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TITLE: Composition, Function, and Role of the Intestinal Microbiome in Pediatric Heart Failure and Heart Transplantation

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14. ABSTRACT

The role of the intestinal microbiome has gained substantial interest as a novel marker for prognosis of disease and as a potential target for therapeutic intervention. There is evidence that the microbiome exerts a fundamental influence on immunity, can be altered in heart failure, and can be further disrupted by immunosuppressive medications. This indicates the potential significant impact that alterations to the intestinal microbiome may play in the care of children who have undergone heart transplant. The composition, function, and role of the intestinal microbiome in children with congenital heart disease or heart transplant is not currently known. The main objective of this research is to characterize and investigate the role of the intestinal microbiome in this population and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. Patient recruitment and stool collection is still ongoing. However, preliminary analyses indicate that there are significant microbial compositional shifts after patients undergo heart transplant. Furthermore, heart transplant patients with post-transplant complications have a significantly different microbial composition compared to those without complications. As this study is prospective in nature, there is still on-going follow up and stool collection to determine associations between specific microbial changes and specific post-transplant complications.

15. SUBJECT TERMS

Intestinal Microbiome, Metabolomics, Pediatric Cardiology, Congenital Heart Disease, Heart Transplant, Diarrhea

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1. INTRODUCTION:

The role of the intestinal microbiome has gained substantial interest as a novel marker for diagnosis and prognosis of disease and as a potential target for therapeutic intervention. There is evidence that the microbiome exerts a fundamental influence on innate and adaptive immunity, can be altered in heart failure, can shift rapidly during intestinal ischemia and reperfusion, and can be further disrupted by immunosuppressive medications. This indicates the potential significant impact that alterations to the intestinal microbiome may play in the care of children who have undergone heart transplant. Furthermore, the success of fecal microbial transplant in patients with *Clostridium difficile* diarrhea has demonstrated that the microbiome is potentially modifiable and indicates the therapeutic potential of microbiome restoration to improve the duration and severity of diarrheal disease. The composition, function, and role of the intestinal microbiome in children and young adults with congenital heart disease or heart transplant is not currently known. Our **long-term goal** is to identify modifiable risk factors and develop innovative treatment strategies to improve outcomes for these patients. The **main objective** of this research is to characterize and investigate the role of the intestinal microbiome in this population and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. Characterizing the intestinal microbiome in this patient population offers significant potential to greatly impact and improve the health outcomes of individuals with congenital heart disease. Improving post-heart transplant outcomes can also ameliorate the supply-and-demand mismatch crisis of donor organ allocation by reducing the need for re-transplantation.

2. KEYWORDS:

Intestinal Microbiome, Metabolomics, Pediatric Cardiology, Congenital Heart Disease, Heart Transplant, Diarrhea

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The three major goals of this project are to:

1) Prospectively document and compare alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults listed for heart transplant to the intestinal microbiome and associated metabolome in healthy, age- and sex-matched controls.
2) Prospectively document and compare alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults before and after placement of a ventricular assist device or heart transplant.
3) Evaluate the association of alterations in the composition and diversity of the intestinal microbiome and associated metabolome in children and young adults with the following post-heart transplant outcomes: diarrhea, systemic infection, coronary allograft vasculopathy, graft rejection, graft failure, and re-transplant or death.

The target enrollment through 12 months was projected to be 140 patients. Currently, 150 patients have been enrolled in the study. This number exceeds our goal of 140 patients to be recruited by 12 months. From these patients, 130 pain/stool diaries and 130 stool samples have been collected. This includes 15 patients with pre-transplant samples who have undergone transplant and from whom sequential post-transplant samples have been obtained. Thirteen of the 150 patients have been supported with a ventricular assist device. All 130 stool samples have undergone DNA extraction, processing, and

sequencing. As stated in the approved SOW, the timeline for performing data analysis is during months 15-18. We are currently performing these analyses.

What was accomplished under these goals?
The major activities that have occurred during this reporting period have included identifying, recruiting, consenting, and enrolling eligible participants. To date, 150 patients have been enrolled in the study. Each patient has filled out a pain/stool diary and nutritional recall survey, and each of these surveys have been entered in to our database. From these 150 patients that have been enrolled, we collected 130 stool samples. This includes samples from 15 patients who have undergone heart transplant during the study period and from whom pre- and post-transplant samples have been obtained. Patient characteristics that are recorded include age, gender, race/ethnicity, type of congenital heart disease or cardiomyopathy, and medications and dosage at time of stool collection. In addition, prospective clinical outcomes are being collected and entered in to our database. Multiple patient outcomes including diarrhea, infection, coronary allograft vasculopathy, graft rejection, graft failure, and death have occurred.

The main objective of this proposal is to characterize and investigate the role of the intestinal microbiome in children that undergo heart transplantation and generate the preliminary data necessary to determine effect estimates that will be used to power large prospective randomized studies of targeted microbial restoration. The target enrollment through 12 months was 140 patients. We have met this objective by recruiting and enrolling 150 patients. Stool collection is still ongoing as is patient recruitment and enrollment. At 12 months, we have collected 130 stool samples.

From stool samples, bacterial DNA is extracted, and the resulting nucleic acid is processed through an Illumina MiSeq 16S sequencing pipeline. Two separate regions of the highly variable areas of the 16S rRNA gene, V1V3 and V4, are targeted for sequencing. Resulting raw sequences are analyzed via the standard analysis pipeline, which utilizes the UPARSE algorithm for clustering of sequences into operational taxonomic units (OTUs) and the SILVA database for taxonomic classification of each OTU. Metabolomic analyses of stool extracts is performed using p180 Kits (Biocrates) on the Ultra-Performance Liquid Chromatography tandem mass spectrometer (AbSciex 6500).

Complete data analysis has not been performed yet. As per the approved SOW, the plan is to perform data analysis during months 15-18. This allows for quality control to analyze the stool samples in large batches. As such, there are no conclusions to provide. However, some preliminary results are included below. Pre-transplant (Pre-HTx) patients have a different composition compared to post-transplant (Post-HTx) patients at both the phyla and genus levels (Figure 1A and Figure 1B), and patients with adverse clinical “events” have a different composition compared to asymptomatic or “routine” patients at both the phyla and genus levels (Figure 2A and 2B). There are also multiple specific microbial genus level compositional differences (Figures 3A and 3B). As this study is prospective in nature, we will continue to follow these patients to determine associations between specific compositional changes and clinical events/adverse outcomes.

We do not anticipate that we will obtain the goal of ~500 stool samples by the completion of the study. One possible reason for this is the current exclusion of patients under 1 year of age. Currently, the majority of our patients who are actively listed and awaiting heart transplant are less than 1 year of age. While we anticipate more patients over 1 year of age to become eligible for the

study, we also do plan to propose an amendment to the protocol to include patients under 1 year of age.

Figure 1A

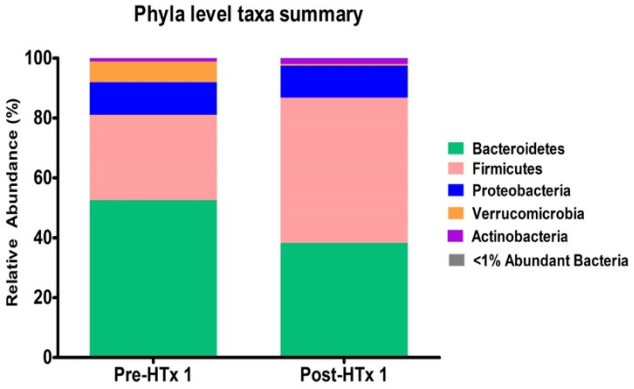


Figure 1B

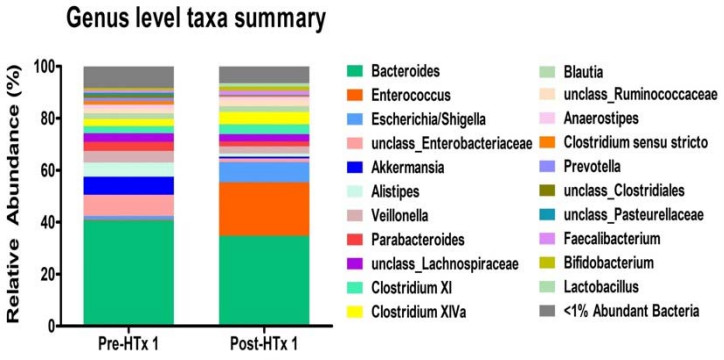


Figure 2A

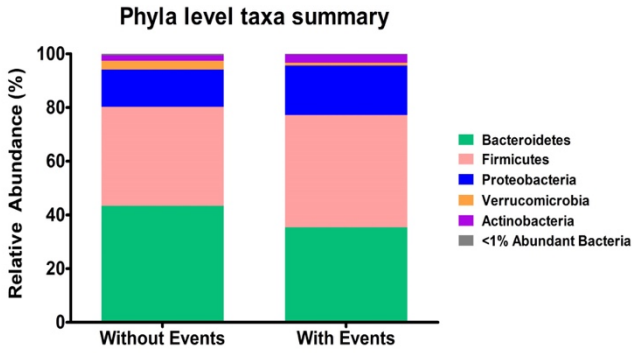


Figure 2B

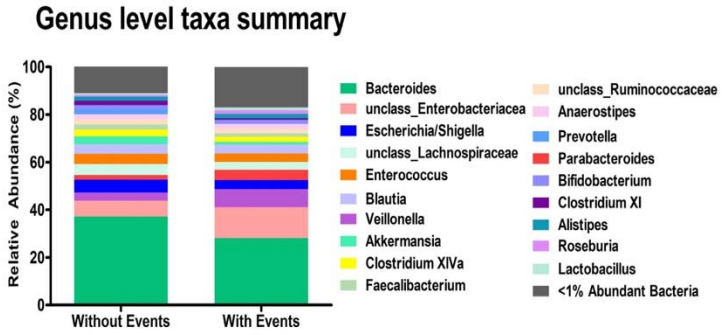


Figure 3A

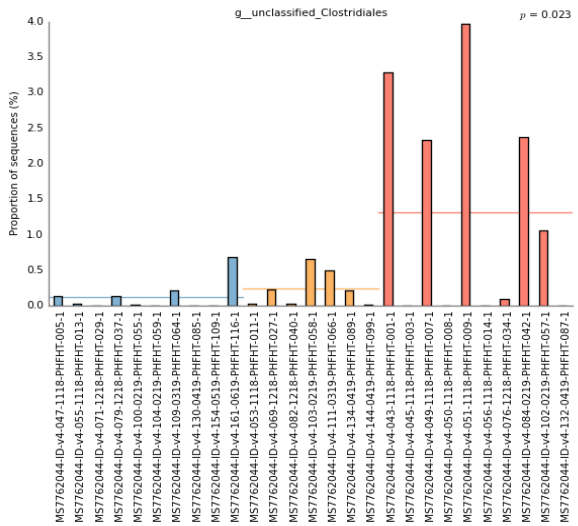
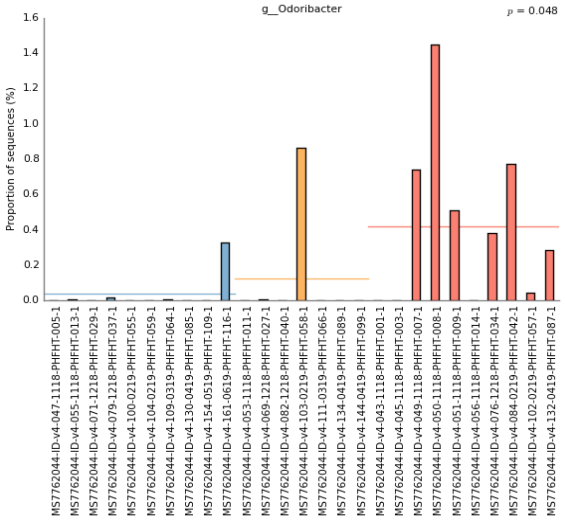


Figure 3B



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?

We will continue to identify, recruit, consent, and enroll eligible participants. In addition, while we anticipate more patients over 1 year of age to become eligible for the study, we also do plan to propose an amendment to the protocol to include patients under 1 year of age.

During the next reporting period, data analysis will be performed, so we plan to provide results and conclusions at the next report.

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

There have been no significant changes in the project or its direction. However, we do plan to propose an amendment to the protocol to include patients under 1 year of age.

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

There have been no problems. Based on the number of samples we have collected, we do not anticipate collecting the projected number of samples by the end of the study period. As such, we

plan to propose an amendment to include patients under 1 year of age which will increase the number of samples we anticipate being able to collect.

Changes that had a significant impact on expenditures

We were able to find and utilize sample collection kits from a different vendor at a much reduced cost, saving us approximately $9300. This will free up additional funds to allow for more detailed and more sophisticated microbiome and metabolomic analysis (approximately $7500). We also plan to disseminate our results at multiple medical meetings and plan to allocate the remaining additional funds for conference travel and publication costs.

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

Significant changes in use or care of human subjects

No changes. As stated above, we do plan to propose an amendment to the protocol to include patients under 1 year of age.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS:

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

  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: Joseph Spinner, MD
Project Role: PI
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0001-9539-6252
Nearest person month worked: 4
Contribution to Project: Dr. Spinner is the project lead & is responsible for the design, implementation, & deliverables.
Funding Support: Baylor College of Medicine Pediatric Cardiology covers salary & protected time for project

Name: Sridevi Devaraj
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0001-9189-7914
Nearest person month worked: 1
Contribution to Project: Dr. Devaraj is a co-investigator responsible for sample extractions, sequencing, & metabolomics testing. She also interprets the statistical analysis performed by the biostatisticians at the TCH Microbiome Center.
Funding Support: None

Name: Ayesha Masood
Project Role: Research Coordinator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: Ms. Masood assists with patient identification, screening, & enrollment. She consents patients, collects records, diaries, samples & surveys, & keeps the database up to date.
Funding Support: None

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.

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS: Not applicable.

QUAD CHARTS: Not applicable.

9. **APPENDICES:** None.