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### Characterization of the Duodenal Microbiome in Children with and without Crohn's disease

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# Characterization of The Duodenal Microbiome in Children with and without Crohn's Disease

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**IRB Number: 14030113**

**Describe role of Submitting/Presenting Trainee in this project (limit 150 words):**

**Dr. Schmidt has been involved with data analysis and interpretation of the processed tissue samples, along with abstract and manuscript development.**

**Background, Objectives/Goal, Methods/Design, Results, Conclusions limited to 500 words**

**Background:**

Complex interactions between the host and the intestinal microbiome have been implicated in the pathogenesis and the potential treatment of many gastrointestinal diseases. The majority of studies in Inflammatory Bowel Disease (IBD), a chronic relapsing and remitting autoimmune disorder that compromises the intestinal mucosa, have focused on characterization of the microbial community in the colon and the distal small intestine, where bacterial species are the most abundant. However, Crohn's disease (CD), a type of IBD, also affects the proximal small intestine (e.g., duodenum), with prevalence of duodenal CD higher in children than adults. Although it is well established that differences in bacterial abundance and composition are observed among different regions in the intestine, the mucosally-adherent microbiome in the pediatric duodenum remains to be characterized and could offer insight into pathogenesis of multiple pediatric disorders affecting the duodenum (e.g., IBD, celiac disease, small bowel bacterial overgrowth).

**Objectives/Goal:**

The objective of this investigation was to characterize the bacterial composition of the mucosally-adherent microbiome in children with and without CD.

## **Methods/Design:**

Fresh-frozen mucosal biopsies were obtained from the terminal ileum (TI) and the duodenum of children undergoing diagnostic evaluation with routine endoscopy at CMH, between 2014 and 2019 (n=856). Biopsies from children with treatment-naïve CD (i.e., no immunomodulators) and age-matched controls without CD (n=81) were assessed for histopathologic inflammation and microbial composition using 16S rRNA sequencing of the bacterial V4 region, via the commercially available Illumina 250-base pair sequencing platform (Novogene; Beijing, China). Bacterial relative abundance, composition and alpha and beta diversity, were compared across tissue types and disease states (Control vs. CD-active vs. CD-inactive) using UniFrac dissimilarity coefficients and LEfSe analysis.

## **Results:**

118 samples (49 duodenal, 69 TI) from 73 children (2-18 years; 35 CD, 38 Control) passed initial quality check, identifying >900 bacterial species. Significant differences in bacterial composition were noted in the duodenum vs. TI, independent of disease status ( $p \leq 0.03$ ), with pairwise weighted UniFrac dissimilarity coefficients ranging from 0.67 to 1.27, and least regional dissimilarity in bacterial composition noted in CD-inactive (UniFrac=0.670). In the duodenum, Pseudomonadales abundance was significantly increased in Controls, and Prevotellaceae in CD-active (linear discriminant analysis score > 4). Although not statistically significant, greatest relative abundance of Bacteroidetes, with minimal abundance of Spirochaetes, was observed in the duodenum of CD-active compared to CD-inactive or Control.

## **Conclusions:**

Our study is the first to characterize the mucosally-adherent microbiome in the pediatric duodenum and confirms that the microbial composition of the proximal small intestine is distinctly different from the distal small intestine. Although our sample size is small (n=49), our findings suggest that the healthy pediatric duodenal microbiome is characterized by an abundance of Pseudomonadales, while the duodenal dysbiosis typical of active duodenal CD is characterized by increased abundance in Prevotellaceae and Bacteroidetes, and decreased Spirochaetes. In the absence of active disease, regional differences in the mucosally-adherent microbiome are diminished in individuals with CD, suggesting that there remains a microbial dysbiosis in individuals with CD independent of disease activity.