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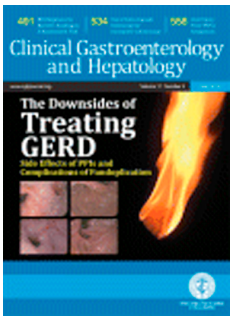

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Microscopic Colitis is Characterized by Intestinal Dysbiosis

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Abbreviations:
AUC – Area Under the Curve
BLAST – Basic Local Alignment Search Tool
BMI – Body Mass Index
EDTA - Ethylenediaminetetraacetic acid
FDR – False Discovery Rate
GIDER – Gastrointestinal Disease and Endoscopy Registry
HMP – Human Microbiome Project
IBD – Inflammatory Bowel Disease
KEGG – Kyoto Encyclopedia of Genes and Genomes
MDS – Multidimensional scaling
MGH – Massachusetts General Hospital
NSAID – Non-Steroidal Anti-Inflammatory Drug
PCoA – Principal Coordinates Analysis

PPI – Proton Pump Inhibitor
PTRC – Peak Trough Ratio Computation
ROC – Receiver-Operating Curve
TNBS – 2,4,6-Trinitrobenzenesulfonic acid
TNF – Tumor Necrosis Factor

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Potential Conflicts of Interest:

- Hamed Khalili has received consulting fees from Abbvie and Samsung Bioepis. Hamed Khalili also receives grant support from Takeda and Pfizer.
- Kyle Staller has received consulting fees from Bayer and Shire, has been a speaker for Shire, and receives grant support from AstraZeneca, Gelesis, and Takeda.
- Jonas Ludvigsson has received grant support from Janssen.
- James Richter has served on the scientific advisory board for Shires and Worldcare Clinical.
- The remaining authors have no conflicts to disclose.

Author Contributions to Manuscript:

- David M. Morgan – Study concept and design, patient enrollment, sample collection and processing, data acquisition, analysis and interpretation of data, drafting manuscript, critically revising manuscript
- Yueming Cao – Study concept and design, patient enrollment, sample collection and processing, data acquisition, analysis and interpretation of data, drafting manuscript, critically revising manuscript
- Kaia Miller – Patient enrollment, sample collection and processing, critically revising manuscript
- Jessica McGoldrick – Patient enrollment, sample collection and processing, critically revising manuscript
- Danielle Bellavance – Patient enrollment, sample collection and processing, critically revising manuscript
- Samantha M. Chin – Patient enrollment, sample collection and processing, critically revising manuscript
- Benjamin Maxner – Patient enrollment, sample collection and processing, critically revising manuscript
- Stefan Halvorsen – Study concept and design, critically revising manuscript
- James M. Richter – Study concept and design, patient enrollment, critically revising manuscript
- Slim Sassi – Study concept and design, critically revising manuscript

- Kristin E. Burke – Patient enrollment, critically revising manuscript
- Joseph C. Yarze – Patient enrollment, critically revising manuscript
- Jonas F. Ludvigsson – Study concept and design, critically revising manuscript
- Kyle Staller – Study concept and design, patient enrollment, critically revising manuscript
- Daniel C. Chung – Study concept and design, patient enrollment, critically revising manuscript
- Hamed Khalili – Study concept and design, patient enrollment, data acquisition, analysis and interpretation of data, drafting manuscript, critically revising manuscript

INTRODUCTION

The critical role of the gut microbiome in microscopic colitis (MC) is evident by the observation that fecal diversion is associated with resolution of mucosal inflammation while restoration of fecal stream is associated with recurrence of disease[1]. Characterization of the composition and function of the gut microbiome in MC could therefore provide insights into disease pathogenesis.

METHODS

We analyzed the microbiome of stool samples from 20 patients during the active (≥ 3 bowel movements/day with Bristol score ≥ 5) and remission (< 3 bowel movements/day with Bristol score ≤ 4) phases of MC, 20 age-and sex-matched healthy controls, and 20 patients with functional diarrhea (Rome IV criteria) using shotgun metagenomics. Sequencing was done on the Illumina HiSeq2500 platform with paired ends (2 x 101 nucleotides) with a depth of 1.9 ± 0.3 Gnt before quality control (QC) and 1.7 ± 0.3 Gnt after. Sequence processing was conducted according to the Human Microbiome Project protocol using the KneadData pipeline. We used MetaPhlAn2 for taxonomic profiling and HUMAnN2 to identify metabolic pathways[2]. We used the Biodiversity Assessment Tools package in R to calculate alpha diversity and estimated the microbial dysbiosis index (MDI) using a previously described method[3]. Unique compositional and functional features were identified using multivariable models[2] while growth dynamics was calculated using peak-to-trough ratio (PTR)[4]. All p values were adjusted for multiple testing using false discovery test and $q < 0.1$ was considered statistically significant.

RESULTS

Baseline characteristics of participants in our study are shown in **Figure 1A**. We observed a significant difference in alpha diversity between active and remission phases in MC

patients (q = 0.031, **Figure 1B**). Although alpha diversity was also lower in active MC compared to healthy controls and functional diarrhea patients, these comparisons did not reach statistical significance (**Figure 1B**). The MDI was found to be significantly higher in active MC compared to healthy controls, functional diarrhea, and remission MC samples (q = 8.11E-6, 6.5E10-4, 7.6E10-4, respectively **Figure 1C**). We considered the possibility that growth dynamics of the gut microbiome as opposed to relative abundances may be more strongly linked to disease status and therefore estimated PTR as a measure of species' growth rate. Compared to healthy controls, there were significant increases in the global (median = 1.24 vs 1.19, q = 0.033, **Figure 1D**) and *Alistipes finegoldii*-specific (median 1.16 vs 1.05, q = 0.065 **Figure 1D**) PTRs in active MC. In contrast, no significant differences were observed in PTRs when comparing active MC to remission or functional diarrhea patients (all q > 0.1).

In multivariable analyses, *Haemophilus parainfluenzae*, *Veillonella parvula*, and *Veillonella unclassified* species were significantly more abundant in MC than in healthy controls while *Alistipes putredinis* had a lower abundance (all q < 0.1). Among these species, the relative abundance of *Alistipes putredinis* was also significantly increased among functional diarrhea patients (q=0.043, **Figure 1E**). We did not identify any differences at the species level when comparing active MC to remission (all q > 0.1). In analyses of functional profiling, the pathways, gluconeogenesis I and Inosine-5'-phosphate biosynthesis III were decreased in active MC compared to healthy controls, while purine deoxyribonucleoside degradation was increased compared to healthy controls, although none reached statistical significance (**Figure 1F**).

DISCUSSION

Here, we demonstrate dynamic changes in diversity and dysbiosis during the active and remission phases of MC, consistent with findings in inflammatory bowel disease (IBD)[5]. Among

compositional differences between MC and healthy controls, the relative abundance of *Alistipes putredinis* appeared to also be significantly different when comparing MC to functional diarrhea patients. The relative abundances of several metabolic pathways including those involved in glucose biosynthesis and oxidative stress were decreased in active MC in return for an increase in purine degradations.

Alistipes species are butyrate-producing bacteria that appear to have anti-inflammatory properties in animal models of colitis[6] and are depleted in new-onset pediatric IBD[7]. In contrast, administration of *Alistipes finegoldii* induces histologic inflammation with preferential colonization and a pro-inflammatory effect in the right colon in animal studies[8]. These prior observations are in large part consistent with our findings on relative abundance and growth dynamics of *Alistipes* species in MC.

We acknowledge several limitations. Our sample size did not allow us to adjust for potential confounders including diet and medications and limited our power to identify more modest compositional and functional associations. In conclusion, we demonstrate that similar to IBD, dysbiosis is the defining feature of the gut microbiome in MC. Larger-scale studies are required to validate our findings and to better characterize dynamic functional changes in the gut microbiome of patients with MC.

REFERENCES:

1. Daferera N, Kumawat AK, Hultgren-Hornquist E, et al. Fecal stream diversion and mucosal cytokine levels in collagenous colitis: A case report. World J Gastroenterol 2015;21:6065-71.
2. Hall AB, Yassour M, Sauk J, et al. A novel Ruminococcus gnavus clade enriched in inflammatory bowel disease patients. Genome Med 2017;9:103.
3. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014;15:382-392.
4. Korem T, Zeevi D, Suez J, et al. Growth dynamics of gut microbiota in health and disease inferred from single metagenomic samples. Science 2015;349:1101-1106.
5. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 2014;146:1489-99.
6. Butera A, Di Paola M, Pavarini L, et al. Nod2 Deficiency in mice is Associated with Microbiota Variation Favouring the Expansion of mucosal CD4+ LAP+ Regulatory Cells. Sci Rep 2018;8:14241.
7. de Meij TGJ, de Groot EFJ, Peeters CFW, et al. Variability of core microbiota in newly diagnosed treatment-naive paediatric inflammatory bowel disease patients. PLoS One 2018;13:e0197649.
8. Moschen AR, Gerner RR, Wang J, et al. Lipocalin 2 Protects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations. Cell Host Microbe 2016;19:455-69.

Figure Legends:

A) Baseline characteristics of study participants; B) Differences in alpha diversity between active MC, remission MC, functional diarrhea, and healthy controls; C) Comparison of MDI between active MC and healthy control, active MC and chronic diarrhea, and active MC and remission MC; D) Comparison of mean global PTR values and PTR values of *Alistipes finegoldii* between active MC and healthy controls; E) Comparison of relative abundances of *Alistipes putredinis* between active MC and healthy control, and active MC and chronic diarrhea F) Comparison of metabolic pathways relative abundances between active MC and healthy controls using multivariate models.

A

Table 1: Baseline characteristics of participants according to disease status*

	Microscopic colitis (n=20)	Healthy control (n=20)	Chronic diarrhea (n=20)
Age (SD), years	63.2 (8.5)	63.2 (8.3)	62.4 (14.5)
Race, white n (%)	19 (95%)	17 (85%)	19 (95%)
Sex, female n (%)	16 (80%)	16 (80%)	13 (65%)
BMI (SD), kg/m^2	24.7 (3.5)	28.2 (6.9)	27.9 (5.5)
Smoking history, n (%)			
Never	11 (55%)	14 (70%)	10 (50%)
Former smoker	5 (25%)	5 (25%)	9 (45%)
Current smoker	4 (20%)	1 (5%)	1 (5%)

*Abbreviations: body mass index (BMI), standard deviation (SD)