IgG Sequencing Identifies Translocating Gut Microbes that Correlate with Differential Cytokine Response and Reduced Bacterial Growth Rates in Post-Infectious Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome (PI-ME/CFS)

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Background

PI-ME/CFS is a debilitating condition with post-exertional malaise, cognitive impairments, and gastrointestinal distress, typically as a result of previous bacterial or viral infection

When gut bacteria escape the intestinal wall, typically because of gut damage, they can cause disease

Immunoglobulin-G (IgG) is a systemic antibody absent in the gut, but binds to microbes upon their departure

Short Chain Fatty Acids (SCFAs), including butyrate, are produced by microbiota and vital to gut health

Hypothesis: Interrogating microbes that cross the intestinal barrier and carry markers of IgG may reveal potential mechanisms of PI-ME/CFS

Methods

- Participants were identified through the **NIH Intramural ME/CFS Study**, enrolled between 2016 and 2019 [NCT 02669212][1]
- Paired stool, serum, and cerebrospinal fluid samples were collected from N=16 **PI-ME/CFS** participants and N=19 **Healthy Volunteers** (HVs)
- **Magnetic beads** separated bacteria into pools of **IgG-bound** and IgG-unbound bacteria from paired stool and serum for “Autologous” IgG-seq[2]
- Pools underwent **16S rRNA gene** sequencing of the V4 region (IgG-seq)
- Reads were processed with **Nephele** (nephele.niaid.nih.gov)
- **Cytokines** from **plasma** and **cerebrospinal fluid** were obtained from the V-PLEX cytokine panel 1 (human) kit (MesoScale.com)
- Bacterial Growth Rates were estimated from paired metagenomic reads using **Growth Rate InDex tool** (GRiD) to obtain a Peak-to-Trough Ratio[3,4]

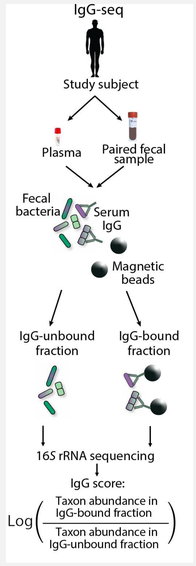


Figure 1. IgG-seq protocol, from Citation 2.

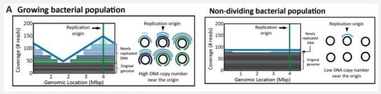


Figure 2. Theory of Growth Rate estimation from metagenomic data, from Citation 3.

Conclusions

PI-ME/CFS is characterized by relatively few taxa that exhibit differential translocation potential

These taxa correlate with both plasma and cerebrospinal fluid cytokines, indicating that translocation of these microbes may initiate a response of the immune system

Preliminary evidence suggests that IgG-targeted microbes have increased metabolic activity in Healthy Volunteers, which was not present in the microbiota from PI-ME/CFS participants

Bacteria Escaping the Gut Barrier Reduce Local **Immune Response** and Have Lower **Growth Rates** in PI-ME/CFS

Gut Microbiota of PI-ME/CFS and Paired IgG-Negative Pools Exhibit **Reduced Diversity**

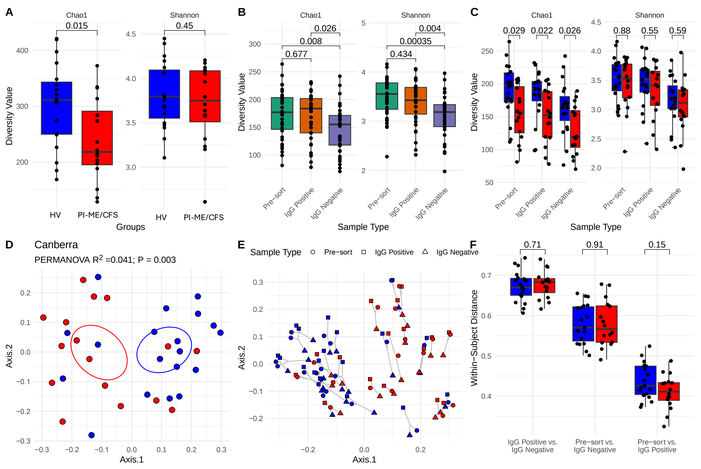
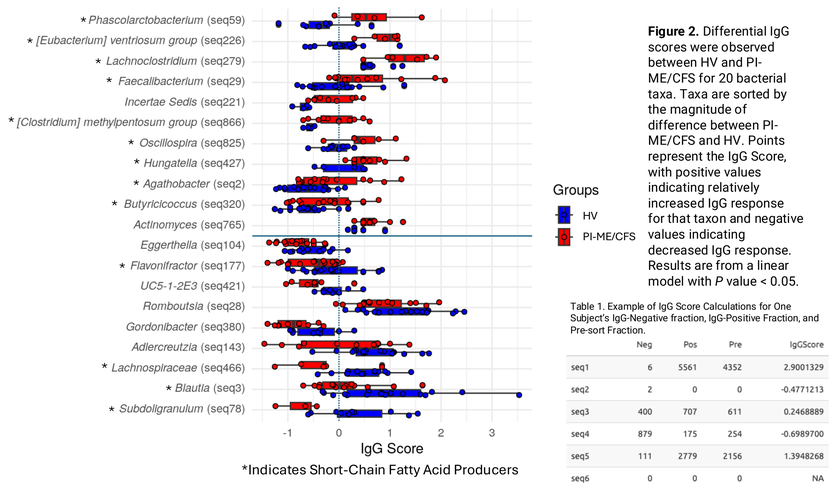


Figure 1. (A) PI-ME/CFS gut microbiota exhibits reduced richness but not diversity. (B) The IgG-negative fraction has reduced richness and diversity compared to the Pre-sort and IgG-Positive fractions (c) This difference occurs in a condition specific manner across all three fractions. (d) Microbiota composition between PI-ME/CFS and HV is distinct. (e) Canberra composition of all three fractions, linking within-subject samples. (f) IgG Negative fractions are compositionally-distinct from Pre-sort and IgG-Positive fractions, with no difference between PI-ME/CFS and HV.

Several Microbiota Members Have Differential **IgG Binding** and Produce SCFAs



*Indicates Short-Chain Fatty Acid Producers

Figure 2. Differential IgG scores were observed between HV and PI-ME/CFS for 20 bacterial taxa. Taxa are sorted by the magnitude of difference between PI-ME/CFS and HV. Points represent the IgG Score, with positive values indicating relatively increased IgG response for that taxon and negative values indicating decreased IgG response. Results are from a linear model with P value < 0.05.

Table 1. Example of IgG Score Calculations for One Subject's IgG-Negative fraction, IgG-Positive Fraction, and Pre-sort Fraction.

	Neg	Pos	Pre	IgGScore
seq1	6	5561	4352	2.9001329
seq2	2	0	0	-0.4771213
seq3	400	707	611	0.2468889
seq4	879	175	254	-0.6989700
seq5	111	2779	2156	1.3948268
seq6	0	0	0	NA

Butyricicoccus spp. Translocation in PI-ME/CFS Negatively Correlates with **Cytokines** in Plasma and CSF

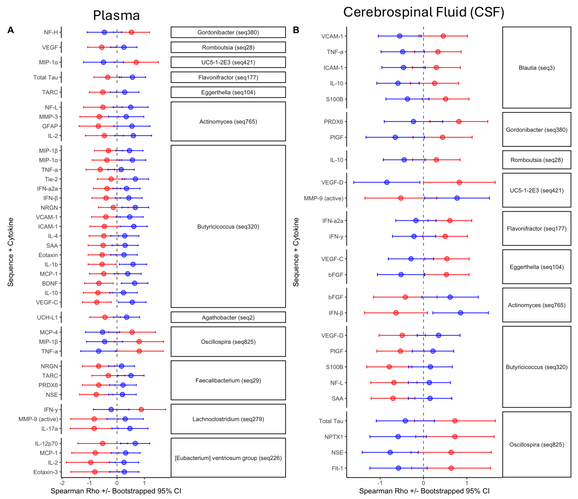


Figure 3. Interaction correlations of **cytokines** with IgG scores in (A) **plasma** and (B) **cerebrospinal fluid**. Correlations were performed within PI-ME/CFS and HV separately using a **non-parametric bootstrap** test. Interactions were identified when there was less than 5% overlap between confidence intervals. Significant taxa-cytokine interactions are **grouped by ASV and sorted by significance** from Figure 2.

Bacteria With Differential Translocation Have Nominally Higher **Metabolic Activity** in Healthy Volunteers

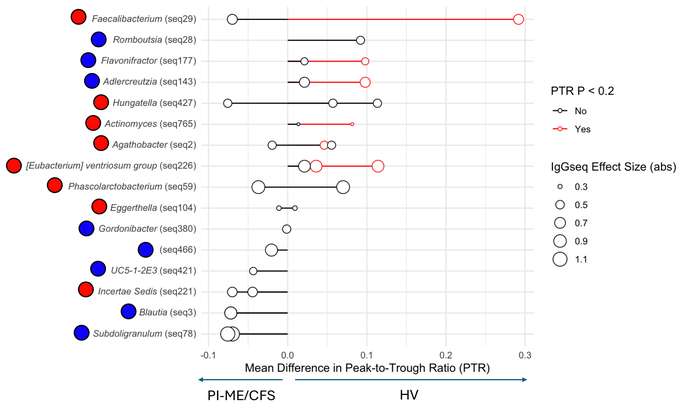


Figure 4. Peak-to-Trough Ratios (PTRs) for taxa with differential translocation are higher in Healthy Volunteers than PI-ME/CFS. Points indicate the **Mean Difference in PTR**, with the IgG effect size (see Figure 2) represented by the size of the circle. A red **outline** indicates that the difference in PTR between groups exhibited modest trends (P < 0.2). PTRs were obtained from **paired metagenomic samples** applied to a curated database of stool genomes. Representative sequences from IgG-seq analysis were mapped to reference genomes to link IgG scores to PTR values. **Circles** to the left of y-axis labels indicate the directionality of IgG-score differences from Figure 2.

Limitations

Amplicon sequencing is limited in its ability to provide species specificity or information about the conserved functions of microbiota with the ability to translocate

In “Autologous” IgG-seq, performed here in which the stool sample and serum sample are derived from the same subject, no bacterial counts in both pools results in a missing value for IgG-seq scores for that taxon and limited techniques are available to recover meaningful values for those taxa.

Confirmation of these findings is difficult – we performed correlative confirmation by employing paired cytokine data and bacterial growth estimates, each with their own limitations

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ktmbiome-niaid.github.io/IgGSeq-in-PI-MECFS

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