assessment planned during the visits including: coordinating for collection of the stool samples, clinical testing planned at the visits. He will maintain informed consent documents.
Funding Support	(effort supported by this grant)

Name	Robert Mikesell
Project Role	Technician
Nearest person month worked	
Contribution to Project	Mr. Mikesell will process blood and stool samples obtained from the patients. These samples will be frozen after collection and they will be thawed when testing will be performed in all the samples together. He has been working with Dr. Piccio for more than 5 years and he is very experienced in all the procedures planned for this application.
Funding Support	(effort supported by this grant)

Name	Kathy Obert
Project Role	Dietician
Nearest person month worked	
Contribution to Project	Mrs. Obert will administer the food diaries and then analyze them to assess calories and nutrients intake. She is a very experienced dietician that has been working with Dr. Piccio for almost 3 years.
Funding Support	(effort supported by this grant)

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 for the other support of the PD/PI.

The subcontract with the Barnes Jewish College was not finalized because Dr. Pamela Newland is currently on medical leave and we do not know if and when she will be available again to work on this study. Therefore, the funding has been used to support another person to assist with recruitment (Giovanni Pastori). A new SOW is submitted with the annual report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

9. CONCLUSIONS

The study is currently enrolling and major tasks planned in year 1 have been achieved.

10. REFERENCES

Not included.

11. APPENDICES:

Updated Statement of work

STATEMENT OF WORK

Site 1:
Washington University School of Medicine (Wash U)
Address for Org#1
PI: Dr. Piccio
Address:
Campus Box 1054
1 Brookings Drive
St. Louis, MO 63130-4862

Site 2:
University of Connecticut Health Center (UCONN)
Address for Org #2
Partnering PI: Dr. Zhou
Address:
263 Farmington Avenue
Farmington
Connecticut, 06030-5335

Site 3
University of Texas Southwestern
Address for Org#3
Partnering PI: Dr. Salter
Address:
5323 Harry Hines Blvd, Dallas, TX 75390,
United States

Specific Aim 1 **Perform a cross-sectional study to determine if the presence of depression in pwMS is associated with specific gut microbiome, gut-derived metabolites or peripheral blood immune profiles.**	**Timeline**	**Site 1**	**Site 2**	**Site 3**
Major Task 1: Obtaining IRB and HRPO approvals, establish the work-flow for enrollment and study visits	Months	Wash U	U Conn	UT Southwestern
Subtask 1. Obtain regulatory approvals from IRB and HRPO	1-5	Dr. Piccio	Dr. Zhou	Dr. Salter
Subtask 2. Setting up the forms in REDCap for all the variables collected during office visits and outcome measures (e.g. microbiome and flow cytometry data)	1-5	Dr. Piccio	Dr. Zhou	Dr. Salter
Subtask 3. Set up meetings with all personnel involved (study coordinator, dietician, technician, post-doc) to establish the work-flow for enrolling patients and study visits	1-5	Drs. Piccio	Dr. Zhou	
Subtask 4. Ordering all the reagents and supplies needed for stool and blood sample collection and processing (eg. all reagents for flow cytometry)	1-5	Dr. Piccio		
Milestone(s) Achieved: Getting everything ready to start patient's enrollment	5	Dr. Piccio		
Local IRB Approval	1-2	Dr. Piccio	Dr. Zhou	

HRPO Approval	2-5	Dr. Piccio	Dr. Zhou	
REDCap forms finalized and ready to collect data;	5	Dr. Piccio	Dr. Zhou	Dr. Salter
All reagents needed ordered and available	5	Dr. Piccio	Dr. Zhou	
Major Task 2: Enrollment of MS subjects in the study, sample collection and collection of clinical data				
Subtask 1. Perform all study visits (~8 subjects/month; total of 150 subjects to enroll at least 120 subjects; 1 study visit/patient)	5-24	Dr. Piccio		
Subtask 2. Collect all stool and blood samples	5-24	Dr. Piccio		
Subtask 3. Collect all the clinical data: body mass index, EDSS, measures of fatigue, sleep, physical activity	5-24	Dr. Piccio		
Milestone(s) Achieved: Enroll all patients, collect all biological specimens and clinical data	5-24	Dr. Piccio		
Major Task 3: Perform microbiome and metabolome analyses				

Subtask 1. DNA extraction, 16S rRNA gene sequencing, metagenomic shotgun sequencing, LC-Mass Spectrometry.	24-30		Dr. Zhou	
Subtask 2. Microbiome and metabolome data analysis	24-30		Dr. Zhou	
Milestone(s) Achieved: Identify the microbiome and metabolites associations with depression	24-30		Dr. Zhou	
Major Task 4: Perform peripheral blood immune phenotyping analyses				
Subtask 1. Perform flow cytometry staining on fresh blood samples	5-24	Dr. Piccio		

Subtask 2. Immuno-phenotyping and cytokine production analyses	24-30	Dr. Piccio	Dr. Zhou	
Milestone(s) Achieved: Identify immune profiles associated with depression	24-30	Dr. Piccio	Dr. Zhou	
Specific Aim 2 **To quantify GABA production in pwMS with or without depression and determine microbiome-immune system interaction in vitro.**				
Major Task 5: To quantify GABA production and determine microbiome-immune system interaction in vitro				
Subtask 1. To quantify GABA levels in whole stool and blood from pwMS with or without depression	24-36		Dr. Zhou	
Subtask 2. Anaerobic culture of taxa of interests and test their GABA production in vitro	24-36		Dr. Zhou	
Milestone(s) Achieved: Determine correlations between GABA production in stool or specific bacteria with depression.	24-36		Dr. Zhou	
Major Task 6: Test microbiome-immune system interaction in vitro				
Subtask 1. Determine immune cell population and cytokine production in coculture of supernatant from whole stool and PBMCs.	30-36		Dr. Zhou	Dr. Salter
Subtask 2. Determine immune cell population and cytokine production in coculture of supernatant from specific bacteria and PBMCs.	30-36		Dr. Zhou	Dr. Salter
Milestone(s) Achieved: Determine anti- or pro-inflammatory effects of GABA producing bacteria and other bacteria strongly correlated with depression	30-36		Dr. Zhou	
Major Task 7: Final analyses and manuscript preparation				
Subtask 1. Analyses will be completed and results compiled in one or more manuscripts	30-36	Dr. Piccio	Dr. Zhou	Dr. Salter
Milestone(s) Achieved: Manuscript preparation and submission	30-36	Dr. Piccio	Dr. Zhou	Dr. Salter

ENROLLMENT TABLE

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Wash U	0	0	25	25	25	25	25	25				
Target Enrollment (cumulative)	**0**	**0**	**25**	**50**	**75**	**100**	**125**	**150**				