Children's National

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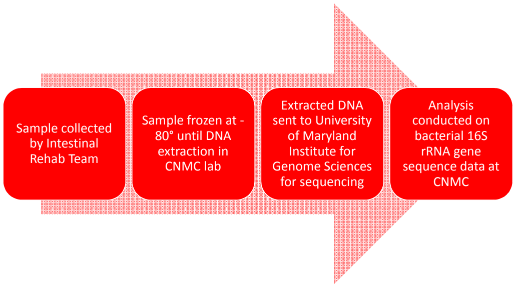
Study Design

- Prospective study using next-generation sequencing (NGS) to characterize the microbiome of patients with Short Bowel Syndrome
- Three patient cohorts:
  - SBS patients without TPN (n = 11)
  - SBS patients with TPN (n = 8)
  - Normal Controls (n = 3)
- All patients between 2-10 years

Study Protocol

- Samples collected as part of routine management.
- No antibiotics for at least two weeks preceding collection.
- Paired jejunal and stool specimens obtained.
- Samples sent to University of Maryland for 16S rRNA sequencing using an Illumina MySeq

Study Workflow



Sample collected by Intestinal Rehab Team

Sample frozen at -80° until DNA extraction in CNMC lab

Extracted DNA sent to University of Maryland Institute for Genome Sciences for sequencing

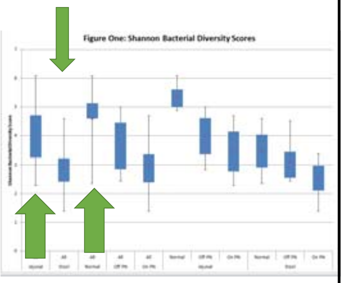
Analysis conducted on bacterial 16S rRNA gene sequence data at CNMC

Shannon Diversity Index

- What is the Shannon Diversity Index?
  - A numerical measure of the diversity, or number of different types of bacteria, in a sample.
  - Very sensitive to change in abundance of rare species
  - Does not measure absolute numbers of bacteria in the sample

Bacterial Diversity Scores

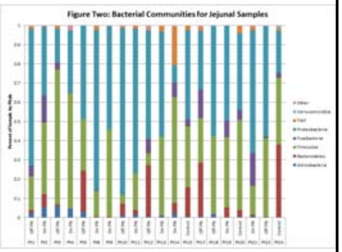
- More bacterial diversity in patients without SBS.
- More diversity in patients off TPN compared to those on TPN.
- Jejunal samples more diverse than stool samples
- No single type of bacteria responsible for these differences.



Jejunal Diversity

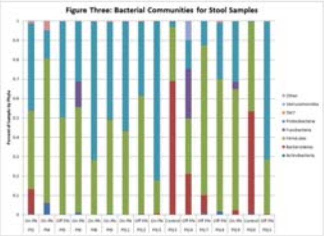
- Elevated jejunal diversity unexpected.
- Prior studies using 16S mostly in biopsy samples.
- Small intestine microbiome highly variable and subject to large temporal variations.
- Limited pediatric studies.
- Additional study needed.
  - Bigger sample size.

Jejunal Bacterial Composition



- Samples dominated by Proteobacteria and Firmicutes phyla.
- *Enterococcus sp.* and *Lactobacillus sp.* most common Proteobacteria observed.
- *Klebsiella sp.* and *Escherichia coli* most common Firmicutes.
- Correlated well with jejunal cultures obtained on same samples.

Stool Bacterial Composition



- Patients without SBS had a greater proportion of Bacteroidetes phyla
- *Bacteroides fragilis* and *Prevotella melaninogenica* most common species observed.
- 16S rRNA sequencing cannot always identify the complete genus and species of a bacteria, if the sequence length is limited.

Future Directions

- Additional studies correlating changes in microbiome to changes in patient clinical status.
- Correlation of nutritional status to changes in intestinal microbiome
- New diagnostics to help diagnosis SBBO and guide appropriate therapy.

Questions?