SARSCOV2 IN STOOLS IN PATIENTS

Gut Pathogens

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Detection of SARS-CoV-2 from patient fecal samples by whole genome sequencing

Andreas Papoutsis ✉, Thomas Borody, Siba Dolai, Jordan Daniels, Skylar Steinberg, Brad Barrows & Sabine Hazan

Gut Pathogens **13**, Article number: 7 (2021) | Cite this article

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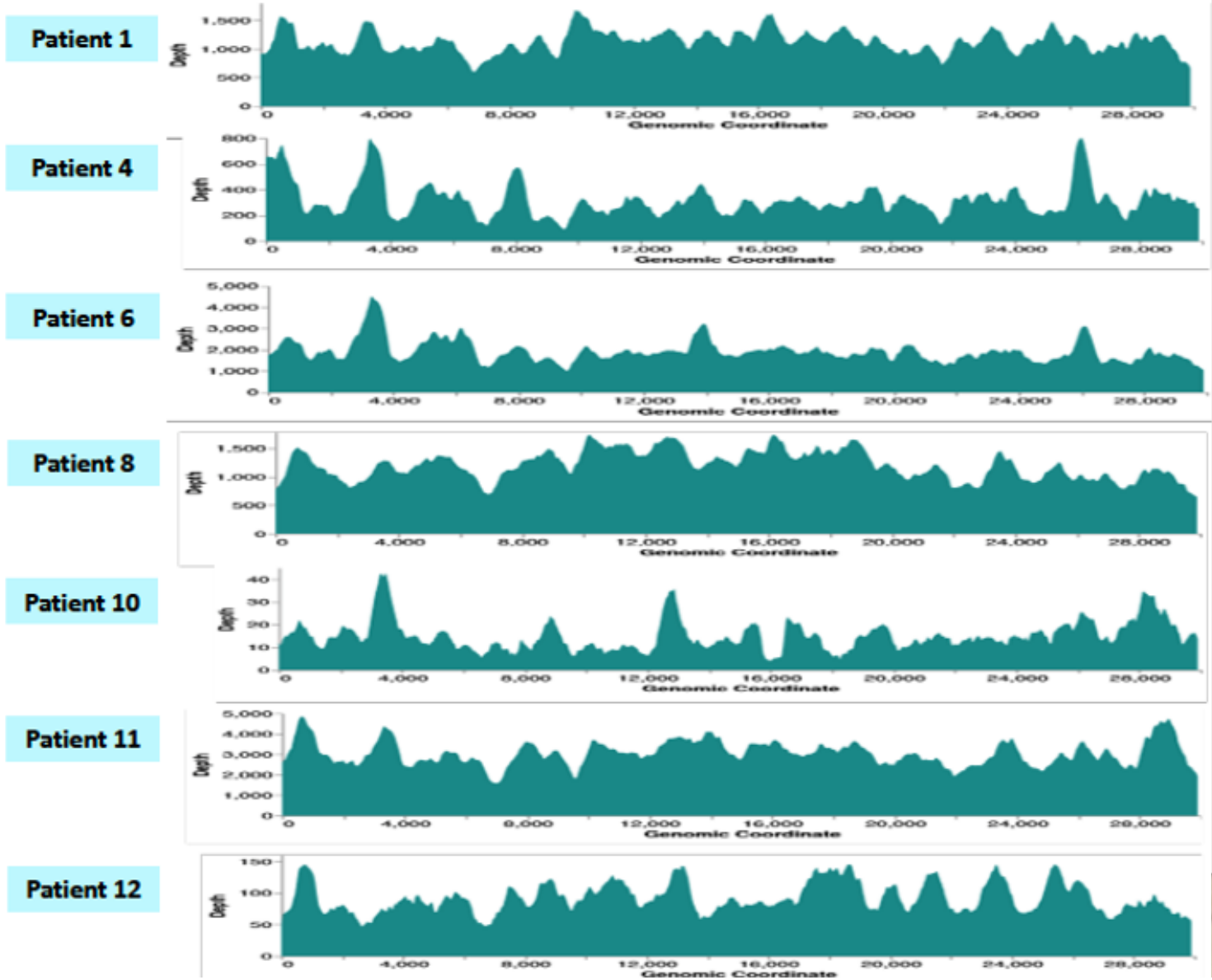


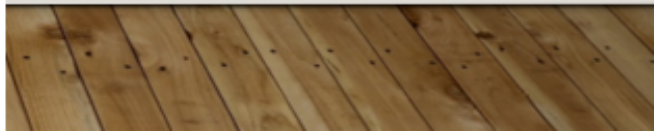
X-Axis: Genomic coordinates

Y-Axis: sequencing dept at specific loci

7/7 showed presence of SARS-CoV2

6/7 had 100% genome coverage

1/7 had 93% genome coverage



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Research Article ▸ J Infect Dis Ther, Vol 10(2) DOI: 10.4172/2332-0877.1000491

SARS-CoV-2 as a Trigger in the Development of Tourette's Like Symptoms

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Case Reports in Gastroenterology

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Case Series

Pre-Existing Microbiome Signature in a SARS-CoV-2 Discordant Family

Sabine Hazan

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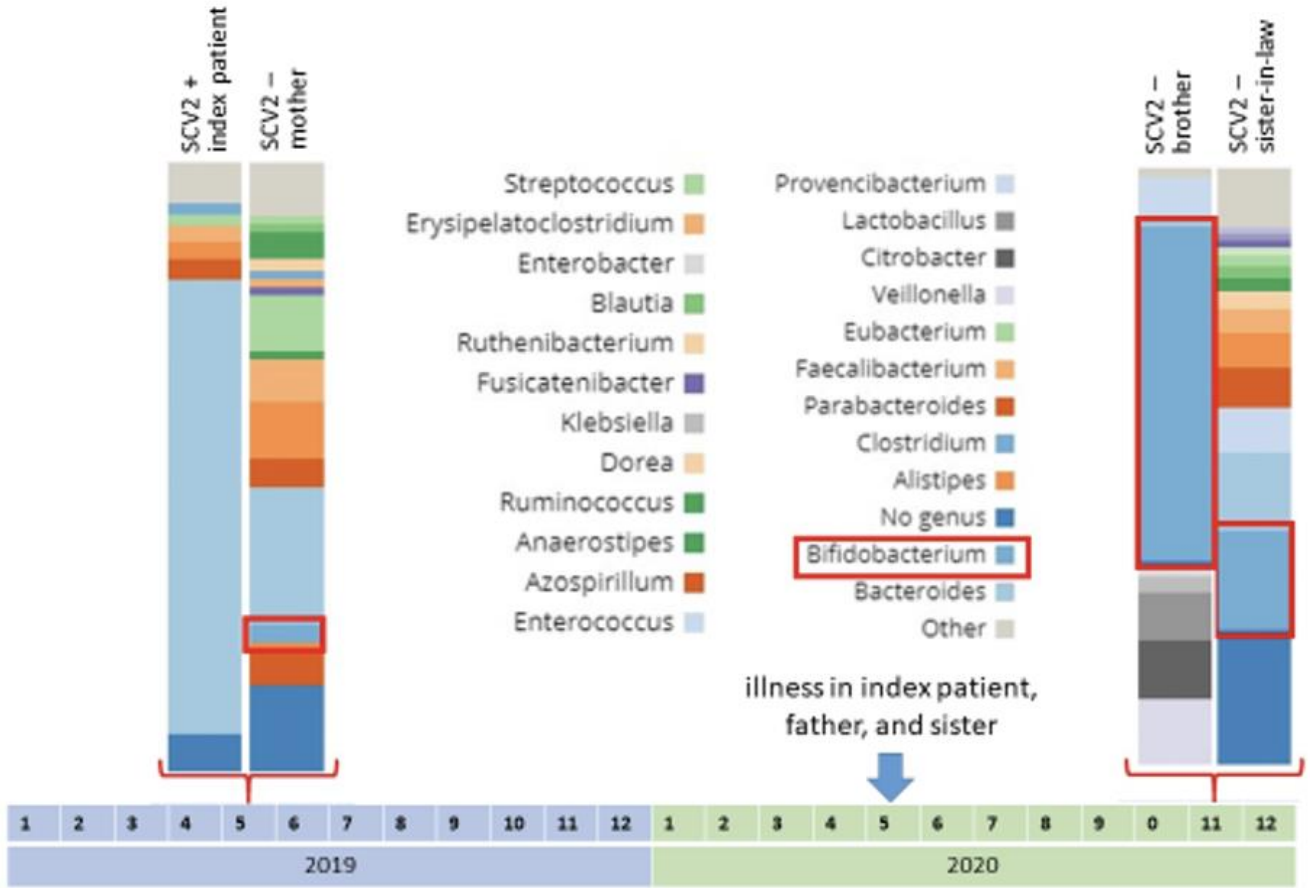
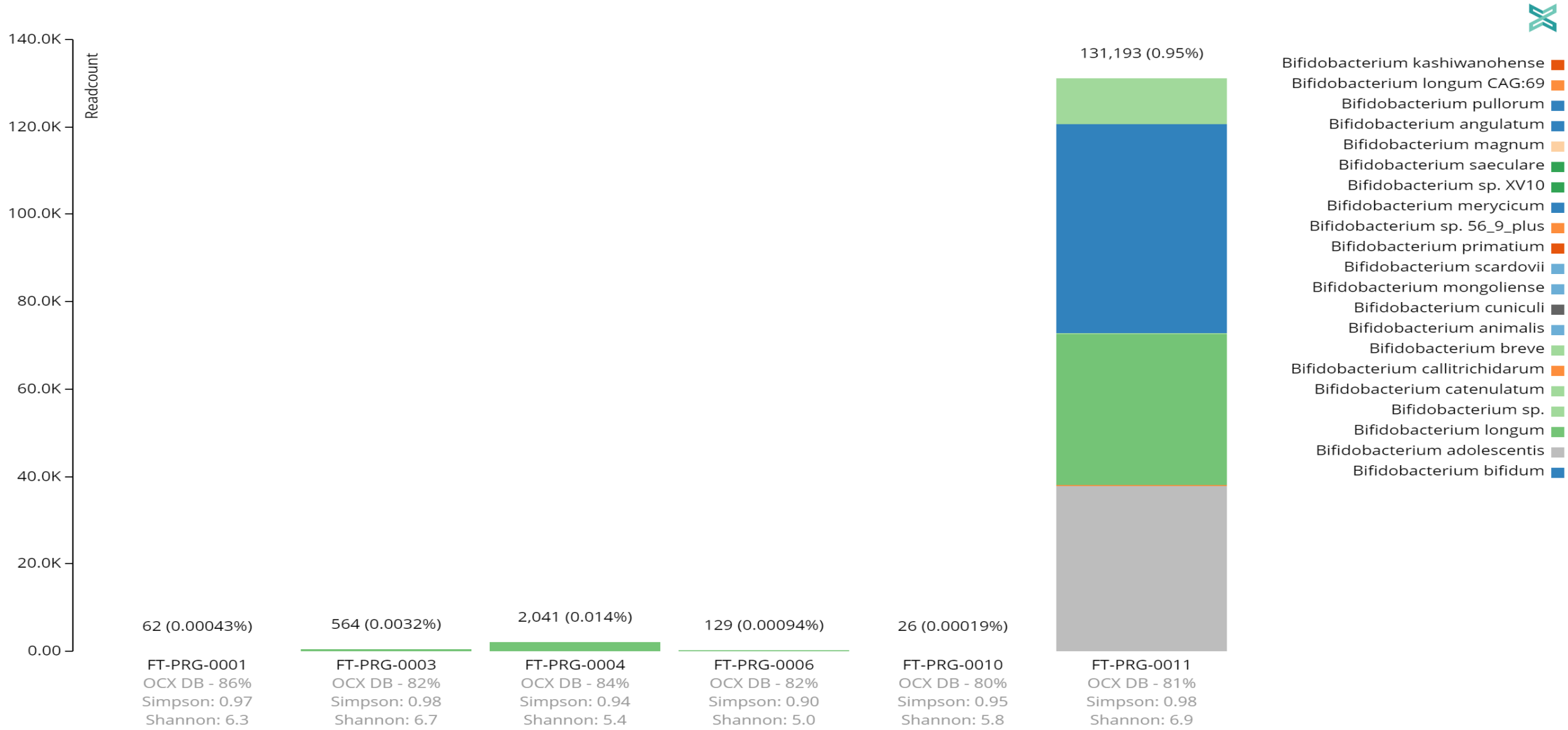
Figure Legends

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Readcount
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129 (0.00094%)
26 (0.00019%)
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FT-PRG-0001
OCX DB - 86%
Simpson: 0.97
Shannon: 6.3
FT-PRG-0003
OCX DB - 82%
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Shannon: 6.7
FT-PRG-0004
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Simpson: 0.98
Shannon: 6.9
Bifidobacterium kashiwanohense
Bifidobacterium longum CAG:69
Bifidobacterium pullorum
Bifidobacterium angulatum
Bifidobacterium magnum
Bifidobacterium saeculare
Bifidobacterium sp. XV10
Bifidobacterium merycicum
Bifidobacterium sp. 56_9_plus
Bifidobacterium primatium
Bifidobacterium scardovii
Bifidobacterium mongoliense
Bifidobacterium cuniculi
Bifidobacterium animalis
Bifidobacterium breve
Bifidobacterium callitrichidarum
Bifidobacterium catenulatum
Bifidobacterium sp.
Bifidobacterium longum
Bifidobacterium adolescentis
Bifidobacterium bifidum

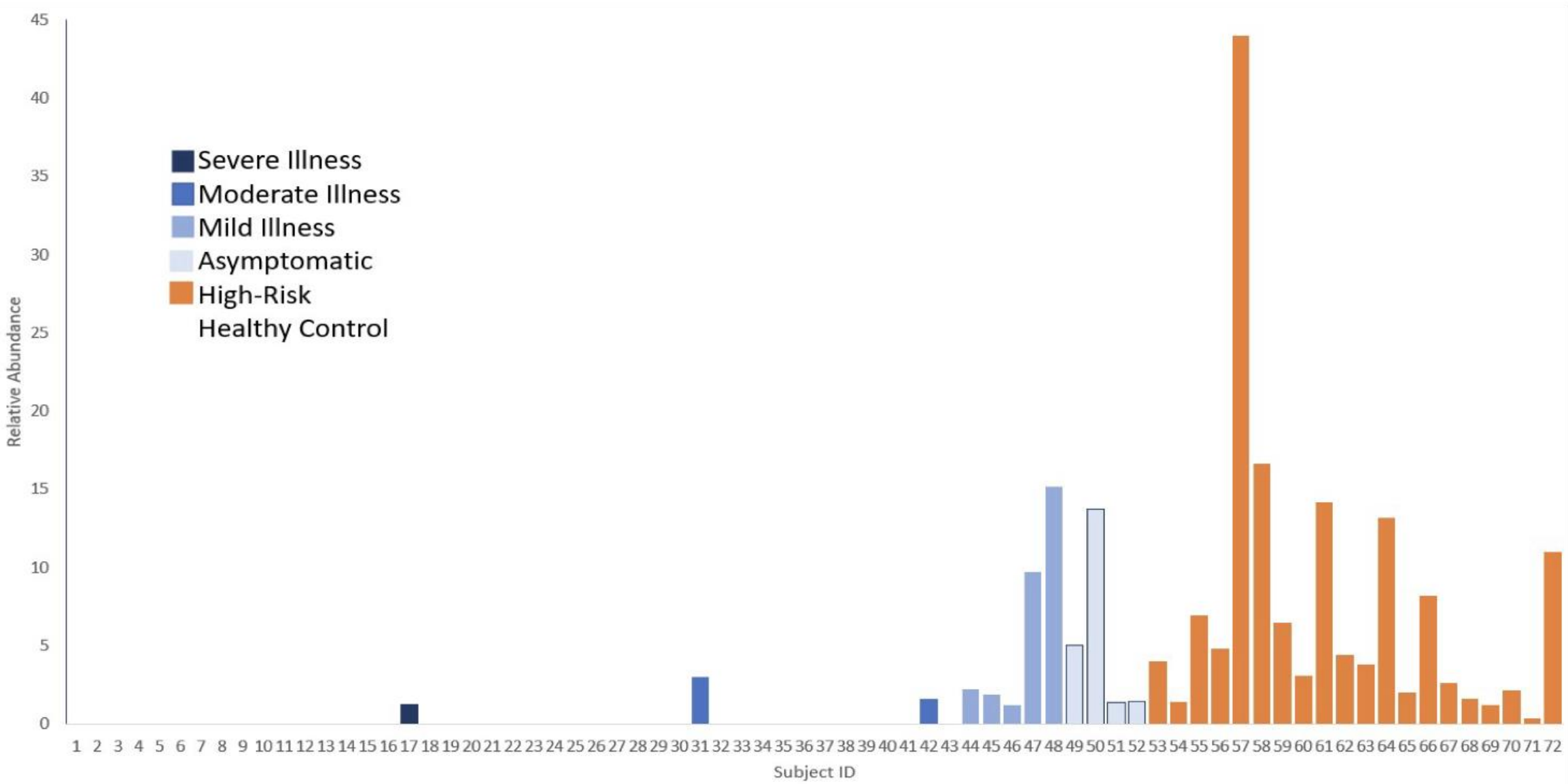
The missing microbes: *Bifidobacterium* and *Faecalibacterium* depletion and loss of microbiome diversity as potential susceptibility markers for SARS-CoV-2 infection and severity

Sabine Hazan, Neil Stollman, Huseyin Bozkurt, Sonya Dave, Andreas J. Papoutsis, Jordan Daniels, Sibasish Dolai, Bradley D. Barrows, Eamonn MM Quigley, Thomas J. Borody

doi: https://doi.org/10.1101/2021.09.02.21262832

Abstract

Posted September 06, 2021.


Severe Illness
Moderate Illness
Mild Illness
Asymptomatic
High-Risk
Healthy Control
Relative Abundance
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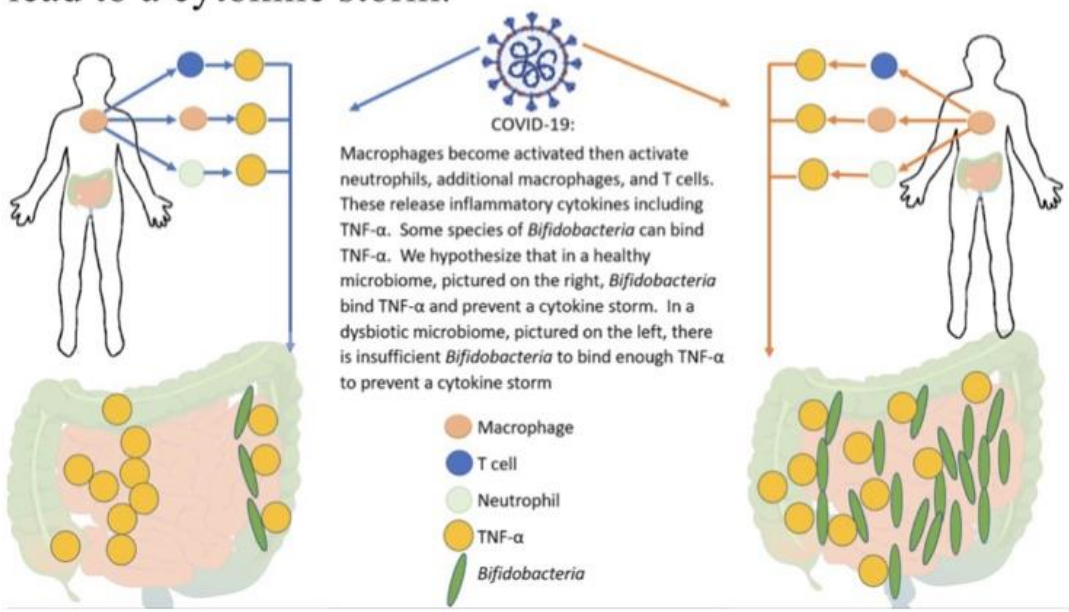
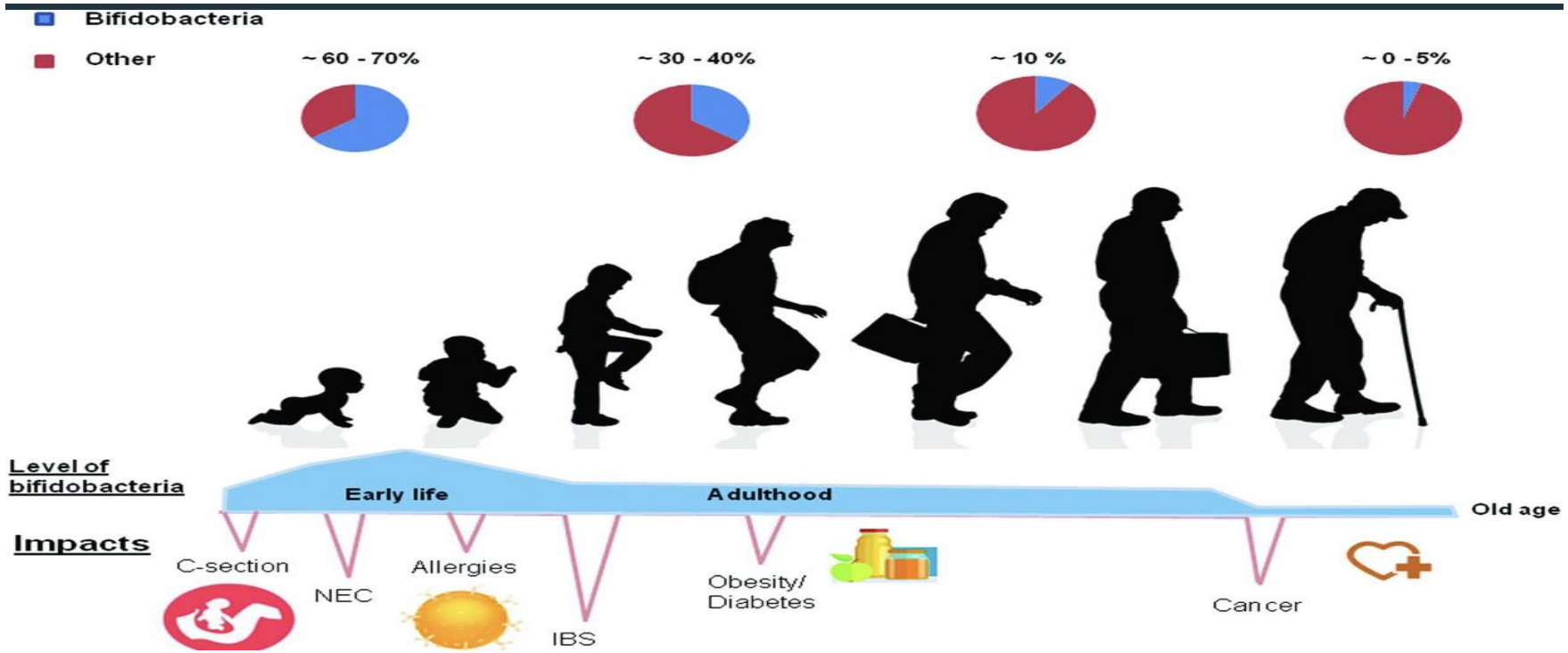
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Subject ID

Figure 5 Proposed mechanism for cytokine storm and immune hyper-response in SARS-CoV-2 positive patients. In individuals infected with SARS-CoV-2, the macrophages become activated; these in turn activate T-cells, additional macrophages, and neutrophils – all of which release cytokines, including TNF-α. *Bifidobacteria,* when present in sufficient numbers, can bind to TNF-α and prevent the subsequent cytokine storm. Therefore, patients with *bifidobacterial* dysbiosis lack this line of defense which may lead to a cytokine storm.



Process of aging



Biodegradation of microplastic by probiotic bifidobacterium

Hüseyin Sancar Bozkurt, Hülya Civelek Yörüklü, Kutsal Bozkurt, Cenk Denktaş, Altan Bozdoğan, Orhan Özdemir and Bestami Özkaya

Published Online: April 19, 2022 • pp 429-443 • https://doi.org/10.1504/IJGW.2022.122435

Loss of Bifidobacteria in Lyme disease: Cause or Effect

Abstract
E0258
(S551)

Sabine Hazan[1], Sonya Davé[2], Amelia Goudzwaard[3], Brad Barrows[1], Thomas J. Borody[4]

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Introduction

- Lyme disease affects nearly 476,000 individuals each year, with annual healthcare costs exceeding 1.3 billion dollars (1).
- Lyme disease is thought to be caused by microbes in the Spirochetes phylum, transmitted by black-legged ticks.
- Nearly 1 in 5 patients with Lyme disease experience GI symptoms including nausea, anorexia, abdominal pain, and diarrhea (2).
- *Bifidobacterium* are known for their beneficial probiotic actions in the human gut microbiome, with reductions implicated in several chronic disease states (3-5).
- The purpose of this study was to investigate the presence of *Bifidobacterium* in fecal samples of patients with Lyme disease compared to healthy controls.

Methods

Fecal samples were assessed for Relative Abundance of genus *Bifidobacterium* in healthy control subjects without Lyme disease (n = 20) compared to patients with Lyme disease (n = 39). The average symptom duration in patients with Lyme disease was 5 years (range of 1 month-20 years). No patients were on antibiotics 2 weeks prior to sample collection, although all treated initially with antibiotics. This study was IRB approved. Metagenomics Next Generation sequencing was performed on fecal samples, where DNA samples were extracted and normalized for library downstream analysis using Shotgun Methodology. The Mann-Whitney test was used to compare *Bifidobacterium* Relative Abundance levels between study groups.

Results

Study Group	Relative Abundance of *Bifidobacterium* (Median, IQR)
Control (n = 20)	4.175 % (1.72-10.27%)
Lyme disease (n = 39)	0.0014% (0.00-0.96%)

Table 1. Relative Abundance of *Bifidobacterium* between study groups.
- $p < 0.001$ for comparing Relative Abundance in Lyme disease patients vs. controls.

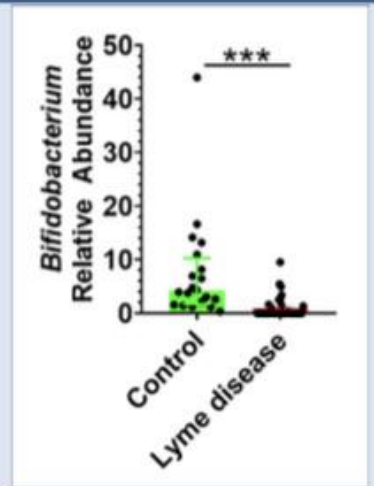

Figure 1. Patients with Lyme disease have significantly reduced Relative Abundance of genus *Bifidobacterium*.

Discussion

- This study is the first to demonstrate low levels of *Bifidobacterium* in patients with chronic Lyme disease.
- Relative Abundance of *Bifidobacterium* was significantly decreased in patients with Lyme disease compared to healthy controls.
- 9/39 (23%) of patient stool samples possessed < 1% Relative Abundance of *Bifidobacterium*.
- Only 1 Lyme patient showed presence of Spirochetes.
- Results suggest further examination into the mechanisms underlying *Bifidobacterium* loss, and the potential for therapeutic restoration of *Bifidobacterium* in patients with Lyme disease.

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5. Yao S, Zhao Z, Wang W, Liu X. *J Immunol Res*. 2021;2021:8030297.

LEVELS OF BIFIDOBACTERIA in LYME

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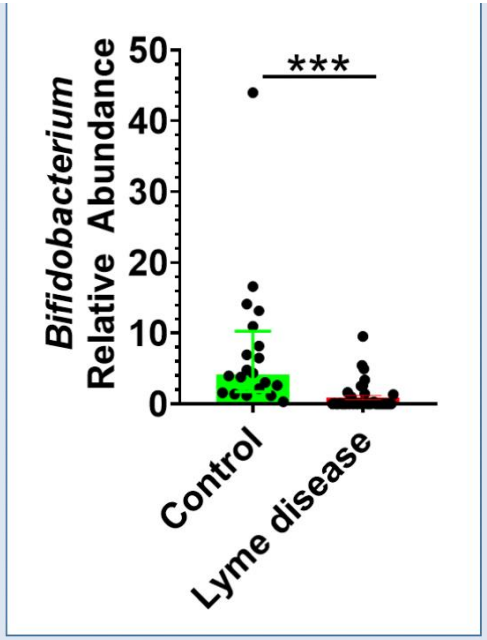

Figure 1. Patients with Lyme disease have significantly reduced Relative Abundance of genus *Bifidobacterium*.

Importance of Bifidobacteria in Crohn's Disease

Sabine Hazan[1], Sonya Davé[2], Brad Barrows[1], Thomas J. Borody[3]

[1]ProgenaBiome, LLC, Ventura, CA, USA, [2]Microbiome Research Foundation, Ventura, CA, USA, [3]Centre for Digestive Diseases, Five Dock, NSW, AUS

Abstract
E0392
(S1010)

Introduction

- Crohn's disease affects approximately 180/10,000 individuals, with a healthcare burden in the billions of dollars (1, 2).
- Etiology of Crohn's disease is believed to involve disruption to the microbiome, immune system processes, and genetic predisposition.
- Fecal transplant and intracolonic administration of certain *Bifidobacterium* species may resolve symptoms and improved mucosal healing (3, 4).
- The purpose of this study was to investigate the presence of genus *Bifidobacterium* in patients with Crohn's disease virgin to treatment, patients with Crohn's disease in treatment, and healthy controls.

Methods

We determined the Relative Abundance of *Bifidobacterium* in two groups of patients with Crohn's disease: treated asymptomatic vs untreated symptomatic, compared to healthy controls. Medications included Humira, Stelara, Remicade, Methotrexate, Enteragam, prednisone, low dose naltrexone, Entevio. No patients were on probiotics prior to stool collection. This study was IRB approved. Metagenomic Next Generation Sequencing was performed on fecal samples, where DNA samples were extracted and normalized for library downstream analysis using Shotgun Methodology. The Kruskal Wallis test was used to compare *Bifidobacterium* Relative Abundance levels between study groups.

Results

Study Group	Relative Abundance of *Bifidobacterium* (Median, IQR)
Control (n=20)	4.18%, 1.72-10.27%
Untreated symptomatic (n =12)	0.05%, 0.00-0.46%
Treated asymptomatic (n=15)	2.35%, 1.16-6.53%

Table 1. Relative Abundance of *Bifidobacterium* between groups
- $p < 0.0001$ for comparing Relative Abundance in untreated symptomatic patients vs. controls.
- $p = 0.0006$ for comparing Relative Abundance in untreated symptomatic vs. treated asymptomatic patients.
- $p > 0.999$ for comparing Relative Abundance in in treated asymptomatic patients vs. controls.

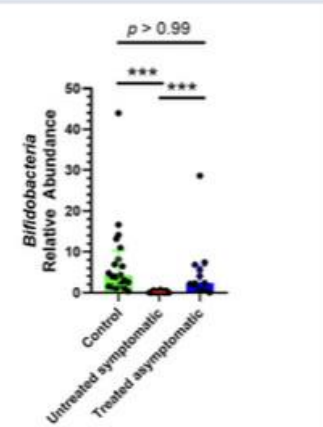

Figure 1. Genus *Bifidobacterium* decreases in abundance in untreated symptomatic Crohn's patients, but is restored in treated asymptomatic patients, compared with controls.

Discussion

- This study is the first to explore the role of monitoring the Relative Abundance of *Bifidobacterium* in assessing treatment success in patients with Crohn's Disease.
- Relative Abundance levels of *Bifidobacterium* were significantly decreased in untreated symptomatic patients vs. health controls, and in untreated symptomatic vs, treated asymptomatic patients.
- Results suggest hope for therapies that predominantly focus on implantation of *Bifidobacterium* or whole stool for Crohn's disease therapy.

References
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4. Paramsothy S, Kamm MA, Kaakoush NO, et al. Lancet. 2017;389(10075):1218-1228.

Bifidobacteria in Crohn’s disease

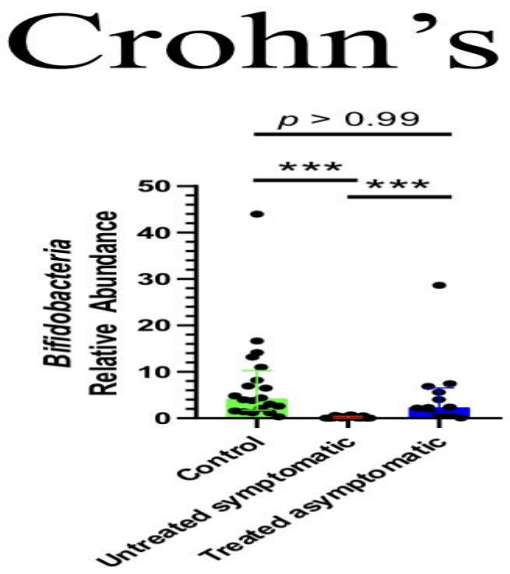

Figure 1: Genus *Bifidobacteria* decreases in abundance in untreated symptomatic Crohn’s patients, but is restored in treated asymptomatic patients, compared with controls.

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Frontiers in Microbiology Sections Articles Research Topics Editorial Board About jc

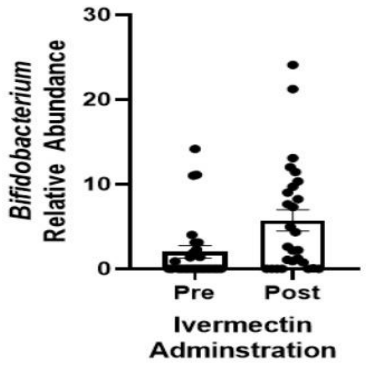
HYPOTHESIS AND THEORY article
Front. Microbiol., 11 July 2022
Sec. Infectious Agents and Disease
https://doi.org/10.3389/fmicb.2022.952321

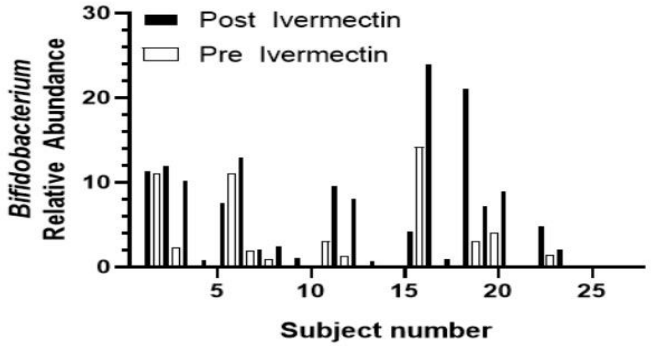
Microbiome-Based Hypothesis on Ivermectin's Mechanism in COVID-19: Ivermectin Feeds Bifidobacteria to Boost Immunity

Sabine Hazan*

Progenabiome, LLC, Ventura, CA, United States

Slide pre and post IVM





Wilcoxon matched-pairs signed rank test	
P value	<0.0001

Messenger RNA SARS-CoV-2 Vaccines Affect the Gut Microbiome

Sabine Hazan[1], Sonya Davé[2], Thomas J. Borody[3]

[1]ProgenaBiome, LLC, Ventura, CA, USA, [2]Microbiome Research Foundation, Ventura, CA, USA, [3]Centre for Digestive Diseases, Five Dock, NSW, AUS

Abstract
E0098
(S227)

Introduction

- Diverse populations of gut bacteria mediate several beneficial effects to health.
- Low *Bifidobacterium* levels have been linked with severe SARS-CoV-2 infection, inflammatory bowel disease, C. *difficile* infection, obesity, and aging.
- Differences in gut microbiome composition can affect immunity to vaccination, yet the effect of mRNA vaccines, for preventing SARS-CoV-2 infection, on the human gut microbiome is largely unknown.
- The purpose of the study was to examine changes in *Bifidobacterium* levels in fecal samples after mRNA SARS-CoV-2 vaccination.

Methods

Fecal matter samples were collected from 34 individuals before and after being vaccinated with Sars-CoV-2 mRNA vaccine; namely BNT162b2 mRNA (Pfizer-BioNTech) or mRNA-1273 (Moderna).

Metagenomic Next Generation Sequencing was performed on fecal samples, where DNA samples were extracted and normalized for library downstream fabrication using Shotgun methodology. The DNA sequences acquired were compared for bacterial species present before and after receiving the vaccine using One Codex database. The Wilcoxon Signed Rank test was used to compare changes in *Bifidobacterium* Relative Abundance over time.

Discussion

- *Bifidobacterium* levels were significantly reduced after receiving mRNA vaccination for SARS-CoV-2.
- This drop in *Bifidobacterium* levels may contribute to observed SARS-CoV-2 infection post vaccination.
- Future studies are needed in order to characterize how *Bifidobacterium* presence in the gut may change over time after SARS-CoV-2 vaccination, the impact on human health, and if these changes occur similarly post-vaccination for other diseases.

Results

Parameter	Value
Total Subjects (N) Males Females	34 15/34 (44.11%) 19/34 (55.88%)
Age (yrs) (mean ± SEM)	55.26 ± 2.65
BMI (kg/m^2) (mean ± SEM)	24.54 ± 0.96
Healthy medical history (%)	4/34 (11.76%)
Relative Abundance of *Bifidobacterium* (median, IQR) Pre-vaccination Post-vaccination	 1.13%, 0.0016-2.52%) 0.64% (0.0015-2.48%

Table 1. Subject characteristics and Relative Abundance of *Bifidobacterium*
- p = 0.0065 for comparing Relative Abundance pre- and post-vaccination.

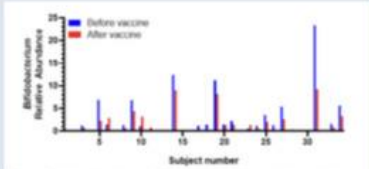


Figure 3. Relative Abundance of *Bifidobacterium* before and after vaccination. *Bifidobacterium* is the only genus whose relative abundances changes with vaccination significantly (p = 0.0065). Blue and red bars indicate median values after and before vaccination, respectively.

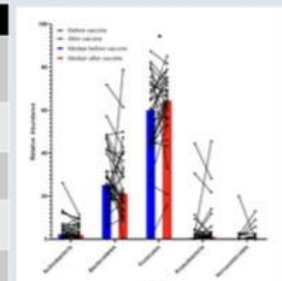


Figure 1. Relative Abundance of various phyla before and after vaccination. Individual points correspond to individual subjects (n=34), and before vs. after vaccination points are connected. Blue and red bars indicate median values before and after vaccine, respectively. * = p < 0.05

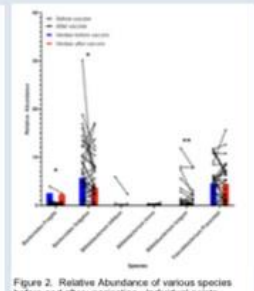


Figure 2. Relative Abundance of various species before and after vaccination. Individual points correspond to individual subjects (n=34), and before vs. after vaccination points are connected. Blue and red bars indicate median values before and after vaccine, respectively. ** = p < 0.01, * = p < 0.05

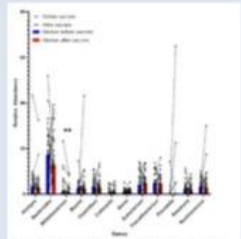


Figure 4. Relative Abundance of various genera before and after vaccination. Individual points correspond to individual subjects (n = 34), and before vs. after vaccination points are connected. Blue and red bars indicate median value before and after vaccine, respectively.
** = p<0.01, * = p<0.05

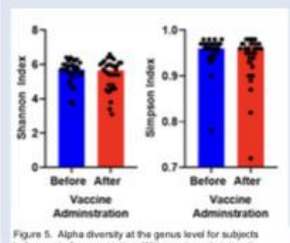


Figure 5. Alpha diversity at the genus level for subjects before and after vaccination. With vaccine administration, there was no significant change in A. Shannon index (p = 0.5441) or B. Simpson index (p = 0.0769).

Persistent Damage to the Gut Microbiome following Messenger RNA SARS-CoV-2 Vaccine

Sabine Hazan[1], Sonya Davé[2], Thomas J. Borody[3]

[1]ProgenaBiome, LLC, Ventura, CA, USA, [2]Microbiome Research Foundation, Ventura, CA, USA, [3]Centre for Digestive Diseases, Five Dock, NSW, AUS

Abstract
E0141
(S2108)

Introduction

- The human gut microbiome is an essential determinant of human health.
- *Bifidobacterium* decline is associated with inflammatory bowel disease, obesity, neurological disorders, *C. difficile* infection and severe COVID-19 (1-3).
- Long-term effect of messenger RNA vaccines for SARS-CoV-2 on the human gut microbiome is unknown.
- The purpose of this study was to explore longitudinal changes in the Relative Abundance of *Bifidobacterium* after mRNA SARS-CoV-2 vaccination.

Methods

We longitudinally recorded the Relative Abundance of *Bifidobacterium* in four subjects before receiving a mRNA vaccine (Pfizer or Moderna) for SARS-CoV-2, approximately one post-vaccination, as well as 6-9 months post-vaccination. Additional SARS-CoV-2 vaccines were given during that period, totaling 2 to 3 doses. Samples were collected at the time points mentioned. No dietary changes or new medications were introduced throughout the study period. Metagenomic next generation sequencing-based methods were applied to samples obtained from fecal collection. DNA was extracted, and the library prepped, enriched and sequenced on an Illumina Nextseq 550 system. This study was IRB approved.

Results

Subject	Change in Relative Abundance of *Bifidobacterium* (% of pre-vaccine level)	
	1 month post-vaccine	6-9 months post-vaccine
1	38%	15%
2	258%	0%
3	49%	35%
4	90%	60%

Table 1. Change in Relative Abundance of *Bifidobacterium* after SARS-CoV-2 mRNA vaccination.

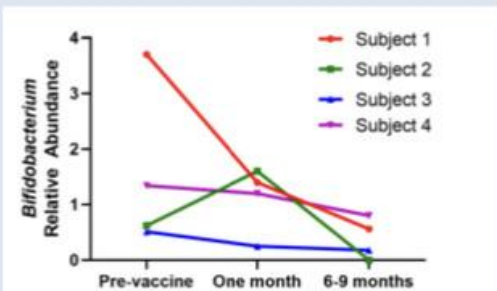


Figure 1. Decline in Relative Abundance of *Bifidobacterium* after SARS-CoV-2 mRNA vaccination.

Discussion

- At 1 month post-vaccination, 3 of 4 subjects experienced a decrease in Relative Abundance of *Bifidobacterium* below pre-vaccination levels.
- At 6-9 months post-vaccination, all subjects experienced a decrease in Relative Abundance of *Bifidobacterium* below pre-vaccination levels.
- No subjects exhibited significant post-vaccine complications.
- The lasting decrease in *Bifidobacterium* levels may contribute to SARS-CoV-2 infection post vaccination.
- Gut dysbiosis after mRNA SARS-CoV-2 vaccination may be a future indication for restoration of *Bifidobacterium* via oral or fecal transplant routes.

References

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LOSS OF *BIFIDOBACTERIUM* IN ADULTS WITH A HISTORY OF INVASIVE CANCER

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3. PROGENABIOME LLC, VENTURA, CA, UNITED STATES.

Introduction

Cancer is a family of diseases characterized by uncontrolled growth and spread of abnormal cells. A contributor to contributes to the pathogenesis of multiple cancers is chronic inflammation (1), and gut dysbiosis is associated with an increased inflammatory state (2). Commensal bacteria, particularly *bifidobacterium*, appear to provide antitumor protection (3) and may be a novel source of cancer therapeutics. However, associations between gut microbiome composition and cancer occurrence are poorly understood. The purpose of this study was to compare the gut microbiome in adults with and without a history of cancer.

Methods

- Participants
  - Control: adults without a history of cancer
  - Aggressive: adults with invasive cancer
  - Non-aggressive: adults with non-invasive cancer.
- Determined relative abundances of *Bacteroides, Faecalibacterium prausnitzii*, and *Bifidobacterium*
  - Metagenomic Next Generation Sequencing performed on participant fecal samples
  - DNA samples extracted and normalized for library downstream analysis using Shotgun Methodology
- Statistics: Kruskal-Wallis tests and Dunn's multiple comparison tests for overall and between-group differences in the relative abundances of each bacterial

Figure 1. Patients with aggressive cancer had lower *Bifidobacterium* abundance

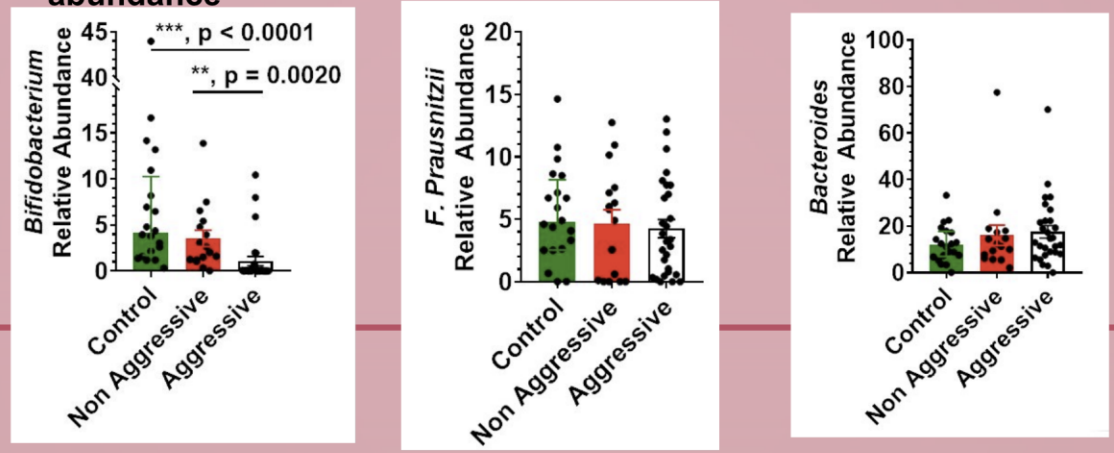

Table 1. Relative Abundance of Bacterial sp.

Study Group	Relative Abundance of *Bifidobacterium* (Median, IQR)	Relative Abundance of *F. prausnitzii* (Median, IQR)	Relative Abundance of *Bacteroides* (Median, IQR)
Control (n = 20)	4.175, 1.723-10.270	4.765, 2.548-8.158	11.780, 6.885-17.760
Aggressive (n = 28)	0.001, 0.000-10.270	3.303, 0.626-7.557	13.820, 8.219-22.250
Non-aggressive (n = 16)	2.338, 1.206-5.268	5.341, 0.026-7.433	11.860, 6.529-17.790

Results

- Sixty-four participants (20 controls, 28 with aggressive cancer, and 16 individuals with non-aggressive cancer)
  - Cancer sites included: appendix, bladder, breast, colon, endometrial, lung, ovarian, prostate, testicular, thyroid, tongue, and uterus, along with lymphomas and unspecified cancer.
- Relative abundance of *Bifidobacterium* was significantly lower in individuals with aggressive cancer
  - vs. controls ($p < 0.0001$)
  - vs. a non-aggressive cancer ($p = 0.0020$).
- Relative abundance of *Bifidobacterium* was similar between controls and individuals with non-aggressive cancer ($p = 0.692$).
- Relative abundances of *Bacteroides* and *F. prausnitzii* were similar between study groups ($p = 0.4535$ and 0.6201, respectively).

Discussion

Adults with a history of invasive cancer appear to have significantly reduced levels of *Bifidobacterium* compared to adults with a history of non-invasive cancer or no history of cancer. These preliminary results suggest an association between the loss of an important anti-inflammatory and antitumor component of the gut microbiome and invasive cancer. Further research is needed to clearly elucidate how the presence or absence of specific microflora may influence cancer pathogenesis and treatment response, as well as the immunotherapeutic potential of *Bifidobacterium*.

References

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