Microbial Signature of Pediatric Crohn's Disease: Differentiation from Functional Gastrointestinal Disorders and Association with Increased Disease Activity

Ryan Zanganeh[1], Scott C. Thomas[2], Fangxi Xu[2], Samantha Hwang[2], Leonardo Trasande[3,4], Jeremiah Levine[5], Amr M. Moursi[6], Deepak Saxena[2,7,8]

1 College of Dentistry, New York University College of Dentistry; New York, NY; 2 Department of Molecular Pathobiology, New York University College of Dentistry; New York, NY;
3 Department of Pediatrics, NYU Grossman School of Medicine; New York, NY; 4 Department of Population Health, NYU Grossman School of Medicine, New York, NY;
5 Division of Pediatric Gastroenterology and Hepatology, New York University Langone Health; New York, NY; 6 Department of Pediatric Dentistry, NYU College of Dentistry; New York, NY;
7 Department of Surgery, New York University Grossman School of Medicine; New York, NY; 8 Perlmutter Cancer Institute, New York University Langone Medical Center; New York, NY.

Introduction

Crohn's Disease (CD) constitutes a complex and chronic inflammatory disorder affecting the gastrointestinal tract, attributed to the interplay between dysregulated immune responses and the intestinal microflora[1]. It has been demonstrated that the incidence and prevalence of CD are increasing, with the highest rates occurring in developed nations[2].

Of concern, the incidence of pediatric CD has significantly increased, a trend not similarly observed in patients with ulcerative colitis[3]. Furthermore, special considerations need to be made in the pediatric population, given the tendency for a more severe phenotype in those with earlier onset disease and the unique lifelong burden these patients may experience, with potential negative effects on growth, psychological/emotional function, and body habitus[4,5].

More recently, the critical role of the microbiome in the development of intestinal dysbiosis has gained increasing attention, specifically, the enrichment of pro-inflammatory bacteria, the depletion of beneficial bacteria, and reduced bacterial richness and diversity[6]. Despite these insights, accurately distinguishing CD from Functional Gastrointestinal Disorders (FGIDs) remains challenging, particularly given the limitations of current diagnostic methods.

Furthermore, the Pediatric Crohn's Disease Activity Index (PCDAI) is a tool to assess disease severity by incorporating subjective reporting of symptoms, presence of extra-intestinal manifestations, physical exam findings, and laboratory data[7].

Objectives

- Compare fecal microbiome profiles of treatment-naïve pediatric CD patients versus FGID patients.
- Analyze differences in bacterial diversity and abundance at phylum and genus levels.
- Correlate microbiome alterations with disease severity using PCDAI.

Methods

Participants:
- 43 treatment-naïve pediatric CD patients.
- 139 age-, sex-, race-, ethnicity-, and insurance-matched FGID controls.
- CD diagnosed by clinical, laboratory, imaging, and endoscopic criteria.
- FGID diagnosed using Rome criteria; reviewed by two independent physicians to confirm diagnosis.

Sample Collection:
- Fecal samples collected at time of diagnosis before any treatment.
- Samples immediately stored at -80°C for microbiome analysis.

Microbial DNA Extraction & Sequencing:
- DNA extracted using QIAamp PowerFecal DNA kit.
- 16S rRNA gene sequencing targeted V3-V4 regions.
- Sequencing performed on Illumina MiSeq platform (2×300 bp paired-end reads).

Bioinformatics and Data Processing:
- Raw reads denoised and quality-filtered with DADA2 via QIIME2 pipeline.
- Taxonomy assigned using SILVA 16S rRNA database.

Statistical Analysis:
- *Alpha diversity:* Compared by Wilcoxon rank-sum test.
- *Beta diversity:* Analyzed by PERMANOVA.
- *Differential abundance:* Wilcoxon test and LEfSe analysis.
- *Correlations with PCDAI:* Visualized with Cytoscape.

Results

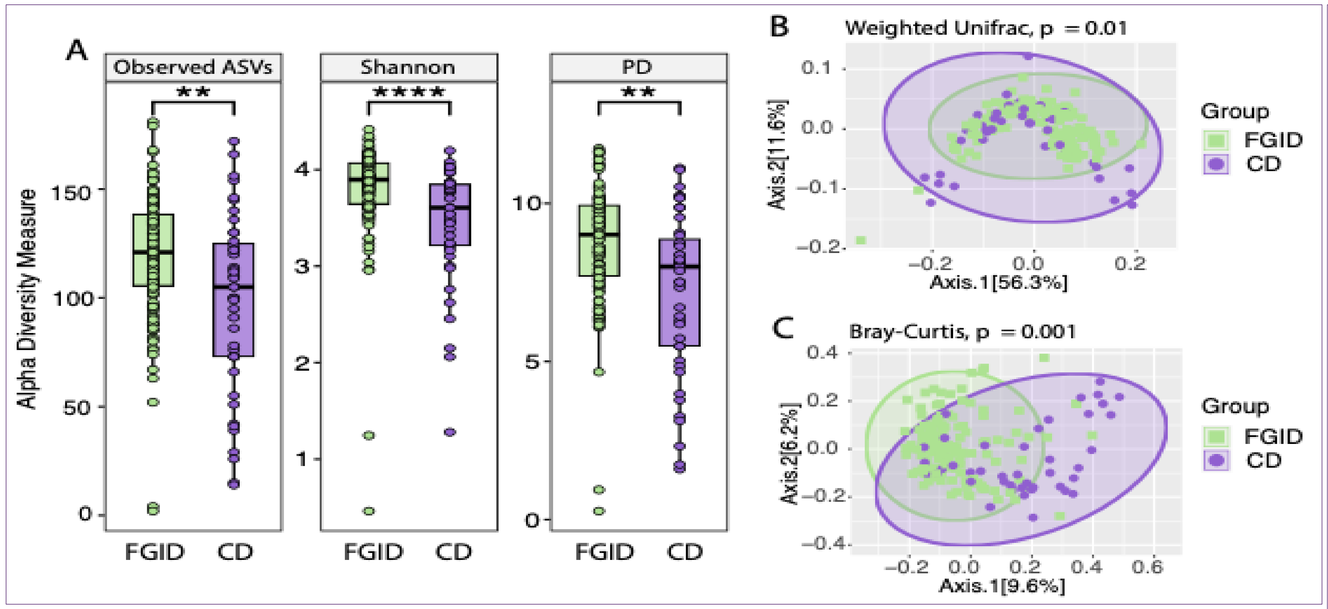

Figure 1. Reduced alpha diversity and distinct beta diversity in CD compared to FGID controls. *Alpha diversity was significantly lower in CD patients (Shannon Index $p < 0.0001$, Faith's PD $p < 0.01$, Observed ASVs $p < 0.01$). Beta diversity showed significant differences between groups (Weighted UniFrac $p = 0.01$, Bray-Curtis $p = 0.001$).*

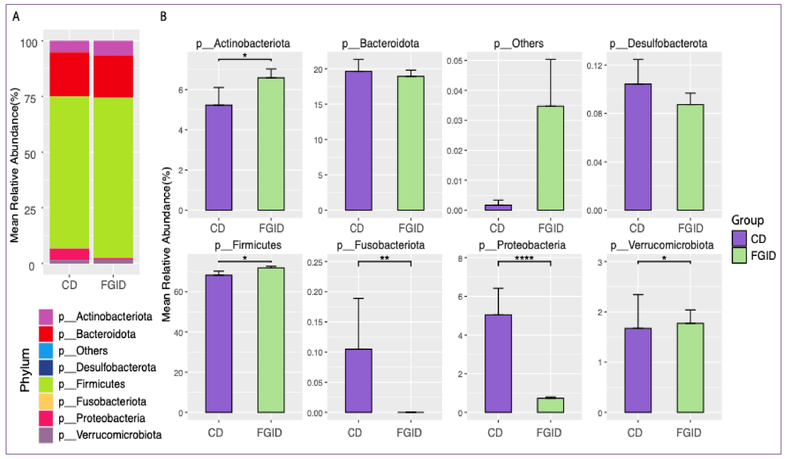

Figure 2. Phylum-level differences in fecal microbiota between CD and FGID patients. *CD patients had increased Fusobacteria ($p < 0.01$) and Proteobacteria ($p < 0.0001$), and decreased Firmicutes ($p < 0.05$), Verrucomicrobia ($p < 0.05$), and Actinobacteria ($p < 0.05$).*

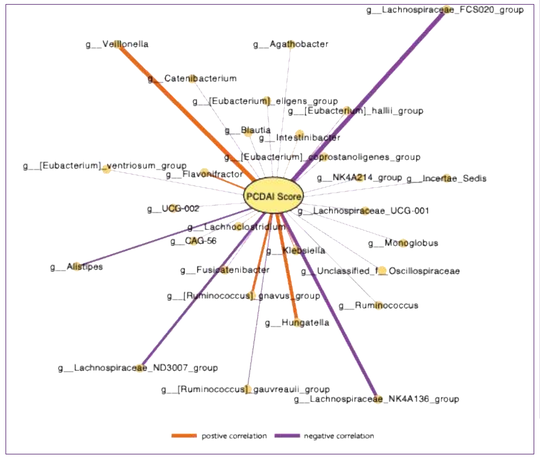

Figure 3. Significant associations between specific bacterial genera and disease severity (PCDAI scores) in CD patients. *Higher PCDAI scores were strongly associated with increased Veillonella ($p < 0.01$) and Hungatella ($p < 0.05$), and decreased Lachnospiraceae FCS020 group ($p < 0.001$) and NK4A136 group ($p < 0.01$).*

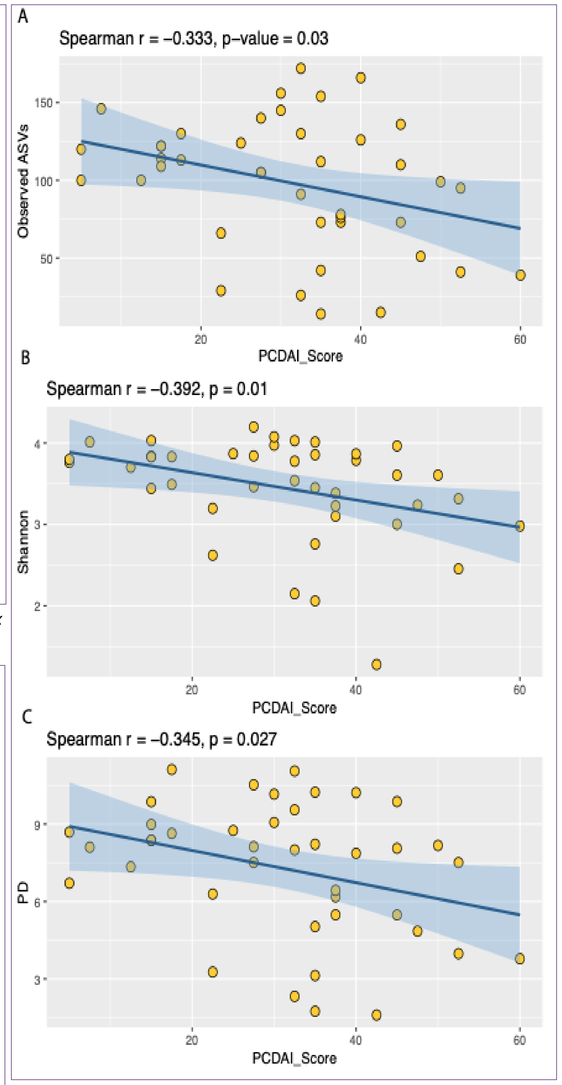

Figure 4. Significant negative correlations between alpha diversity measures and disease severity (PCDAI scores). *Observed ASVs ($r = -0.333$, $p = 0.03$), Shannon Index ($r = -0.392$, $p = 0.01$), and Faith's PD ($r = -0.345$, $p = 0.027$) were significantly negatively correlated with PCDAI scores in CD patients*

Conclusions

Our study demonstrates significant differences in fecal microbiome composition between pediatric patients with CD compared to a FGID control group. Notably, we found that a decrease in microbial alpha diversity was associated with worsening disease severity. The enrichment of pro-inflammatory genera such as *Veillonella* and *Hungatella* as well as the depletion of protective genera such as *Lachnospiraceae* allow for a greater understanding of the microbiomes' role in CD pathogenesis and progression. Our findings support the potential for fecal microbiome analysis to be used alongside current diagnostic tools in CD diagnosis and/or management in future hopes of using specific microbiome-targeted therapies for pediatric patients with CD to improve their clinical outcomes.

References

1. Sartor RB. Mechanisms of Disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3(7):390-407.
2. Benchimol EI, Fortinsky KJ, Gozdyra P, Van den Heuvel M, Van Limbergen J, Griffiths AM. Epidemiology of pediatric inflammatory bowel disease: a systematic review of international trends. *Inflamm Bowel Dis.* 2011;17(1):423-439.
3. Malaty HM, Fan X, Opekun AR, Thibodeaux C, Ferry GD. Rising incidence of pediatric Crohn's disease in Texas. *South Med J.* 2010;103(7):683-686.
4. Griffiths AM. Specificities of inflammatory bowel disease in childhood. *Best Pract Res Clin Gastroenterol.* 2004;18(3):509-523.
5. Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis.* 2014;8(10):1179-1207.
6. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe.* 2014;15(3):382-392.
7. Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a Pediatric Crohn's Disease Activity Index. *J Pediatr Gastroenterol Nutr.* 1991;12(4):439-447.