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Title: Metagenomics analysis reveals unique gut microbiota signature of slow-transit constipation

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Conflicts of Interest:

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Abbreviations: BMI, body mass index; IBS-C, irritable bowel syndrome with constipation; NMDS, non-metric multidimensional scaling; PERMANOVA, Permutational multivariate analysis of variance analysis; STC, slow-transit constipation

Data, analytic methods, and study materials will be made available to other researchers upon reasonable request.

ABSTRACT

Introduction: Altered gut microbiota may play a role in slow-transit constipation (STC). We conducted a study of gut microbiota composition and functionality in STC using metagenomic analyses.

Methods: We assembled a clinical cohort of 24 patients with STC physiology age- and sex-matched to 24 controls. We performed shotgun metagenomic sequencing followed by prediction of metabolite composition from functional profiles.

Results: In a middle-aged (mean 55.3 years), predominantly female cohort, there were no significant differences in α diversity indices, but permutational multivariate analysis of variance analysis showed significant between-group differences (R^2=0.050, p<0.001) between STC patients and controls. *Gordonibacter pamelaeae*, *Bifidobacterium longum*, *Firmicutes bacterium* CAG 94, and *Anaerotruncus colihominis* were more abundant in STC, while *Coprococcus comes* and *Roseburia intestinalis* were more abundant in controls. Gut-derived metabolites varying in STC relative to controls were related to bile acid and cholesterol metabolism.

Discussion: We found a unique metagenomic and metabolomic signature of STC.

INTRODUCTION

Alterations of human gut microbiota and products of its metabolism may play a critical pathophysiological role in slow-transit constipation (STC) (1). However, there is limited data on the compositional and functional characteristics of the gut microbiome in patients with STC, with most studies limited to genus-level data without species-level resolution (2-5). We conducted a cross-sectional study describing the gut microbiota composition and functionality in patients with STC relative to healthy individuals using metagenomic analyses. We aimed to determine if there was a metagenomic signature of STC as well as explore functional capabilities of this metagenome as potential clues to pathophysiology.

METHODS

We assembled a cohort of 24 patients with Rome IV-defined chronic constipation prospectively recruited during consultations at Massachusetts General Hospital with STC physiology subsequently confirmed by radiopaque marker testing and rectal evacuation disorders excluded by anorectal manometry with balloon expulsion testing. All patients completed questionnaires assessing relevant demographics, disease characteristics, dietary patterns, and potential confounders (see Supplement). Stool samples were self-collected by STC patients as well as 24 age- and sex-matched, healthy controls. All participants were required to have abstained from antibiotics for 3 months prior to stool collection, and stool samples were collected prior to initiation of any laxative therapy. Shotgun metagenomic sequencing was performed on the Illumina HiSeq platform. Raw sequencing data was processed with bioBakery workflows (6). Briefly, after quality controls of sequence reads using KneadData, microbial taxonomic classifications and their relative abundance were generated using MetaPhlAn 3.0 followed by HUMAnN 3.0 for constructing metabolic pathways. MelonnPan was then used to predict metabolite composition from functional profiles. A *q*-value (false discovery rate-corrected *p*-value) of 0.25 was considered statistically significant consistent with prior studies (see Supplement).

RESULTS

Participant characteristics are shown in **Table 1**. Mean age of the subjects was 55.3 years and most were female. Although there was no significant difference in Bristol stool scale or dietary pattern between groups, STC patients were more likely to use laxatives and antibiotics (all $p < 0.001$). Notably, STC patients had significantly lower weight and body

mass index (BMI) compared to controls. There were no significant between group differences in α diversity indices (Shannon's index and Simpson's 1-D index)(**Figure 1A**). We calculated the β diversity using Bray-Curtis distance between any pair of samples and used NMDS ordination plots to visualize the samples based on species abundance from metagenomic data (**Figure 1B**). Permutational multivariate analysis of variance analysis (PERMANOVA) showed significant group differences, indicating there was a clear separation of the samples between two groups (R^2=0.050, p<0.001).

Microbial species that did not surpass the minimum prevalence (10% of samples) and relative abundance (0.01%) threshold were excluded from analyses. After filtering samples, a total of 127 microbial species were included. Group differences in relative abundance were tested using a multivariate linear mixed model, controlling for BMI as an important confounder that varied between groups. Six species were significantly different between groups as shown in **Figure 1C**. *Gordonibacter pamelaeae*, *Bifidobacterium longum*, *Firmicutes bacterium* CAG 94, and *Anaerotruncus colihominis* were significantly more abundant in STC than in healthy controls (q=0.072, 0.142, and 0.142, respectively), while *Coprococcus comes* and *Roseburia intestinalis* were more abundant in healthy controls compared to STC (q=0.148 and 0.072, respectively).

By performing correlation analyses between constipation parameters and the gut microbiota, we found that there was a positive correlation between *Coprococcus comes* and bowel frequency (R=0.31; p=0.032, **Figure 2**). Meanwhile, there were negative correlations between *Anaerotruncus colihominis* and bowel frequency (R=-0.34, p=0.16), and between *Bifidobacterium longum* and stool consistency (R=-0.3, p=0.041). Reads were also assigned to metabolic pathways and were compared between the groups using multivariable analysis. In total, 2 pathways differed significantly between groups (**Figure 1D**). Formaldehyde assimilation II was significantly increased (q=0.002), while L-ornithine biosynthesis II was significantly decreased in STC compared with healthy controls (q=0.215).

Using a predictive metabolomic approach, 24 gut-derived metabolites in microbial communities were obtained (**Figure 1E**). Lithocholate, caproic acid, taurine and cholestenone were more abundant in STC than in healthy controls, while N-acetylputrescine, C16-0-LPC, nicotinic acid, diacetylspermine, imidazole propionate, ADMA-SDMA, arachidonic acid, creatine, glucurote, docosapentaenoate, dimethyllysine, nicotinate, docosahexaenoic acid, erythronic acid, threosphinogosine, pantothenate, N-acetylhistidine, trimethyllysine, bilirubin, and hypoxanthine were more abundant in healthy controls (**Figure 1F**).

DISCUSSION

Here, we characterized stool microbial composition and their related functions in STC based on shotgun metagenomic sequencing with prediction of gut-derived metabolomics. Notably, our results are consistent with a previous study reporting reduction in the butyrate-producing bacterium *Roseburia intestinalis* and increase in probiotic genera *Bifidobacterium* in STC patients compared to HC (2). However, this result was not STC-specific and has also been reported in chronic constipation (7) and functional constipation (8). Butyrate is a fecal short-chain fatty is known to inhibit mucin secretion (9) and stimulate colonic water and electrolyte absorption (10); decreased butyrate levels are known to be associated with longer transit time and therefore could drive STC physiology (1).

In the view of the functional capabilities of the gut microbiome, our results showed that metabolic pathways related to methane oxidation were increased while pathways related to biosynthesis of L-ornithine, known to have beneficial effects on the liver, were significantly lower in STC patients (11). Healthy controls (relative to STC), had significantly higher levels of the metabolite arachidonic acid, which is known to promote growth and repair of neurons in the brain and is related to skeletal muscle growth, and nicotinic acid, a water-soluble vitamin whose derivatives play essential roles in energy metabolism of the cell. The predicted abundances of metabolites including bile acids (lithocholate) and amino acids related to bile acid conjugation (taurine) and cholesterol metabolism (cholesterone) were increased in STC. It is not surprising that functional changes in STC patients were related to factors associated with bile acid metabolism—known modulators of colonic transit—and increased levels of these secondary bile acids have been seen in patients with irritable bowel syndrome with constipation (IBS-C) (12, 13).

There are several limitations to this study. Small sample size did not allow us to adjust for additional confounders outside of BMI such as medications and diet. Since it is difficult to obtain stool samples from constipated patients without laxatives, it was also difficult to completely rule out changes in gut microbiome induced by laxatives. Additionally, the predicted metabolic pathways used in this study are not perfect surrogates for measured metabolites, which are the gold standard. Larger studies are required to validate our findings.

In conclusion, we found significant compositional and functional characteristics associated with STC. Data reported in this study could provide a scientific basis for future development of diagnostic and treatment modalities for STC patients.

Supplementary material--http://links.lww.com/CTG/B202

REFERENCES

1. Muller M, Hermes GDA, Canfora EE, et al. Distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans with slow colonic transit. Am J Physiol Gastrointest Liver Physiol 2020;318:G361-G369.
2. Tian H, Chen Q, Yang B, et al. Analysis of Gut Microbiome and Metabolite Characteristics in Patients with Slow Transit Constipation. Dig Dis Sci 2021;66:3026-3035.
3. Yu T, Ding Y, Qian D, et al. Characteristics of fecal microbiota in different constipation subtypes and association with colon physiology, lifestyle factors, and psychological status. Therap Adv Gastroenterol 2023;16:17562848231154101.
4. Wolf PG, Parthasarathy G, Chen J, et al. Assessing the colonic microbiome, hydrogenogenic and hydrogenotrophic genes, transit and breath methane in constipation. Neurogastroenterol Motil 2017;29:1-9.
5. Parthasarathy G, Chen J, Chen X, et al. Relationship Between Microbiota of the Colonic Mucosa vs Feces and Symptoms, Colonic Transit, and Methane Production in Female Patients With Chronic Constipation. Gastroenterology 2016;150:367-79 e1.
6. Beghini F, McIver LJ, Blanco-Miguez A, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. Elife 2021;10.
7. Yarullina DR, Shafigullin MU, Sakulin KA, et al. Characterization of gut contractility and microbiota in patients with severe chronic constipation. PLoS One 2020;15:e0235985.
8. Mancabelli L, Milani C, Lugli GA, et al. Unveiling the gut microbiota composition and functionality associated with constipation through metagenomic analyses. Sci Rep 2017;7:9879.
9. Barcelo A, Claustre J, Moro F, et al. Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon. Gut 2000;46:218-24.
10. Binder HJ, Mehta P. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. Gastroenterology 1989;96:989-996.
11. Butterworth RF, McPhail MJ. L-ornithine L-aspartate (LOLA) for hepatic encephalopathy in cirrhosis: results of randomized controlled trials and meta-analyses. Drugs 2019;79:31-37.
12. Vijayvargiya P, Busciglio I, Burton D, et al. Bile Acid Deficiency in a Subgroup of Patients With Irritable Bowel Syndrome With Constipation Based on Biomarkers in Serum and Fecal Samples. Clin Gastroenterol Hepatol 2018;16:522-527.
13. Shin A, Camilleri M, Vijayvargiya P, et al. Bowel functions, fecal unconjugated primary and secondary bile acids, and colonic transit in patients with irritable bowel syndrome. Clin Gastroenterol Hepatol 2013;11:1270-1275.e1.

Table 1: Subject characteristics

Characteristics	HC (n=24)	STC (n=24)	p-value
Sex (female, %)		22 (91.7%)	
Age at stool sample collection (years)		55.3 (9.5)	
Body mass index (kg/m^2)	28.7 (6.8)	23.7 (4.3)	0.004*
Weight (kg)	77.0 (15.8)	65.6 (13.5)	0.010*
Height (cm)	164.8 (8.1)	166.2 (7.9)	0.479
Bristol stool scale[†]	3.7 (1.3)	3.5 (1.8)	0.738
Number of bowel movements per week	6.1 (1.7)	2.6 (2.1)	<0.001*
Dietary pattern[‡]			0.217
Western standard	13 (54.2%)	10 (41.7%)	
Low red meat (<3times/month)	10 (41.7%)	9 (37.5%)	
No red meat	0 (0.0%)	4 (16.7%)	
Vegetarian	1 (4.2%)	1 (4.2%)	
Yogurt consumption[‡]	14 (58.3%)	15 (62.5%)	1
Regular alcohol consumption[‡]	13 (54.2%)	15 (62.5%)	0.770
Disease-modifying drug exposure[‡]			
Laxatives (any recent use)[§]	3 (16.7%)	20 (83.3%)	<0.001*
Proton pump inhibitors	1 (4.2%)	5 (20.8%)	0.190
H2 blocker	0 (0.0%)	3 (1.3%)	0.233
Antacids	1 (4.2%)	2 (8.3%)	1
Antibiotic use in past 4 years	5 (20.8%)	19 (79.2%)	<0.001*

Data are shown as mean (standard deviation) or frequency (percentage)

*p value less than 0.05 was considered statistically significant

[†]Bristol stool form of the bowel movement submitted for metagenomic sequencing

[‡]Chi-square test

[§]In response to the question: "How often do you use a laxative (such as softeners, bulking agents, fiber supplements or suppositories)?" Any response outside of "Never" would count as a positive response.

FIGURE LEGENDS

Figure 1. Fecal microbiota community structure. (A) Differences in α- diversity between slow transit constipation (STC), and healthy controls (HC). (B) NMDS ordination plot of β-diversity using Bray-Curtis distance shows the differences between samples and groups. (C) Bacterial species with significant difference between groups (D) Metabolic pathways with significant difference between groups (E) Relative abundances of predicted metabolites with significant difference between groups. (F) Predicted metabolites with significant difference between groups

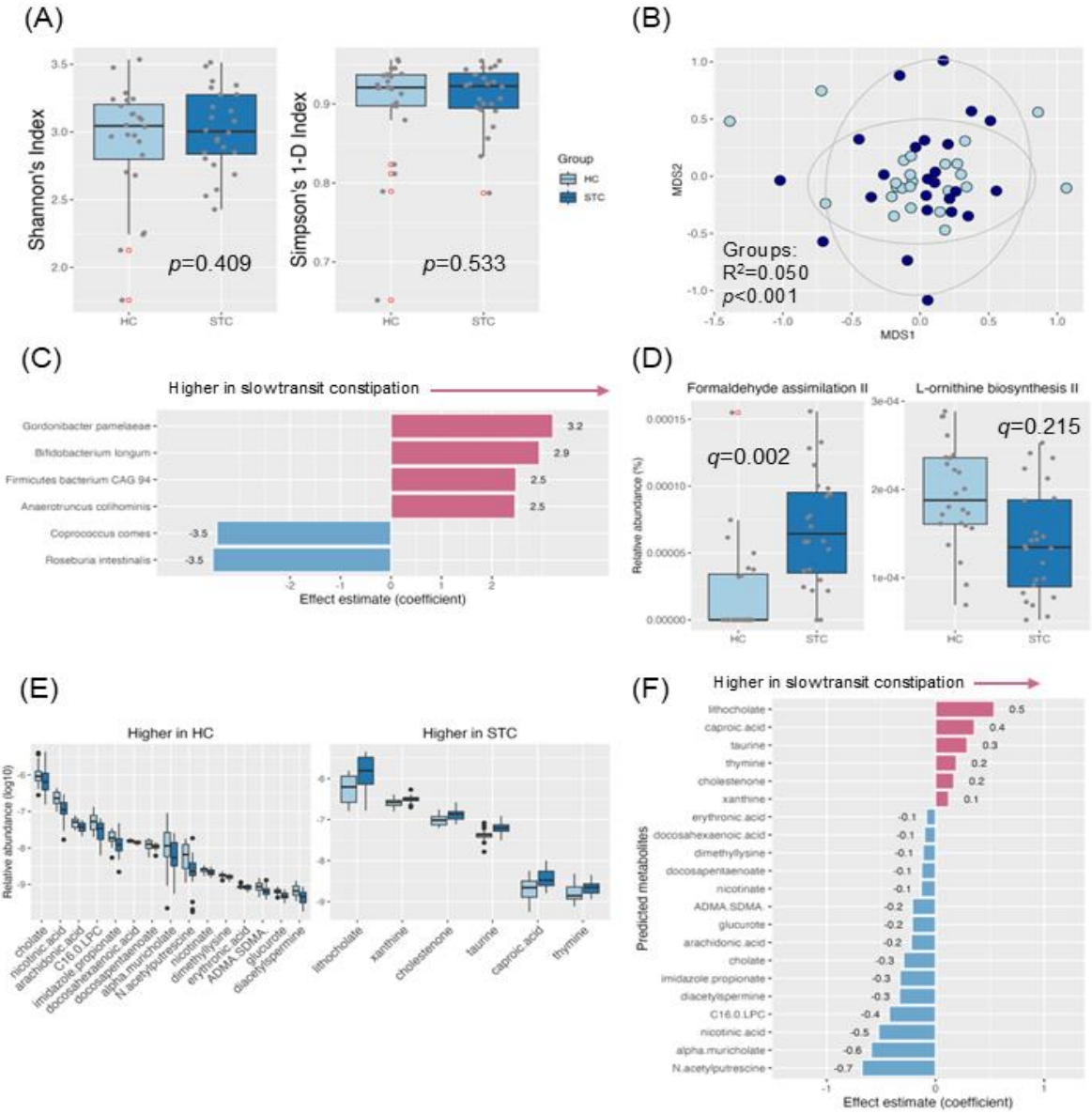

Figure 2. Bacterial species significantly correlated with presence of specific slow-transit constipation parameters.