RESEARCH LETTERS

Pilot Study of Fecal Mitochondrial DNA in Inflammatory Bowel Disease Patients Demonstrates a High Sensitivity Assay With Likely Capacity to Differentiate Between Disease and Histologic Remission

Inflammatory bowel disease (IBD) is an autoimmune disease with multiple manifestations, although typically centered around inflammatory changes in the intestines. Incidence rates of pediatric IBD continue to increase annually, with the fastest increases occurring in areas that have recently modernized their infrastructure.[1] The condition comes with a significant burden of care. Therapeutic challenges involve optimizing therapy despite the common discordance between histologic and symptom severity as well as assessing disease state based on the interpretation of currently available inflammatory markers.[1]

IBD is driven by a combination of genetic susceptibility, microbial interactions, and initial inflammatory triggers and can be difficult to monitor with serum laboratory tests. Inflammatory markers are often not sensitive due to the primarily epithelial nature of the inflammatory changes, leading to patients with normal serum markers of inflammation who nonetheless have significant disease activity.[2] Current practice is to monitor disease activity via a combination of disease scores and recurrent endoscopic evaluations to determine histologic remission, as mucosal healing has been strongly associated with decreased risk of relapses and of long-term complications.[3,4] Patients may be in clinical remission (asymptomatic, remission scores on the pediatric ulcerative colitis [UC] activity index and pediatric Crohn's disease [CD] activity index), while still having active disease at a histologic level.[2] The currently accepted gold standard biomarker (fecal calprotectin [FC], which measures a byproduct of neutrophil activity) does not reliably determine histologic status. Calprotectin has other limitations in small intestinal disease, as bacteria digest and degrade it, making small intestinal disease or proximal colitis sometimes appear less significant on the basis of marker assay alone.[2] This leaves endoscopic evaluation as the only consistent way to establish true remission, as patients in histologic remission have a higher risk of negative outcomes and more severe disease reactivation.

While it has been demonstrated that cell death due to intestinal inflammation results in elevated host deoxyribonucleic acid (DNA) in stool,[5,6] we theorized that measuring a conserved fragment of human mitochondrial DNA (mtDNA) would provide increased sensitivity due to the greater copies per cell. Our prior work (Zhu, Wallach et al, *ES&T Letters*, 2022)[7] found that concentrations of human mtDNA distinguished between norovirus presence and the inflammatory state of active symptomatic norovirus, likely reflecting the increased epithelial extrusion and apoptosis known to occur during intestinal inflammatory states. In this study, we assessed its utility as a disease monitoring tool in pediatric inflammatory bowel disease.

We recruited pediatric and young adult patients (aged 5–22 years) at the pediatric gastroenterology clinic of State University of New York Downstate Health Sciences University with planned endoscopic procedures and collection of stool samples for fecal calprotectin. Stool samples were collected using at-home collection kits, stored at ∼4 °C until patient appointment, and stored at −80 °C upon receipt. Calprotectin was measured by enzyme-linked immunosorbent assay via Quest Diagnostics Clinical Laboratory as μg per gram of stool. We extracted DNA in biological triplicates using the Qiagen DNeasy PowerSoil Pro following the manufacturer's instructions and quantified mtDNA copies using hCYTB484,[8] an assay targeting a human-specific region of the cytochrome b gene, on the Qiagen QIAcuity digital polymerase chain reaction (PCR) system. We normalized copies of the mtDNA marker to milligrams of stool. The Minimum Information for Publication of Quantitative Digital PCR Experiments[9] for this study is available in the supplemental materials. To assess severity of disease, we used the relevant disease severity score (pediatric UC activity index/pediatric CD activity index) to differentiate disease severity and the Robarts Histopathology Index (RHI), which has been shown to be an applicable histologic assay for both UC and CD to establish histologic remission (RHI < 3).[10,11] We used nonparametric tests (Kendall's tau) to assess correlations between RHI and fecal mtDNA as well as calprotectin to account for nonlinearity observed in the data.

We recruited 13 patients, 7 UC and 6 CD (Table 1). Our results (Figure 1A) suggest that concentrations of fecal mtDNA in children and young adults with IBD increase with increasing severity of disease, with several orders of magnitude difference between histological remission vs moderate to severe cases. Despite both fecal calprotectin and fecal mtDNA having strong correlations to RHI (Kendall's tau approximately 0.7 for both calprotectin and mtDNA), concentrations of fecal mtDNA were more distinct between cases of histological remission vs mild and moderate severity than that of fecal calprotectin (Figure 1A). This demonstrates, in these samples, that while fecal mtDNA and fecal calprotectin have similar correlations with histopathology scoring across the range of disease severity, fecal mtDNA

Table 1. Summary Table of Individual Patients From This Study

	Age, y	Gender	Race	IBD subtype	Disease location at Dx	Medication	Clinical activity	Histologic remission	Fecal calprotectin (μg/g)	Fecal mtDNA (copies/mg)
1	12	M	Black	UC	Transverse, descending colon, and rectum	Not applicable	Remission	Yes	6.30E+01	8.94E+02
2	14	M	Black	CD	Ileocolonic	Humira	Moderate	No	1.13E+03	7.48E+04
3	9	M	Black	UC	Transverse, descending colon, and rectum	Not applicable	Moderate	No	2.93E+03	9.70E+05
4	16	M	White	UC	Pancolitis	Not applicable	Moderate	No	1.82E+03	4.49E+05
5	15	M	Black	CD	Cecum, rectum	Humira	Mild	No	2.40E+02	2.46E+05
6	12	M	Black	UC	Transverse, left colon, and rectum	Remicade	Remission	Yes	1.50E+01	2.46E+02
7	17	M	White	UC	Cecum, transverse, descending colon, sigmoid, and rectum	Mesalamine	Remission	No	9.00E+00	2.07E+04
8	17	M	White	UC	Pancolitis	Vedolizumab, Mesalamine	Mild	No	1.15E+03	7.82E+04
9	19	F	White	CD	Terminal ileum	Not applicable	Mild	No	1.10E+01	6.58E+03
10	16	M	Black	CD	Cecum, left colon, and rectum	Stelara	Mild	No	3.30E+02	5.02E+04
11	11	M	Black	UC	Transverse, descending colon, and rectum	Vedolizumab	Severe	No	3.37E+03	9.52E+05
12	12	F	Black	CD	Terminal ileum, colon	Infliximab	Remission	No	N/A	1.33E+04
13	16	M	Black	CD	Terminal ileum, sigmoid colon	Infliximab	Remission	Yes	5.70E+01	8.77E+03

may offer additional resolution in the lower and intermediate values in which there is difficulty in interpreting concentrations of calprotectin.[2]

Our findings suggest that assay of fecal mtDNA offers substantial potential as a reliable and sensitive biomarker for assessing IBD activity. The mtDNA assay utilizes now widespread PCR technology, can be completed in-house at higher speed and lower cost than enzyme-linked immunosorbent assay[12], and due to the high dynamic range of the assay, appears to be more accurate at low disease severity states. Our study demonstrates at least equal overall fidelity to disease state as FC; however, as FC has had challenges in identifying histologic remission,[3] the clinical implications of higher accuracy at low disease states are substantial. The ability to noninvasively differentiate between clinical and histologic remission for even a subset of patients will improve burden of care, allow providers to have improved insight into disease state, and likely drive improved outcomes. As mtDNA reflects current state rates of cell death and extrusion, it is also highly likely that mtDNA levels will be much more responsive to treatment-induced changes, improving clinician agility in care by being able to assess outcomes in days rather than weeks. Additionally, previously reported work does not show elevation in healthy children at early ages[13] as calprotectin does. While generalizability of our findings is limited due to the small sample size, the effect size between median concentrations from histologic remission and active groups (at least 10 times difference) justifies investigation with more samples. Our study is limited by a pediatric population, but our prior work has not shown alterations to baseline concentrations of mtDNA by age in children,[13] and work in adults has demonstrated elevated concentrations with inflammatory processes like noroviral infection.[7] Accordingly, further work in adults is

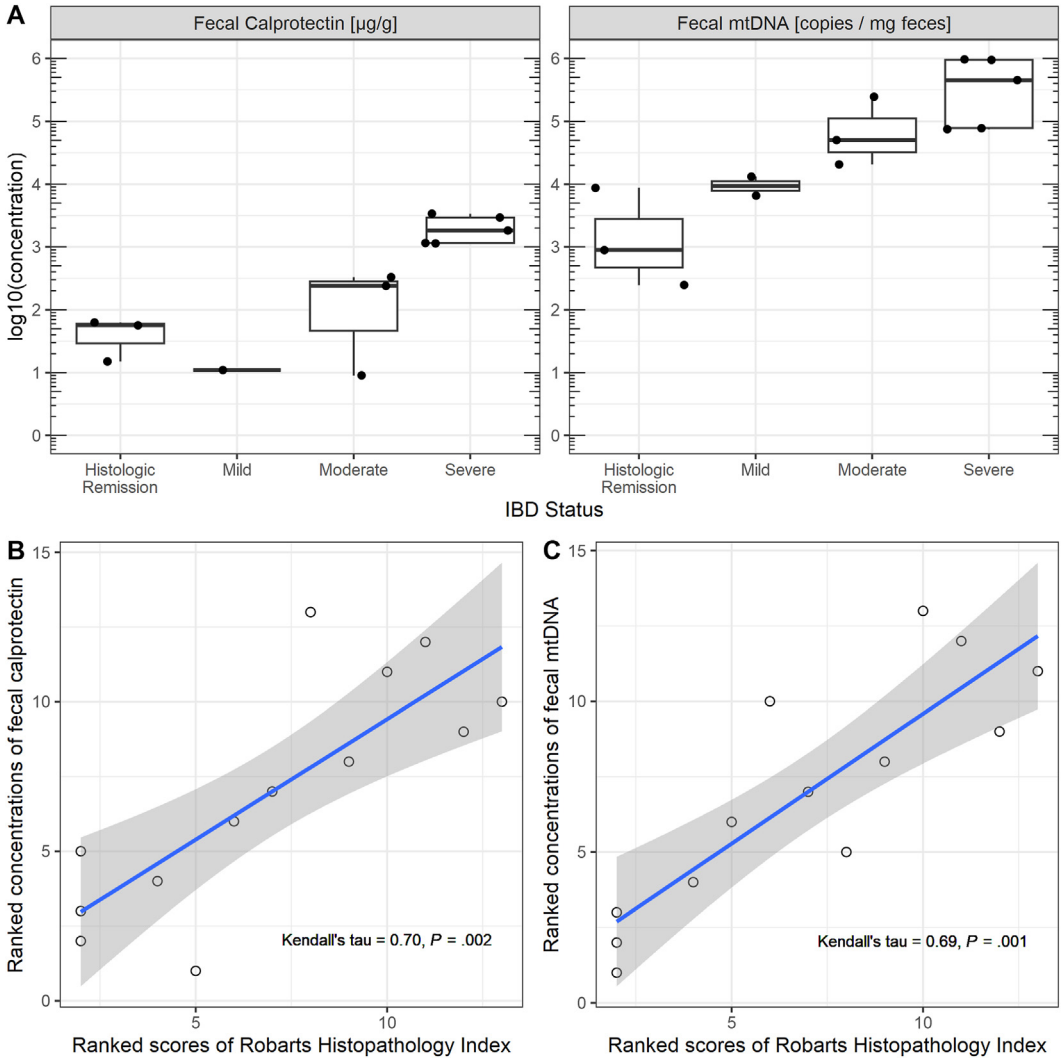

Figure 1. Top row box plots (A) showing concentrations of fecal calprotectin (left) and fecal mtDNA (right) with increasing severity of IBD from histologic remission to severe disease from samples from 13 IBD patients. Boxplots display the median of distributions as the middle horizontal line and the 25th and 75th percentiles as the bottom and top of the box, respectively. Bottom row scatter plots showing nonparametric correlations between scoring from the Robarts Histopathology Index and fecal calprotectin (B) and fecal mtDNA (C) from the same 13 samples.

indicated, with our assay likely to translate well. In this study, mtDNA measurements across biological triplicates did not vary greatly; future work into understanding shedding patterns and rates would help contextualize concentrations. While our current dataset is too small to specifically assess mtDNA in small bowel disease, ongoing work has noted elevated levels of fecal mtDNA in upper gastrointestinal diseases such as H. pylori. As DNA is degraded in the gastrointestinal tract, we do expect attrition from upper gastrointestinal sources, but the sheer quantity of mtDNA suggests viable signal will remain, based on prior work identifying detectable DNA survival even after murine oral consumption.[14] While this will create some confounding effects with regard to disease monitoring that will require additional work, it seems quite likely that small intestinal disease would reliably elevate levels, further strengthening the potential utility of this assay.

In short, this is a promising new technology for the management of IBD, with the potential to improve care decision-making curves and decrease endoscopic burden for patients. Further study is necessary to determine exact populations and disease states that will benefit.

KEVIN ZHU[1,*]
THOMAS WALLACH[2,*]
SHAGUN SHARMA[2]
VIVIAN VEGA[2]
MEREDITH PITTMAN[3]
JOE BROWN[1]

[1]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina
[2]Division of Pediatric Gastroenterology, Department of Pediatrics, SUNY Downstate Health Sciences University, Brooklyn, New York
[3]Department of Medicine, Maimonides Medical Center, Brooklyn, New York

Correspondence:
Address correspondence to: Dr. Thomas Wallach, MD. Division of Pediatric Gastroenterology, Department of Pediatrics, SUNY Downstate Health Sciences University, 450 Clarkson Avenue, MSC 49, Brooklyn, New York 11203. e-mail: thomas.wallach@downstate.edu.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2025.100622.

References

1. Park J, Jeong GH, Song M, et al. The global, regional, and national burden of inflammatory bowel diseases, 1990-2019: a systematic analysis for the global burden of disease study 2019. Dig Liver Dis 2023;55:1352–1359.
2. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: an update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) initiative of the international organization for the study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. Gastroenterology 2021;160:1570–1583.
3. D'Amico F, Bonovas S, Danese S, et al. Review article: faecal calprotectin and histologic remission in ulcerative colitis. Aliment Pharmacol Ther 2020;51:689–698.
4. Yoon H, Jangi S, Dulai PS, et al. Histologic remission is associated with lower risk of treatment failure in patients with Crohn disease in endoscopic remission. Inflamm Bowel Dis 2021;27:1277–1284.
5. Huang AD, Luo C, Pena-Gonzalez A, et al. Metagenomics of two severe foodborne outbreaks provides diagnostic signatures and signs of coinfection not attainable by traditional methods. Appl Environ Microbiol 2017;83:e02577-16.
6. Jiang P, Lai S, Wu S, et al. Host DNA contents in fecal metagenomics as a biomarker for intestinal diseases and effective treatment. BMC Genom 2020;21:348.
7. Zhu KJ, Suttner B, Knee J, et al. Elevated fecal mitochondrial DNA from symptomatic norovirus infections suggests potential Health relevance of human mitochondrial DNA in fecal source tracking. Environ Sci Technol Lett 2022;9:543–550.
8. Zhu K, Suttner B, Pickering A, et al. A novel droplet digital PCR human mtDNA assay for fecal source tracking. Water Res 2020;183:116085.
9. Huggett JF. The digital MIQE Guidelines update: minimum information for publication of quantitative digital PCR Experiments for 2020. Clin Chem 2020;66:1012–1029.
10. Sherlock ME, Benchimol EI. Classification of inflammatory bowel disease in children. In: Mamula P, Grossman AB, Baldassano RN, et al, eds. Pediatric inflammatory bowel disease. Cham: Springer International Publishing, 2017:181–191.
11. Pai RK, Lauwers GY. Measuring histologic activity in inflammatory bowel disease: why and how. Adv Anat Pathol 2022;29:37–47.
12. Yang Z, Clark N, Park KT. Effectiveness and cost-effectiveness of measuring fecal calprotectin in diagnosis of inflammatory bowel disease in adults and children. Clin Gastroenterol Hepatol 2014;12:253–262.e2.
13. Zhu KJ, Lai A, Holcomb D, et al. Fecal mitochondrial DNA as a potential biomarker for environmental enteropathy. Gastroenterology 2023;164:S189–S190.
14. Xing R, Liu H, Qi X, et al. Measuring the process and rate of exogenous DNA degradation during digestion in mice. Sci Rep 2022;12:6463.

***These two authors contributed equally to this publication**

Abbreviations: **CD, Crohn's disease; DNA, deoxyribonucleic acid; FC, fecal calprotectin; IBD, inflammatory bowel disease; mtDNA, mitochondrial deoxyribonucleic acid; PCR, polymerase chain reaction; UC, ulcerative colitis**

Most current article

2772-5723
https://doi.org/10.1016/j.gastha.2025.100622

Received July 16, 2024. Accepted January 20, 2025.

Conflicts of Interest:
These authors disclose the following: Thomas Wallach, Kevin Zhu, and Joe Brown have obtained a patent on the work described herein. The remaining authors disclose no conflicts.

Funding:
The authors report no funding.

Ethical Statement:
The study was approved by the SUNY Downstate IRB (#2045855-1).

Data Transparency Statement:
Data, methods, and study materials are available on request from Dr Wallach. Data will only be available with a data use agreement.

Reporting Guidelines:
Not applicable for this article type.