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Enteric Virome and Bacterial Microbiota in Children with Ulcerative Colitis and Crohn's Disease

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Abstract

Objectives: We examined the fecal enteric virome and bacterial community composition of children with Crohn's disease (CD), ulcerative colitis (UC), and healthy controls to test the hypothesis that unique patterns of viral organisms and/or presence of bacterial pathogens may be identified that could contribute to the pathogenesis of pediatric inflammatory bowel disease (IBD).

Methods: Fecal samples from 24 children (mean 12.2 years) with CD (n=7) or UC (n=5) and similar aged controls (n=12) were processed to determine individual viromes. Viral sequences were identified through translated protein sequence similarity search. Bacterial microbiota were determined by sequencing of the V4 region of the 16S rRNA gene.

Results: Only a few human viruses were detected, so virome analyses focused on bacterial viruses. The relative abundance of *Caudovirales* was greater than that of *Microviridae* phages in both IBD and healthy controls. Caudovirales phages were more abundant in CD (mean 80.8%) than UC (48.8%) (p=0.05) but not controls. The richness of viral strains in *Microviridae* but not *Caudovirales* was higher in controls than CD (p=0.05) but not UC cases. No other measure of phage abundance, richness, or Shannon diversity showed significant difference between the two IBD and control groups. Bacterial microbiota analysis revealed that IBD diagnosis, albumin, hemoglobin, erythrocyte sedimentation rate, and probiotic supplementation correlated to the composition of gut bacterial microbiota.

Conclusions: Minor patterns in gut virome and bacterial community composition distinguish pediatric IBD patients from healthy controls. Probiotics are associated with bacterial microbiota composition. These exploratory results need confirmation in larger studies.

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Keywords

Inflammatory Bowel Disease; pediatric; enteric biome; microbiota; probiotics; virus; bacteria; phage; Caudovirales; Microviridae

Introduction:

Pediatric inflammatory bowel disease (IBD) is a chronic illness of childhood with an increasing incidence worldwide (1). Although current research suggests a multifactorial pathogenesis including genetic, environmental, and autoimmune factors, the etiology of IBD remains an enigma (2–5). The enteric microbiome, which includes bacteria, fungi and viruses, has been purported to play a role in the pathogenesis of IBD (6). Distinct gut microbial communities have been demonstrated in patients with IBD, but the origins and underlying causes of these patterns remains unknown. Enteric viruses combined with other factors such as the microbiome, host genetics, and immune status are hypothesized to be modulating factors influencing disease severity by impacting intestinal and immune development and chronic inflammatory responses (7).

The enteric virome is being increasingly studied, with the supposition that it will provide insight into the pathogenesis and disease course of IBD. Published reports focus on describing the components of the enteric virome, investigating if any common differences exist among IBD patients. Norman *et al* recently characterized the enteric virome of three adult IBD cohorts (n=12 controls; n=18 Crohn's disease; n=42 ulcerative colitis) and showed that adult IBD patients exhibited increased relative sequence abundance of *Caudovirales* bacteriophages compared with normal controls (8). Wang, et al, focused on intestinal tissue RNA rather than fecal samples, demonstrating a difference in the abundance of reads matching mammalian viruses and endogenous retrovirus sequences of adult IBD patients versus healthy controls (10 IBD patients; 5 controls) (6). A recent study of fecal microbiota transplantation (FMT) showed that phage richness in UC patients did not differ from healthy controls at any time point before or after FMT. However, eukaryotic viral richness was higher in patients with UC compared with healthy controls and in non-responders versus responders of FMT (9). Eukaryotic viral reads matched mainly members of the *Anelloviridae* and *Picobirnaviridae* known to infect humans, *Virgaviridae* infecting plants, and *Circoviridae* of unknown tropism. Documentation of alterations of the microbiota in adult patients with IBD may provide a basis for targeting future therapies, monitoring disease state, and/or assessing response to medical therapy.

We investigated the fecal virome and bacterial composition in children with CD, UC and age-matched healthy controls to determine if pediatric patients with IBD have a unique pattern associated with disease. While both adult- and pediatric-onset IBD share many characteristics, disease phenotypes remain distinct, making it essential to specifically evaluate this question in children.

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Materials and Methods:

Subjects

Patients 5 to 17 years of age seen at the UCSF Benioff Children's Hospital in San Francisco pediatric gastroenterology outpatient facilities from January 1 2015 to June 30 2015 were identified for inclusion and screened by one research assistant (E.S.). Fifty subjects (n=29 IBD; n=21 healthy age-matched controls) consented for the study; ultimately 24 (n=7 CD, n=5 UC; n=12 control) provided fecal samples. All IBD patients had a confirmed diagnosis of UC or CD by a UCSF pediatric gastroenterologist at least 6 months prior to enrollment. Diagnostic information included at least one endoscopy and colonoscopy with biopsies.

Control subjects were excluded if they had a 1st degree relative with IBD, medical history of arthritis or uveitis within 6 months of enrollment, diarrheal illness within 4 weeks prior to enrollment, or had a current or past history of immunosuppression. Additionally, if a control subject had a colonoscopy within the last year, he/she must not have macroscopic or histologic findings consistent with a diagnosis of IBD. Exclusion criteria for all subjects included exposure to antibiotics within four weeks prior to enrollment or a positive *Clostridium difficile* stool test or co-infection within four weeks prior to enrollment.

At the time of consent, subjects and their guardians were provided a stool collection kit with instructions, asking for samples to be frozen after collection and returned in person or shipped via next day delivery service. Samples were batched for processing.

Biological samples and viral metagenomics

Fecal samples from 12 children with IBD (n=5 ulcerative colitis and n=7 Crohn disease) and from 12 healthy, non-IBD subjects were collected and stored at −80°C. Fecal suspensions were vigorously vortexed and clarified by 15,000 × *g* centrifugation for 10 minutes. 200 μL of fecal supernatant was filtered through a 0.45-μm filter (Millipore) to remove bacterium-sized particles. The filtrate was treated with a mixture of DNase and RNase enzymes to digest unprotected nucleic acids. Viral nucleic acids protected from digestion within viral capsids were extracted using a Maxwell 16 viral nucleic acid purification kit (Promega, Madison, USA). Reverse transcription into cDNA used a primer containing a fixed 18-bp sequence plus a random nonamer at the 3′ end (GCCGACTAATGCGTAGTCNNNNNNNNN). The 2nd strand DNA was generated using Klenow Fragment (New England Biolabs, Ipswich, USA). Fifteen cycles of PCR were performed after adding the fixed 18-bp portion primer (GCCGACTAATGCGTAGTC) according to protocol N1 (10). The resulting PCR products were used to construct a DNA library using the Nextera XT Sample Prep Kit (Illumina, San Diego, USA). The library was then sequenced using the MiSeq Illumina platform with 250 base paired end reads. Viral sequences were identified through translated protein sequence similarity search (BLASTx) to annotated viral proteins available in GenBank's databases. This protocol has been shown to amplify both RNA and DNA viral genomes (10).

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Phage analysis

The MiSeq data were compared to annotated phage proteins available in GenBank's databases using BLASTx. An E-value of 10^{-5} was the cutoff value for significant hits, and the phage sequences were identified. Sequence based classification therefore allowed reads with translated protein similarity (threshold BLASTx E value 10^{-5}) to any of the currently sequenced viruses in GenBank to be identified. A recognized limitation of this approach is that sequences from viruses without detectable protein sequence similarity to any of all the viral proteins in GenBank are not recognized (11–13). In each fecal sample, phages were sorted based on their number of reads, and the 20 most abundant phages in each individual sample were selected. Phages that share >80% identity at the amino acid level over >80% of their entire genomes were considered the same virus. Based on this criterion, the large collection of phages for the total of 24 fecal samples was collapsed into the small database comprising of 107 unique phage genomes (Supplemental table 1). The MiSeq data were then compared to this database of most abundant phages. Based on BLASTx output, reads were classified into different phage strains based on the taxonomic origin of the best-hit (lowest E-score) sequence match. The relative abundance of phages was determined by the number of matching sequence reads (divided by the total number of reads to adjust for slight differences in library size), while the richness of phages was measured by the number of different phage strains. Shannon's diversity measures both the richness and distribution of phages within each viral taxon. Data were visualized and tested for statistical significance using Kruskall Wallace and Dunn's post-hoc tests in R. Illumina sequence information has been deposited in GenBank under BioProject ID PRJNA391511.

Bacterial analysis

In a 96-well Mo-Bio Powersoil HTP Bead Beating Plate, 300 uL of stool was combined with 500 uL of CTAB DNA extraction buffer (5% hexadecyltrimethylammonium bromide, 250 mM phosphate buffer, 1M NaCl) and incubated at 65 °C for 15 minutes, homogenized in a Retsch MM 400 plate shaker at speed 20 for 20 minutes, then centrifuged for 5 minutes at 2000 × *g*. Samples were pipet-mixed with 300 uL of phenol-chloroform-isoamyl alcohol (25:24:1) in a sterile 96-well plate and centrifuged for 30 minutes at 3000 × *g*. The aqueous phase was transferred to a new plate, and 500 uL of CTAB buffer was added to the bead-beating plate for a second extraction. Samples were pipet-mixed with 600 uL chloroform and centrifuged for 30 min at 3000 × *g* to remove residual phenol. The aqueous phase was transferred to a deep-well plate, pipet-mixed with 2 volume-equivalents of polyethylene glycol buffer (30% PEG 6000, 1.6 M NaCl), incubated overnight at 4 °C, and centrifuged for 60 min at 3000 × *g*. Pellets were washed twice with 70% ethanol, re-suspended in 100 uL sterile water and quantified for DNA content.

The V4 region of the bacterial 16S rRNA gene was amplified by combining 10 ng of DNA template with a PCR master mix (0.2 mM dNTP mix, 0.56 mg/ml BSA, 0.4 uM Illumina adapter sequence-tagged forward primer, 0.025 U/uL Taq DNA polymerase) and 0.4 uM barcode-tagged reverse primers in triplicate 25 uL reactions (30 cycles: 98° C for 2 min, 98 °C for 20 sec, 50 °C for 30 sec, 72 °C for 45 sec; extension: 72 °C for 10 min). Amplicons were purified with the Sequal Prep kit (Invitrogen), quantified, and pooled to an equimolar concentration of 5 nM. The library was purified and concentrated with Agencourt

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AMPure XP SPRI beads (Beckman-Coulter), quantified for Illumina-tagged amplicon with the KAPA SYBR FAST qPCR assay (KAPA Biosystems), diluted to 2nM and combined with equimolar PhiX (40% total volume). Sequencing was performed on the Illumina NextSeq 500 Platform on a 153bp × 153bp run.

Sequencing reads were assembled with FLASh v.1.2.7, demultiplexed, and quality-filtered (Q-score >30) in QIIME 1.8. After discarding singletons, chimeras were removed and operational taxonomic units (OTUs) were formed from the remaining sequences by clustering at 97% sequence identity to the most abundant representative sequence in USEARCH. Taxonomy was assigned using the most current Greengenes database (May 2013 version). Taxa with fewer than 3 sequences in total were removed. The resulting OTU table was rarefied to an even depth of 85,486 reads per sample. To maximize similarity between the original and rarefied OTU tables, random subsampling was performed at this predefine depths 100 times, and the most representative subsampled community was selected based on the minimum Euclidean distance to the other OTU vectors generated for each sample.

Alpha-diversity indices were computed in QIIME, assessed using Wilcoxon rank-sum test, and visualized with ggplot2 package (R). Unweighted uniFrac dissimilarity matrices were generated in QIIME and visualized by principal component analysis with *ggplot2* (R). Variables were assessed for their relationship to bacterial beta-diversity by permutational analysis of variance (PERMANOVA) using the Adonis function (*vegan* package) in the R environment; comparisons of $p<0.05$ were considered statistically significant.

Enriched taxa were identified using a "three model" approach where Poisson, negative-binomial, and zero-inflated negative-binomial models were applied to each sequence read counts for each taxon, individually, and the best-fit model that minimized the Akaike information criterion value (AIC) was selected for each taxon. To avoid spurious associations between bacterial taxa and disease status, the OTU table was de-noised by removing taxa detected in less than 25% of the samples prior to the analysis. To correct for multiple testing, the false-discovery rate was calculated for each taxon; a q-value of <0.10 was considered significant.

Ethical Considerations:

This study was approved by the UCSF/BCHSF Committee for Human Research. All parents and patients provided signed consents and assents, respectively.

Results:

Subject characteristics are in Table 1. Thirteen (54%) were male; mean (SD) age was 12.2±3.3 years, and BMI was 19±4.3 across all three groups. Characteristics and management of IBD varied among the UC and CD children (Tables 1a, 1b). Five subjects were receiving oral probiotic supplementation at the time of stool collection (n=2 CD, n=1 UC, n=2 control). Unknown to us, one age-matched control subject had started a five-day course of antibiotics at the time of stool collection. We included the patient's sample given the very short exposure to the antibiotic and lack of diarrhea. The non-IBD control subjects

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presented to the gastroenterology clinic for the following indications: constipation (9), epigastric abdominal pain (2), and dysphagia (1) (Table 1c). All participants were passing soft, well-formed stool at time of collection.

Viral relative-abundance and richness within phage taxa differ between subject groups.

There were 8.3×10^{-6} sequence reads generated in the MiSeq run with 250bp paired-end reads. For eukaryotic viruses, we detected low prevalence of nucleic acids of dsRNA picobirnaviruses (133 reads in 2 cases and 1 control), ssDNA parvovirus bufavirus (15 reads in 1 control), ssDNA anellovirus (10 reads in 1 case), ssDNA circo-like virus (4 reads in 1 case), and ssDNA gyrovirus (1 read in 1 control). Phages belonging to both ssDNA *Microviridae* family and dsDNA *Caudovirales order* were detected in feces of all subjects. Because of the low number and frequency of detection of eukaryotic viral sequences, we focused our analysis on sequences of bacterial viruses. The relative abundance of *Caudovirales*, richness, and Shannon diversity was consistently higher than that of *Microviridae* in both IBD and control groups. The relative-abundance of *Caudovirales* phage was significantly higher in CD (mean 80.8%) compared with UC (mean 48.8%) patients (Fig. 1a, Dunn's test; P=0.05) and trended higher in CD compared with controls (mean 55.0%; Dunn's test; P=0.07). While the number of *Microviridae* phage detected in controls was significantly higher than those found in CD patients and trended higher compared with UC patients (Fig. 1b, Dunn's test; P=0.05, P=0.1, respectively), no detectable difference was found in Shannon's Diversity Index across the three subject groups within *Microviridae* or *Caudovirales* (Fig. 1c).

Disease status, dominant bacterial taxon and probiotic supplementation relate to gut bacterial microbiota composition.

Since sufficient sequencing depth (n=85,486 reads per sample) was only obtained for 9 of the 12 IBD patients, these participants were collapsed into a single IBD group. Between-group bacterial alpha diversity was compared using richness, Pielou's evenness, and Shannon's Diversity indices (Supplemental Figure 1). There were no significant differences in alpha-diversity between healthy subjects and IBD patients (Supplemental Figure 1a-c, Wilcoxon rank-sum test; $P>0.05$), though control subjects trended towards richer bacterial gut microbiota compared with IBD subjects. PERMANOVA analysis was used to examine whether variation in bacterial community composition was explained by clinical variables, including probiotic supplementation and markers of inflammation. Of the factors examined, IBD diagnosis (yes/no, Supplemental Figure 2), multiple markers of health status (albumin, hemoglobin and erythrocyte sedimentation rate), and probiotic supplementation were significantly related to the composition of the gut bacterial microbiota (Table 2). Although antibiotic treatment is a potential confounding factor, the control subject taking antibiotics at the time of sample collection clustered with the remaining controls, indicating that the administration of antimicrobials did not dramatically shift the composition of the microbiota of this participant. Of note, the dominant bacterial family explained the greatest degree of gut microbiota compositional variance (Figure 2), while dominant phage detected in these samples did not significantly relate to community composition. These data suggest that the health status of pediatric participants in our study was related to the composition of their gut

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microbiota, and that probiotic supplementation influences gut bacterial microbiota composition.

To further identify specific bacterial taxa that discriminate IBD patients from healthy subjects, we performed an analysis of taxon relative abundance across these groups. (Supplemental Tables 3a and 3b) In contrast to healthy children who exhibited significant enrichment of *Bacteroides*, *Ruminococcus* and *Blautia*, IBD patients were significantly enriched for *Haemophilus* and *Streptococcus;* the former has been associated with early onset disease (14).

Discussion

Prior studies focused on the etiology of IBD have established differences in bacterial diversity between IBD patients and controls, and predominance of specific microbiota in those with IBD (8). However, the microbiome is a dynamic environment impacted by viral composition as well. Our study characterized the enteric virome and bacterial microbiota in children with IBD, and compared their results with an age-matched control group, uncovering only minor differences in the virome and bacterial community composition between the IBD and the control groups. Exploring the baseline viral and bacterial compositions is paramount in investigating intestinal dysbiosis in IBD patients.

Novel characteristics of the microbiome in IBD have previously been established including paucity of overall diversity with increased abundance of select microbiota members (15). In adult studies, comparisons between UC and Crohn's patients and of both groups of IBD patients compared with healthy controls have established disease-specific patterns in the enteric virome namely a greater abundance of *Caudovirales* relative to *Microviridae* phages in IBD than controls and a greater diversity of *Caudovirales* phages in IBD than controls (8). Here we found that a greater abundance, richness, and diversity of Caudovirales relative to *Microviridae* was present in all three groups studied irrespective of disease status.

In pediatrics, *Pasteurellaceae* (*Haemophilus* sp.), *Veillonellaceae*, *Neisseriaceae*, and *Fusobacteriaceae* predominate in the microbiome in treatment-naïve Crohn's patients (16). Further work also suggests that particular microbiota patterns can be predictive of severity of disease. For example, a decrease in *Faecalibacterium prausnitzii* can be associated with recurrence of disease in Crohn's patients following ileal resection (14, 17)

The driving forces underlying the composition of the microbiome are also multifactorial. A recent evaluation of the neonatal microbiota in children at risk for atopy identified an inter-kingdom gut microbial co-evolution over the first year of life (18). Additionally, in mouse models, gut microbiome supplementation with *Lactobaccillus johnsonii* promoted protection against viral respiratory infection (19), suggesting that the gut microbiome plays an integral role in both immune function and viral interactions.

We obtained samples from 24 pediatric patients with IBD and healthy controls. As this was an exploratory study, sample size limited our ability to elucidate differences and patterns. Our intent was to evaluate a general pediatric IBD population. Enrolled patients vary based on several factors, including medications, disease location, age, and disease severity,

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although specific disease activity was not recorded. Recent studies have begun to suggest that medications used in treatment may be beneficial in part due to their impact on enteric microbiota (20, 21). Given various pressures on the microbiome, recruiting patients with similar disease phenotype and medication exposures might improve our capability to identify differences. Another limitation is recruitment of our control population from general gastroenterology clinic rather than from the general population. Controls were screened for colitis and other disorders related to IBD, and none had macroscopic or microscopic evidence of IBD. By recruiting from the same subspecialty clinic, we were able to standardize our collection instructions and methods including the geographic recruitment area. Collectively, these limiting factors – small sample size, heterogeneity of IBD cases, and control subjects being drawn from a gastroenterology clinic – may explain our ability to only detect a statistically significant trend in lower bacterial richness in IBD cases relative to controls. Additionally, only stool samples were analyzed in this study; further studies would be enhanced by also including rectal mucosal samples.

The statistically significant differences identified here were the *Caudovirales* relative-abundance between CD and UC patients, finding a significantly lower number of strains within the *Microviridae* phage family in CD patients compared with healthy controls, and a significant difference in bacterial community composition between pediatric IBD patients and non-IBD, healthy, age-matched controls. Previous studies in adults, also analyzing low numbers of patients and cases, report distinct virome patterns in IBD patients compared with household and geographical controls (6, 8), possibly reflecting differences between children and adults and/or use of different starting materials (colon tissue RNA versus feces). The borderline p-values for the observed differences indicate that conclusions regarding relevance to IBD be considered tentative. Bacterial microbiota exhibited significant relationships with disease status and with multiple objective markers of host health, indicating that composition and function of bacterial communities in the gut may influence such attributes. While these results lend further support to the role of gastrointestinal bacterial and viral communities in IBD pathogenesis, larger studies are necessary to account for confounding factors such as age and duration of disease, and more in-depth studies are needed to identify mechanisms by which the gut microbiome may modulate immune function in pediatric patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

1. Kim DH, Cheon JH. Pathogenesis of Inflammatory Bowel Disease and Recent Advances in Biologic Therapies. Immune Netw 2017;17:25–40. [PubMed: 28261018]

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2. Adamiak T, Walkiewicz-Jedrzejczak D, Fish D, et al. Incidence, clinical characteristics, and natural history of pediatric IBD in Wisconsin: a population-based epidemiological study. Inflamm Bowel Dis 2013;19:1218–23. [PubMed: 23528339]
3. Douglas GM, Hansen R, Jones CM, et al. Multi-omics differentially classify disease state and treatment outcome in pediatric Crohn's disease. Microbiome 2018;6:13. [PubMed: 29335008]
4. Jakobsen C, Paerregaard A, Munkholm P, et al. Pediatric inflammatory bowel disease: increasing incidence, decreasing surgery rate, and compromised nutritional status: A prospective population-based cohort study 2007–2009. Inflamm Bowel Dis 2011;17:2541–50. [PubMed: 21381152]
5. Eszter Muller K, Laszlo Lakatos P, Papp M, et al. Incidence and paris classification of pediatric inflammatory bowel disease. Gastroenterol Res Pract 2014;2014:904307. [PubMed: 24778643]
6. Wang W, Jovel J, Halloran B, et al. Metagenomic analysis of microbiome in colon tissue from subjects with inflammatory bowel diseases reveals interplay of viruses and bacteria. I nflamm Bowel Dis 2015;21:1419–27.
7. Cadwell K The Virome in Host Health and Disease. Immunity 2015;42:805–13. [PubMed: 25992857]
8. Norman JM, Handley SA, Baldridge MT, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 2015;160:447–60. [PubMed: 25619688]
9. Conceicao-Neto N, Deboutte W, Dierckx T, et al. Low eukaryotic viral richness is associated with faecal microbiota transplantation success in patients with UC. Gut 2018;67:1558–9. [PubMed: 29066574]
10. Li L, Deng X, Mee ET, et al. Comparing viral metagenomics methods using a highly multiplexed human viral pathogens reagent. J Virol Methods. 2015;213:139–146. [PubMed: 25497414]
11. Hurwitz BL, U'Ren JM, Youens-Clark K. Computational prospecting the great viral unknown. FEMS Microbiol Lett 2016;363.
12. Krishnamurthy SR, Wang D. Origins and challenges of viral dark matter. Virus Res 2017;239:136–42. [PubMed: 28192164]
13. Roux S, Hallam SJ, Woyke T, et al. Viral dark matter and virus-host interactions resolved from publicly available microbial genomes. Elife 2015;4.
14. Vindigni SM, Zisman TL, Suskind DL, et al. The intestinal microbiome, barrier function, and immune system in inflammatory bowel disease: a tripartite pathophysiological circuit with implications for new therapeutic directions. Therap Adv Gastroenterol 2016;9:606–25.
15. Zhou M, He J, Shen Y, et al. New Frontiers in Genetics, Gut Microbiota, and Immunity: A Rosetta Stone for the Pathogenesis of Inflammatory Bowel Disease. Bio Res Int 2017;2017:8201672.
16. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 2014;15:382–92. [PubMed: 24629344]
17. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–6. [PubMed: 18936492]
18. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med 2016;22:1187–91. [PubMed: 27618652]
19. Fonseca W, Lucey K, Jang S, et al. Lactobacillus johnsonii supplementation attenuates respiratory viral infection via metabolic reprogramming and immune cell modulation. Mucosal Immunol 2017;10:1569–80. [PubMed: 28295020]
20. Morgan XC, Tickle TL, Sokol H, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 2012;13:R7. [PubMed: 22293038]
21. Levine A, Kori M, Kierkus J, et al. Azithromycin and metronidazole versus metronidazole-based therapy for the induction of remission in mild to moderate paediatric Crohn's disease : a randomised controlled trial. Gut. 2018 PAP

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What is known:

- Reports document differences in bacterial diversity between adult and pediatric inflammatory bowel disease (IBD) patients and controls, and adult patients exhibit viral expansion.
- Little information is available regarding the enteric virome in children with IBD.

What is new:

- A greater abundance, richness, and diversity of *Caudovirales* relative to *Microviridae* was found in children with Crohn's disease, ulcerative colitis and non-IBD controls irrespective of disease status.
- Minor patterns in gut virome and bacterial community composition distinguish pediatric IBD patients from healthy controls.
- Probiotics are associated with bacterial microbiota composition in children with IBD.

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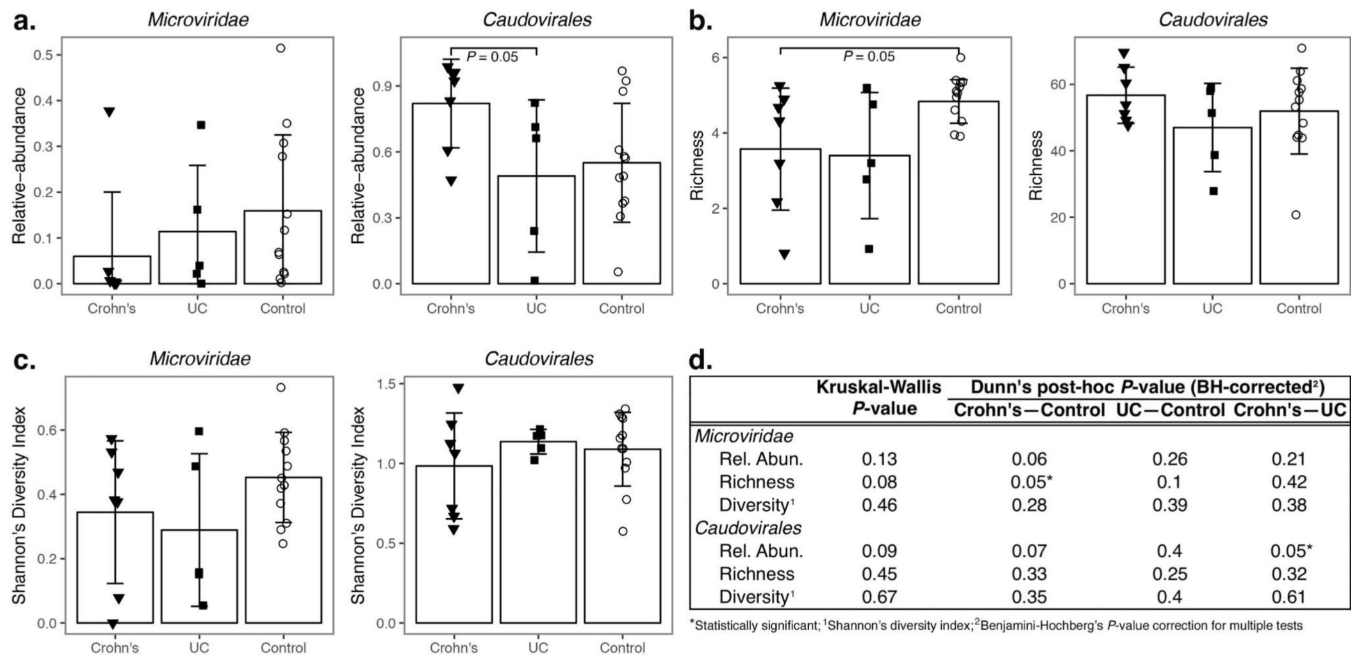

	Kruskal-Wallis *P*-value	Dunn's post-hoc *P*-value (BH-corrected²)		
		Crohn's—Control	UC—Control	Crohn's—UC
Microviridae				
Rel. Abun.	0.13	0.06	0.26	0.21
Richness	0.08	0.05*	0.1	0.42
Diversity¹	0.46	0.28	0.39	0.38
Caudovirales				
Rel. Abun.	0.09	0.07	0.4	0.05*
Richness	0.45	0.33	0.25	0.32
Diversity¹	0.67	0.35	0.4	0.61

*Statistically significant; ¹Shannon's diversity index; ²Benjamini-Hochberg's *P*-value correction for multiple tests

Figure 1:

Caudovirales relative-abundance is greater in CD compared with UC patients, and *Micro*viridae richness is reduced in CD versus control populations. Box height represents the group mean and error bars signify +/− 1 standard deviation. See text for details.

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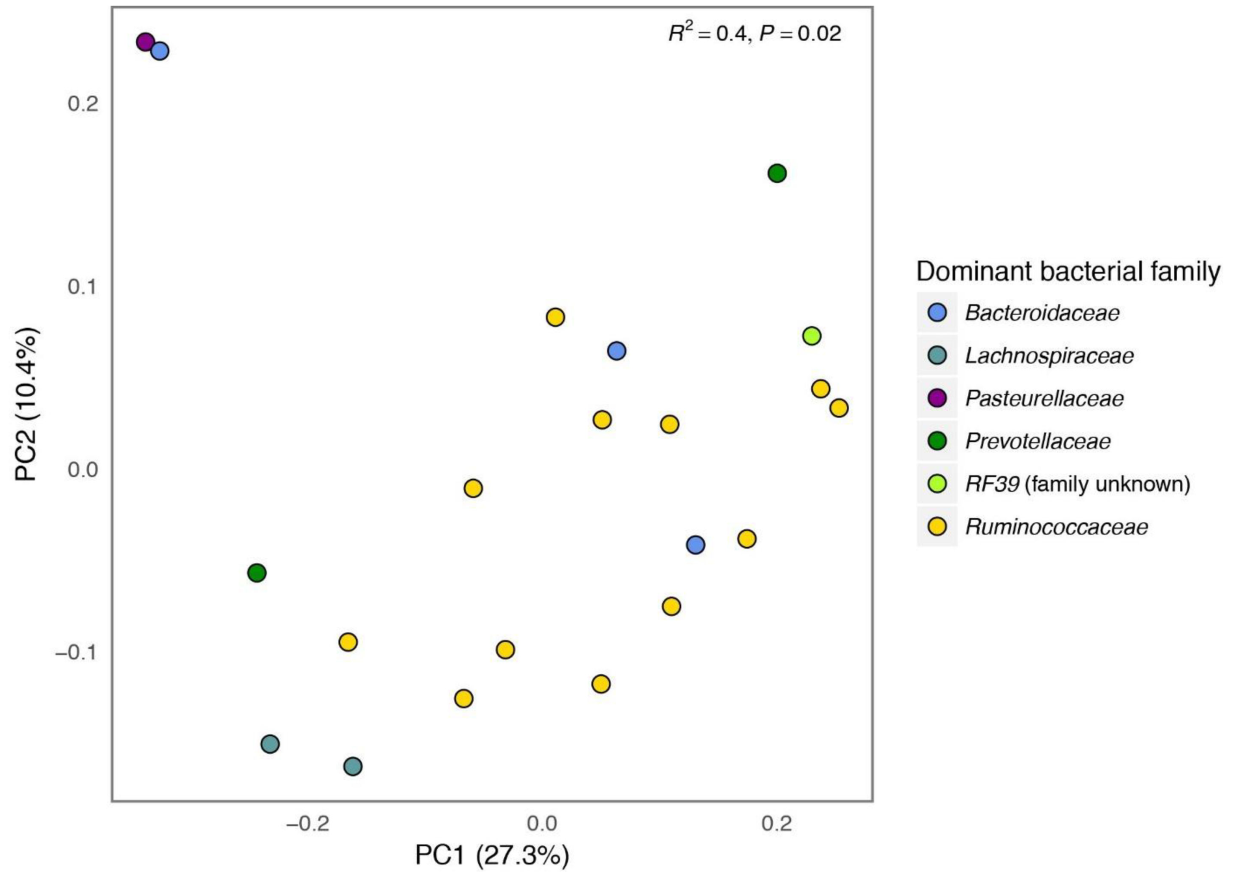

Figure 2:

Dominant bacterial family explains a large proportion of variance in pediatric fecal microbiota composition.

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Table 1a.

Characteristics of Patients and Control Subjects Crohn's Disease Patients

Subject	Age (years) at time of stool collection	IBD Medications	Acid Suppression (Y/N)	Disease Classification at time of stool collection	
				Location (macroscopic)	Behavior
1	14.9	Infliximab	No	Ileocolonic	Inflammatory
2	12.2	Budesonide Methotrexate	Yes	Ileocolonic Upper GI Findings	Inflammatory
3	8.5	Infliximab Methotrexate 5ASA	No	Ileal	Inflammatory
4	16.6	Infliximab	No	Colonic	Penetrating
5	15.6	Budesonide Methotrexate 5ASA	Yes	Ileocolonic	Inflammatory
6	15.0	Methotrexate 5ASA	Yes	Ileocolonic	Inflammatory
7	14.5	Infliximab	No	None	Inflammatory

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Table 1b.

Ulcerative Colitis Patients

Subject	Age (years) at time of collection	IBD Medications	Acid Suppression (Y/N)	Disease Location
8	10.9	5ASA	No	Pancolitis
9	17.3	5ASA TPN VSL3	Yes	Pancolitis
10	15.0	5ASA	No	Pancolitis
11	12.9	5ASA	No	Pancolitis
12	16.8	Azathioprine 5ASA	No	Pancolitis

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Table 1c.

Control Subjects

Subject	Age at time of collection (years)	GI Medications	Acid Suppression (Y/N)	Symptom/Complaint
13	10.6	Polyethylene glycol Senna glycoside Cyproheptadine	No	Constipation
14	8.2	Omeprazole Lactobacillus	Yes	Epigastric abdominal pain
15	13.4	Bisacodyl Magnesium citrate Polyethylene glycol	No	Constipation
16	10.6	Multivitamin Omeprazole Polyethylene glycol Citalopram Melatonin	Yes	Constipation
17	9.5	Acetaminophen Ondansetron Omega3 Lactobacillus	No	Abdominal pain
18	14.8	Omeprazole Polyethylene glycol	Yes	Constipation
19	13.3	Omeprazole Polyethylene glycol	Yes	Constipation
20	8.0	Polyethylene glycol Fiber Senna glycoside	No	Constipation with fecal incontinence
21	7.6	Polyethylene glycol Omeprazole	Yes	Constipation
22	7.8	Lansoprazole Ibuprofen	Yes	Dysphagia
23	6.6	Multivitamin Senna glycoside Polyethylene glycol	No	Constipation
24	13.1	Azithromycin* Polyethylene glycol Senna glycoside Docusate Methyl cellulose Fiber	No	Constipation

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Table 2.

Bacterial Beta-diversity.

Variable	n	R^2	p-value
Dominant Bacterial Family	21	0.40	0.02
Dominant Phage Family	21	0.26	0.14
IBD Diagnosis	21	0.12	0.03
Probiotic Supplementation	21	0.13	0.03
Erythrocyte Sedimentation Rate	13	0.25	0.02
Hemoglobin	13	0.23	0.02
Albumin	11	0.24	0.04