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**Title**: Fecal Microbiota Transplant Improves Cognition in Hepatic Encephalopathy and its Effect Varies by Donor and Recipient

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**Summary**

**Background and Aims**: Early data suggests fecal microbiota transplant (FMT) may treat hepatic encephalopathy (HE). Optimal FMT donor and recipient characteristics are unknown. We assessed the safety and efficacy of FMT in patients with prior overt HE, comparing 5 FMT donors. **Methods**: We performed an open-label study of FMT capsules, administered 5 times over 3 weeks. Primary outcomes were change in Psychometric HE Score (PHES) and serious adverse events. Serial stool samples underwent shallow shotgun metagenomic sequencing. **Results**: Ten patients completed FMT administration and 6-month follow-up. MELD score did not change after FMT (14 vs. 14, $P$=0.51). Thirteen minor adverse events and 3 serious adverse events (2 unrelated to FMT) were reported. One SAE was extended-spectrum beta-lactamase *E. coli* bacteremia. PHES improved after 3 doses of FMT (+2.1, $P$<0.05), after 5 doses of FMT (+2.9, $P$=0.007), and 4 weeks after the 5$^{th}$ dose of FMT (+3.1, $P$=0.02). Mean change in PHES ranged from -1 to +6 by donor. Two taxa were identified by random forest analysis and confirmed by linear regression to predict PHES: *Bifidobacterium adolescentis* (adjusted $R^2$ = 0.27) and *Bifidobacterium angulatum* (adjusted $R^2$ = 0.25), both short-chain fatty acid [SCFA] producers. Patients who responded to FMT had higher levels of *Bifidobacterium*, as well as other known beneficial taxa, at baseline and throughout the study. The FMT donor with poorest cognitive outcomes in recipients had the lowest fecal SCFA levels. **Conclusions**: FMT capsules improved cognition in HE, with an effect varying by donor and recipient factors (NCT03420482).

**Keywords**: Fecal microbiota transplant, microbiome, hepatic encephalopathy, cirrhosis

**Background**

Hepatic encephalopathy (HE) is a common complication of cirrhosis characterized by neuropsychiatric and motor dysfunction. HE leads to poor quality of life and increased mortality.(1-3) Currently available HE treatments have limited efficacy and carry risk of diarrhea, dehydration and patient discomfort.(4) More effective and better tolerated therapies are needed to prevent overt HE episodes and treat subclinical HE that persists after overt episodes.

Growing evidence links the gut microbiome to HE pathogenesis.(5) Microbiome-targeted therapies could treat HE by influencing host-microbiome metabolism (including ammonia generation), improving intestinal barrier function, and decreasing systemic immune activation.(6)

Fecal microbiota transplant (FMT) is the transfer of processed stool from a healthy donor to a recipient, with well-documented efficacy for the treatment of recurrent *C. difficile* infection.(7) Two randomized controlled trials have confirmed the safety of FMT enema and oral FMT capsules in patients with recurrent HE.(8, 9) A single dose of FMT capsules from one donor improved cognitive function on one psychometric test but not another, and did not change the fecal microbiome. Patients with cirrhosis require 2-3 times more oral FMT capsules than noncirrhotic patients to treat recurrent *C. difficile* infection, so may also require additional FMT to overcome resident microbial dysbiosis and treat HE.(10) The ideal FMT donor and number of doses to treat HE remains unknown.

We conducted an open-label trial to assess the safety and efficacy of multiple doses of oral FMT capsules to improve cognitive function in patients with a history of overt HE and compared the efficacy of different FMT donors. Secondarily, we aimed to identify recipient microbiome and metabolic features that predicted cognitive improvement with FMT.

## Methods

*Study Patients*

Eligible patients were at least 18 years old, carried a diagnosis of cirrhosis, had at least one prior episode of overt HE, were taking both lactulose and rifaximin at least daily, and were not recently on additional antibiotics or consuming alcohol. Outpatients were enrolled from a single academic center. Only patients with ongoing neurocognitive dysfunction were enrolled, defined as Psychometric Hepatic Encephalopathy Score (PHES) of less than 0. In November 2019, after a serious adverse event (SAE) related to FMT, the following exclusion criteria were added: MELD > 17, history of low-protein ascites, and history of spontaneous bacterial peritonitis.(11) Complete inclusion and exclusion criteria are detailed in **Supplementary Table 1**.

*Study Design and Procedures*

The study protocol was approved by the local institutional review board and an Investigational New Drug application was filed with the FDA (IND 17895). Ten patients were enrolled in this open-label pilot study of FMT. Once enrolled, patients received 15 oral FMT capsules on days 1, 2, 7, 14, and 21 (**Figure 1**). The dosing schedule was based on the authors' prior study showing that patients with cirrhosis often require 4-6 doses of FMT capsules to achieve successful *C. difficile* treatment.(12) Pre-treatment antibiotics were not used to avoid confounding.

FMT donors were healthy adults with normal body mass index, selected through a previously published, rigorous screening process.(13, 14) FMT capsules were generated using established protocols, approved by the local institutional review board and the FDA.(14) Donated fecal matter was blenderized, sieved, centrifuged, suspended in sterile saline with 40% glycerol, and double encapsulated with acid-resistant capsule. On average, 15 capsules contained 24g of fecal matter. Processing was performed under ambient air, and capsules were stored in –80 °C until use. The plan was for 5 donors to provide stool for FMT for 2 patients each. Patients received FMT derived from one donor. However, due to FMT capsule availability, one donor supplied FMT to 3 patients and another donor supplied to 1 patient.

*Efficacy and Safety Assessments*

Safety was assessed at 8 time points until 6 months after FMT, and cognitive function 4 times over the study period. Stool and serum were obtained for sequencing, inflammatory markers, and metabolomic analysis at 4 time points (**Figure 1**).

Clinical efficacy was primarily assessed by change in PHES. The PHES is a validated assessment tool specifically designed for HE trials to test cognitive and psychomotor processing speed and visuomotor coordination (copyright by Hanover Medical School).(15-17) Prior work has demonstrated no learning effect, or improving scores, when tests are 14 days apart in patients with cirrhosis and a history of overt HE.(18) Any potential learning effect was mitigated by using 4 different PHES versions. A secondary efficacy outcome was assessed by change in the EncephalApp Stroop Test, also validated in HE.(18) SF-36 was performed to assess quality of life.

Adverse events were recorded and graded based on the Common Terminology Criteria for Adverse Events (CTCAE) V.4.03. The definition of a SAE is outlined in the Code of Federal Regulations Title 21 (312.32).

*Statistical Analysis*

Efficacy data was analyzed by intention-to-treat. The primary outcome was change in PHES from day 1 to one week after the last day of FMT. Secondary outcomes included the number of adverse events and change in Stroop Test results, SF-36, venous ammonia level, and microbiome features.

We planned to perform a paired t-test to compare PHES scores if the data was normally distributed, and a Wilcoxon Rank Sum test if not normally distributed. This testing strategy was also used for the continuous secondary outcome variables.

Post-hoc we categorized patients as responders vs. non-responders. Responders had an improvement in PHES score from day 1 to one week after the last FMT and did not have an episode of overt HE in 6 months of follow up.

During the COVID-19 pandemic, most study visits were converted to virtual video visits. Due to the remote nature of those study visits, two patients could not provide serum samples for inflammatory biomarker analysis or Stroop Test results at some time points.

All authors had access to the study data and reviewed and approved the final manuscript.

*Stool Analysis*

Fresh stool was collected at 4 time points and kept at 4°C for < 24 hours before being stored at -80°C. All samples were analyzed in a single batch at the completion of the study. For full microbiome analysis details see **Supplementary File**. Samples were analyzed using the SHOGUN pipeline.(19) Every input sequence was compared to every reference sequence in Diversigen's Venti database using fully gapped alignment with BURST. Statistical analyses of microbiome data were performed in R (R Core Team, 2017). The HMP package(20) was used to determine group mean relative abundance values by fitting the sample relative abundances to a Dirichlet-

multinomial distribution using a maximum likelihood method. Alpha diversity was calculated as the Shannon index.(21) Beta diversity was calculated using the Bray-Curtis dissimilarity index and mapped onto two-dimensional space using multidimensional scaling.(20) Feature selection was performed with the R package Boruta.(22) Linear regression using the R stats package was used to determine significant associations of taxa identified as important with PHES. Antimicrobial resistance genes in the data set were identified by alignment of the FASTQ files to MEGARes 2.0.(23)

*Inflammatory Biomarkers*

Cytokine profiling of serum samples were performed on a Luminex 12-plex plate.

**Results**

Of 132 patients screened, 10 patients with cirrhosis and a history of overt HE were enrolled between May 2018 and May 2020 (**Figure 2**). All 10 patients received 5 doses of 15 FMT capsules and completed study activities through 6 months of follow up. Median age was 61 years (range 53 – 72), 6 (60%) were male, 4 (40%) had alcohol-associated cirrhosis, 3 (30%) had non-alcoholic steatohepatitis cirrhosis, and 4 (40%) had undergone transjugular intrahepatic portosystemic shunt (TIPS) (**Table 1**). Median MELD at screening was 14 (range 9 – 18).

*Safety*

Mean MELD score did not change from baseline to after the 3$^{rd}$ dose of FMT (14 vs. 14, $P$=0.34), after the 5$^{th}$ dose of FMT (14 vs. 14, $P$=0.51), and 4 weeks after the 5$^{th}$ dose of FMT (14 vs. 14, $P$=1.0, **Supplementary Figure 1**).

Thirteen minor adverse events were reported by patients (**Supplementary Table 2**), including nausea, bloating, fatigue, and constipation. Four were judged as possibly related to FMT.

Three SAEs occurred during the study. One occurred prior to the administration of FMT. One SAE was transmission of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* bacteremia through FMT, documented in detail in a prior report.(11) The bacteremia was diagnosed 17 days after the patient's 5$^{th}$ dose of FMT. The patient was treated with piperacillin–

tazobactam and then 14 days of meropenem when organism sensitivities were known. His clinical condition remained stable after discharge. A follow-up stool sample was negative for ESBL-producing organisms. The third serious adverse event occurred 12 weeks after the final dose of FMT. The patient was admitted after missing at least one dose of lactulose with fatigue, slurred speech, and was found to have a urinary tract infection, electrolyte abnormalities, and acute kidney injury. She was diagnosed with probable precipitated overt HE, which was deemed unrelated to FMT.

*Cognitive Changes with Fecal Microbiota Transplant*

Compared to baseline, PHES improved after 3 doses of FMT (+2.1, $P<0.05$), after 5 doses of FMT (+2.9, $P=0.007$), and 4 weeks after the 5$^{th}$ dose of FMT (+3.1, $P=0.02$; **Figure 3A**). For reference, improving from 33 seconds to 15 seconds on the number-connection test can improve the PHES by 1 point. Mean change in PHES ranged from -1 to +6 by donor. The mean improvement in PHES did not vary by history of TIPS (TIPS: +2.5 vs. no TIPS: +3.2, $P=0.72$; **Figure 3B**). Raw scores of 3/5 PHES sub-tests improved after 5 doses of FMT (**Supplementary Figure 2**).

Compared to baseline, Stroop test results did not improve after 3 doses of FMT (14.5 seconds improved, $P=0.40$), but trended towards improvement after 5 doses of FMT (34.3 seconds improved, $P=0.06$) and 4 weeks after the 5$^{th}$ dose of FMT (19.1 seconds improved $P=0.05$).

The Physical Component Summary ($P=0.77$) and Mental Component Summary ($P=0.64$) of the SF-36 did not change after 5 doses of FMT.

*Unplanned Antibiotic Administration*

Two patients received unplanned non-rifaximin antibiotics during the study period. Removing patients with non-rifaximin antibiotics from the analysis did not meaningfully change the primary analysis: PHES improved after 5 doses of FMT (+2.6, $P=0.02$).

*Microbiome Changes with Fecal Microbiota Transplant*

There was no significant change in alpha diversity between baseline and subsequent post-FMT days (**Supplementary Figure 3**). In beta-diversity analysis, patients did not clearly remodel towards the donors over time (**Supplementary Figure 4**).

*Taxa that Predict Cognitive Outcomes*

In a random forest analysis, 22 variables were deemed important in predicting PHES (**Figure 4**). Of these, 6 variables were found to be significantly associated with PHES by linear regression (**Table 2**). Two taxa were positively associated with PHES scores, *Bifidobacterium adolescentis* and *Bifidobacterium angulatum*, both SCFA-producers. Two taxa were negatively correlated with PHES scores, *Enterobacter asburiae* and *Bifidobacterium breve*, though the significant association with *Bifidobacterium breve* disappeared when one outlier patient was removed (**Supplementary Figure 5**).

*Comparing Fecal Microbiota Transplant Donors*

FMT donors did not vary by age (24-34 years old) or diet type (all omnivores), but did vary in their impact on recipient cognitive changes, secondary to primary bile acid ratios, and total normalized SCFA levels. Donor D was associated with the worst cognitive outcomes, as well as the lowest secondary to primary bile acid ratio and normalized SCFA level (**Supplementary Table 3**). Donor microbiomes generally shared the same genera, but varied by relative abundance (**Supplementary Figure 6**).

*Comparing Fecal Microbiota Transplant Responders and Non-Responders*

The 7 patients who clinically responded to FMT (improved PHES and no overt HE at 6 months) differed at baseline from the 3 patients who did not clinically respond to FMT. Bacterial families identified *a priori* as beneficial or harmful in HE were compared between FMT responders and non-responders.(8, 24-27) FMT responders appeared to have a higher abundance of beneficial families at baseline and across study time points, while FMT non-responders had a higher abundance of harmful bacterial families (**Figure 5**). *Bifidobacterium* abundance in particular appeared to be higher in responders at baseline compared to non-responders, as well as over the course of the study.

*Antimicrobial Resistance Genes*

Total antimicrobial resistance genes in patients' fecal microbiome decreased from baseline to 4 weeks after the 5th FMT dose, approaching donor levels (**Supplementary Figure 7**). The prevalence of the rpoB gene (resistance to rifampicin) was high in the cohort at baseline – present in 7/10 subjects. One non-responder appeared to obtain the rifampin resistance gene from their donor; whereas two responders appeared to lose rifampin resistance with FMT (**Figure 6**).

*Metabolite Changes with Fecal Microbiota Transplant*

In the entire group, total normalized SCFA levels did not change after 5 doses of FMT ($P = 0.87$; **Supplementary Figure 8**). SCFA levels rose in 4 of 7 responders and fell in 2 of 3 non-responders. Only three of 10 patients developed an increase in secondary to primary bile acid ratios with FMT, and two of those were clinical non-responders (**Supplementary Figure 9**). Compared to baseline, venous ammonia did not change after 5 doses of FMT (73 μmol/L vs. 75 μmol/L, $P$=0.73; **Supplementary Figure 10**).

*Inflammatory Markers with Fecal Microbiota Transplant*

Compared to baseline, serum TNF-alpha ($P$=0.09), IL-6 ($P$=0.55), and IFN-gamma ($P$=0.30) did not change after 5 doses of FMT (**Supplementary Figure 10**).

**Discussion**

Patients with a history of cirrhosis and overt HE developed improved cognitive function after 5 doses of oral FMT capsules given over 3 weeks. The mean improvement in PHES four weeks after the last FMT dose was 3.1 points – a clinically relevant improvement. In addition, only one (10%) patient experienced an overt HE episode in 6 months of follow-up. Similar patients in other studies experience overt HE at 21% in 3 months or 30-50% in 6 months.(8, 9, 28) Both Stroop scores and PHES improved between 3 and 5 doses of FMT, so it is possible that additional doses provide additional clinical benefit. A history of TIPS did not influence response to FMT.

FMT led to mild and brief gastrointestinal side effects in some patients. FMT also led to a serious adverse event in one patient, ESBL-producing *Escherichia coli* bacteremia, the analysis of which has been published previously.(11) This is not the only report of pathogen transmission via FMT,

with recent reports of Shiga toxin-producing *Escherichia coli* transmitted by FMT.(29) Despite these reports of FMT-transmitted infections, a recent systematic review of 4241 patients found FMT to be overall safe, with a very low rate of microbiota-related serious adverse events.(30) Even when investigating patients with cirrhosis specifically, a multicenter study found FMT to be safe, with no infection-related serious adverse events.(31) FMT donor screening practices continue to evolve and incorporate enhanced screening for potential pathogens, including most recently SARS-CoV-2 virus.(32) Synthesizing available data, it appears that FMT is safe in some patients with cirrhosis, but FMT screening practices must be rigorous, and some sub-groups may warrant exclusion such as those with high MELD, low-protein ascites, or a history of spontaneous bacterial peritonitis.

FMT did not lead to wholesale fecal microbiome remodeling; rather, its therapeutic mechanism may have been through subtle or proximal gut changes in microbial composition and function. First, the microbial changes may have occurred in the proximal bowel, and this study sampled only stool. In a prior study of oral FMT capsules to treat HE, FMT did not change bacterial diversity in sigmoid or stool samples, but did lead to composition and function changes in the proximal bowel mucosal microbiome.(9) Second, even in the distal bowel, it is possible that subtle changes in microbial composition and function influenced clinical outcomes. This study was designed in part to compare the efficacy of different FMT donors, and thus introduced heterogeneity which made summary assessment of microbiome changes challenging. It is possible that individual recipients acquired specific donor taxa which influenced cognitive outcomes, without demonstrating significant changes in alpha and beta diversity. Finally, it is possible that co-administration of rifaximin with FMT blunted microbiome remodeling.

While FMT has been highly effective in the treatment of *C. difficile* infection from nearly any healthy donor, clinical trials of FMT for inflammatory bowel disease have suggested a possible donor effect.(33, 34) In our study, cognitive improvement in FMT recipients appeared to vary by donor. Prior trials of FMT for HE have selected donors based on abundance of potentially beneficial taxa.(8, 9) Despite differences in clinical outcomes by donor, microbiome composition was fairly similar between donors. Ideal FMT donor selection for HE may be more related to microbial function than composition. FMT from Donor D led to the worst recipient outcomes, and

notably had the lowest SCFA levels and secondary to primary bile acid ratios. Both SCFAs and bile acids, via different mechanisms, influence intestinal epithelial health and permeability.(35) Further study in larger cohorts should investigate possible FMT donor effects for this condition,(36) and consider differentiating donors by microbiome metabolic activity as opposed to abundance alone.

Increasingly, recipient factors are being recognized as important in FMT success.(37) We found that patients who responded positively to FMT had a more beneficial baseline microbiome profile. In particular, FMT responders had higher baseline *Bifidobacterium* abundance compared to non-responders. Two *Bifidobacterium* significantly predicted cognitive scores. *Bifidobacterium adolescentis* is known to have beneficial qualities for the host, including increasing SCFA production, increasing tight junction protein production, decreasing intestinal permeability, and dampening systemic immune response.(38-40) Less is known of *Bifidobacterium angulatum*, but it has demonstrated SCFA-producing abilities.(41) In alignment with these findings, total stool SCFA content rose in most FMT responders and fell in most non-responders. Notably, venous ammonia levels did not change with FMT, nor were any of the taxa associated with cognitive scores involved in ammonia metabolism. Further study will be required to explore the role of *Bifidobacterium* in facilitating response to FMT, but the mechanism may involve known synergism between *Bifidobacterium* species and other taxa in fermentation and SCFA generation.(41)

Patients with cirrhosis, and especially those using rifaximin, have high prevalence of the rpoB gene, conferring resistance to rifaximin.(42) Our study found that FMT led to a decrease in total antimicrobial resistance genes in patients, nearly to healthy donor levels. Two FMT responders lost rifampicin resistance with FMT. This data supports a prior finding of decreased rifaximin resistance after FMT in cirrhosis.(43) While the numbers are small, these findings raise the possibility that FMT exerts its effect by re-sensitizing the microbiota to conventional rifaximin therapy.

These results must be interpreted within the context of study design. First, there was no control group in this study; therefore, definitive conclusions about efficacy and safety are not possible.

Our study population was restricted by MELD and antibiotic use, thereby limiting the external validity of our results to sicker populations. Future well-powered, placebo-controlled trials will be required for definitive evaluation of efficacy and safety. Second, FMT donors with high SCFA production should be strongly considered for future trials. Third, future trials should consider stratification or selection by recipient microbiome, including *Bifidobacterium* abundance. Fourth, future FMT studies should strive to performed strain-level sequencing to better understand strain engraftment and impact on clinical outcomes. We did not find wholesale microbiome remodeling, but smaller community or strain-level changes may have occurred. Fifth, the impact of TIPS, cirrhosis etiology, and metabolic disorders could not be explored in detail in this study design, but should be investigated in future studies. Finally, this study does not explore the role of rifaximin after FMT, which will be important to investigate in future work especially for patients who lose rifampicin resistance after FMT.

In conclusion, this study suggests that FMT may be effective in treating HE, and likely safe for select patients with intensive pathogen screening. Microbial manipulation with FMT or a defined consortium of beneficial bacteria may be a way to improve quality of life in patients with cirrhosis. This is the first study to explore donor and recipient factors that may lead to HE improvement with FMT; initial findings which will be studied in future work.

**Figure Legends**

Figure 1: Study design. Patients receive 15 oral FMT capsules on 5 days over 3 weeks. Cognitive testing, serum and stool collections occur at 4 time points. Standard of care with lactulose and rifaximin are continued throughout the study.

Figure 2: Subject enrollment flowchart. *For the first 5 subjects, MELD > 17 was excluded. Per protocol, after the first 5 patients, MELD > 20 was excluded. However, after a serious adverse event, MELD > 17 were again excluded.

Figure 3: Illustration of PHES over time. The first timepoint is prior to FMT delivery, the second timepoint is day 14 (1 week after 3 doses of FMT), the third timepoint is day 21 + 1 week (1 week after the 5$^{th}$ FMT dose), and the fourth timepoint is day 21 + 4 weeks (4 weeks after the 5$^{th}$ FMT dose). 3A: PHES over time for all patients and mean change in PHES by donor. 3B: PHES over time by history of transjugular intrahepatic portosystemic shunt (TIPS).

Figure 4: In a random forest analysis, variables were ranked by importance in predicting PHES. Of the important variables, those bolded and starred were additionally found to be significantly associated with PHES by linear regression.

Figure 5: 5A and 5B: Bacterial families identified *a priori* as beneficial or harmful in HE were compared between FMT responders and non-responders. 5C: Bifidobacterium abundance appeared to be higher in responders compared to non-responders.

Figure 6: The presence of rpoB gene (resistance to rifampicin) over time. Each column is a study subject. R denotes fecal microbiota transplant responders; NR denotes non-responders. + denotes presence of rpoB gene in that subject at that timepoint, - denotes absence.

Supplementary Figure 1: Safety labs over time.

Supplementary Figure 2: PHES sub-test scores over time.

Supplementary Figure 3: Shannon diversity over time.

Supplementary Figure 4: Beta diversity as multi-dimensional scaling plots, genus level.

Supplementary Figure 5: Correlation between specific taxa and PHES.

Supplementary Figure 6: Relative bacterial abundance by fecal transplant donor.

Supplementary Figure 7: Total Antimicrobial Resistance Genes Per Individual.

Supplementary Figure 8: Short-chain fatty acid levels over time.

Supplementary Figure 9: Secondary to primary bile acid ratio over time. Each subject is labeled as an FMT responder or non-responder as defined in the text.

Supplementary Figure 10: Inflammatory biomarkers over time. Units: venous ammonia μmol/L; TNF-Alpha, IL-6, and IFN-Gamma pg/mL.

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**TABLE 1: Patient Characteristics at Baseline**

| Characteristics | Values (N = 10) |
|---|---|
| Age, years | 61 (53, 72) |
| Male sex, n (%) | 6 (60%) |
| MELD score | 14 (9, 18) |
| Etiology of cirrhosis, n (%) | |
| Alcohol | 4 (40%) |
| Non-alcoholic steatohepatitis (NASH) | 3 (30%) |
| NASH and alpha-1 antitrypsin deficiency | 1 (10%) |
| Viral | 1 (10%) |
| Cryptogenic | 1 (10%) |
| Body mass index | 30.5 (21, 39) |
| Diabetes diagnosis, n (%) | 7 (70%) |
| Number of patients with OHE episode in prior 6 months, n (%) | 3 (30%) |
| Transjugular intrahepatic portosystemic shunt in place, n (%) | 4 (40%) |
| Presence of ascites, n (%) | 7 (70%) |
| History of hepatocellular carcinoma, n (%) | 1 (10%) |
| Active on liver transplant waitlist, n (%) | 5 (50%) |
| Total bilirubin, mg/dL | 2.0 (1.4, 4.4) |
| Creatinine, mg/dL | 0.8 (0.50, 1.31) |
| International normalized ratio | 1.4 (1.1, 1.9) |

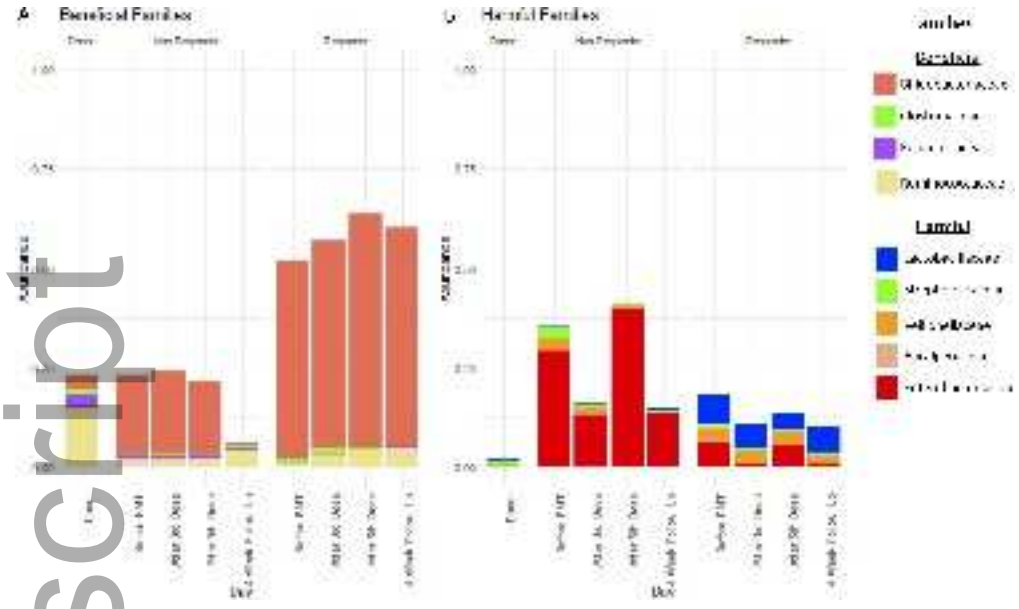
Data are presented as median (range) unless mentioned otherwise. MELD denotes model for end-stage liver disease; NASH non-alcoholic steatohepatitis; OHE overt hepatic encephalopathy.

**TABLE 2: Variables Significantly Associated with PHES**

| Attribute | Mean Importance (Random Forest) | Correlation with PHES (Regression) | Adjusted $R^2$ |
|---|---|---|---|
| Stroop Test | 5.17 | Negative* | N/A |
| Bifidobacterium adolescentis | 3.98 | Positive | 0.27 |
| Bifidobacterium angulatum | 3.41 | Positive | 0.25 |
| Sex | 3.31 | N/A | N/A |
| Enterobacter asburiae | 3.29 | Negative | 0.19 |
| Bifidobacterium breve | 2.97 | Negative | 0.39 |

*Higher Stroop Test results (On + Off Time in seconds) is associated with poorer cognition, whereas the inverse is true of PHES where higher score is associated with better cognition.

PHES denotes psychometric hepatic encephalopathy score



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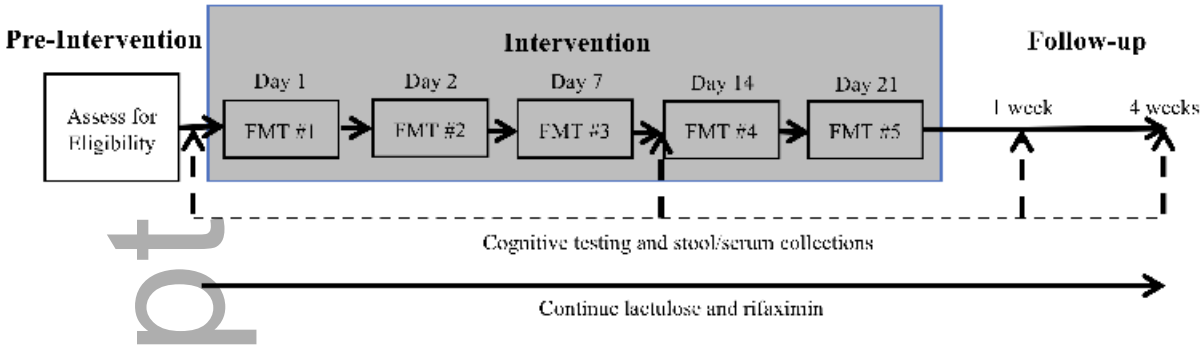


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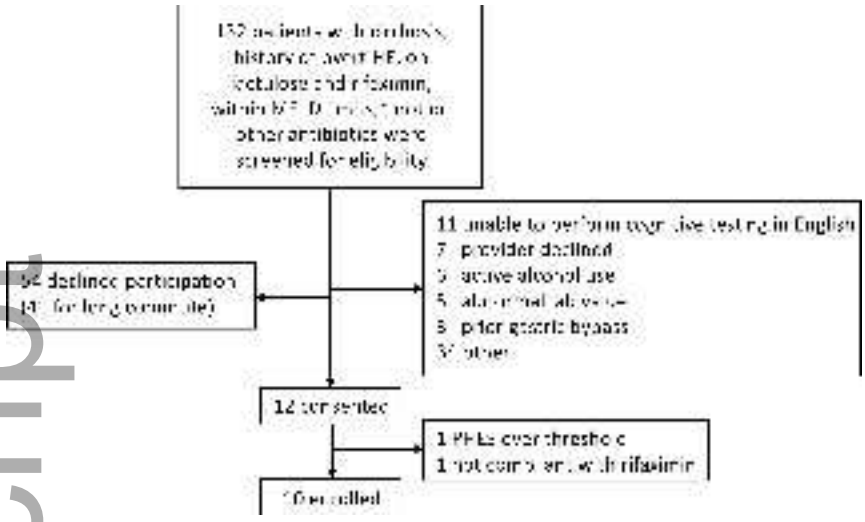


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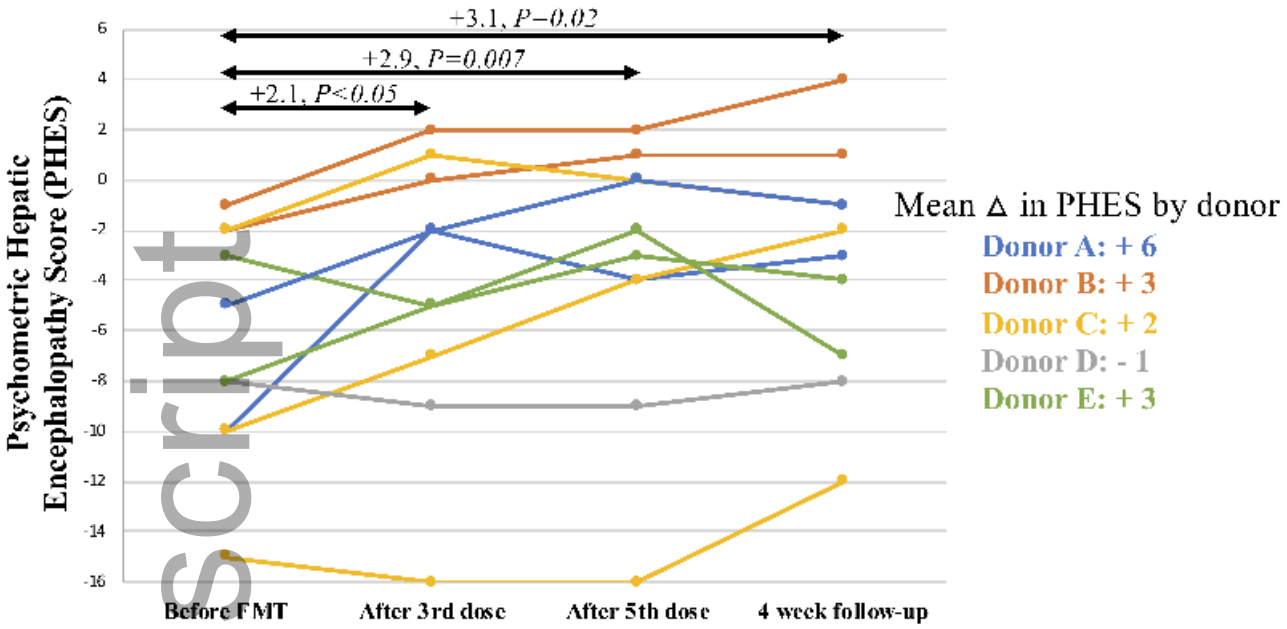


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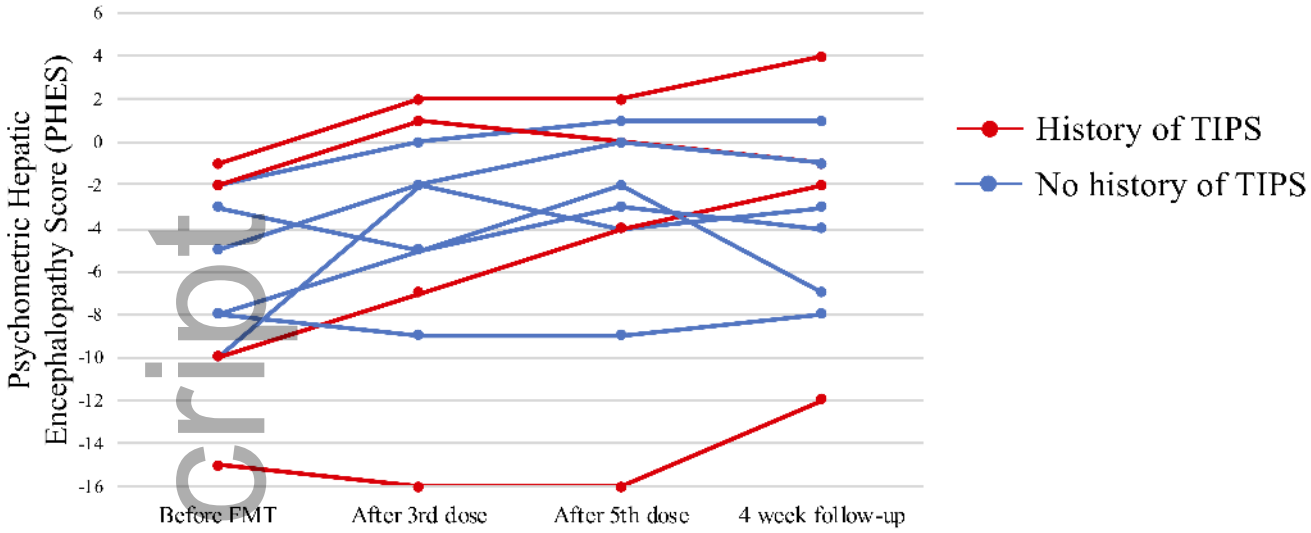


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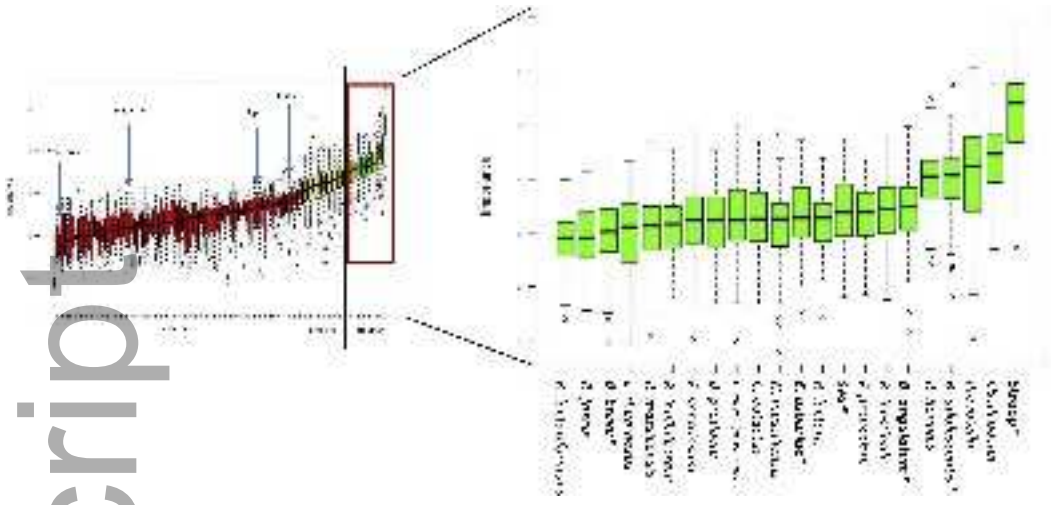

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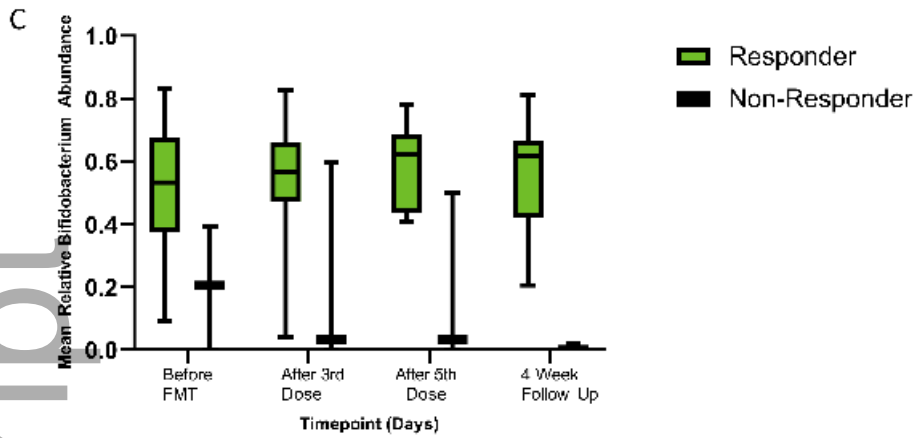


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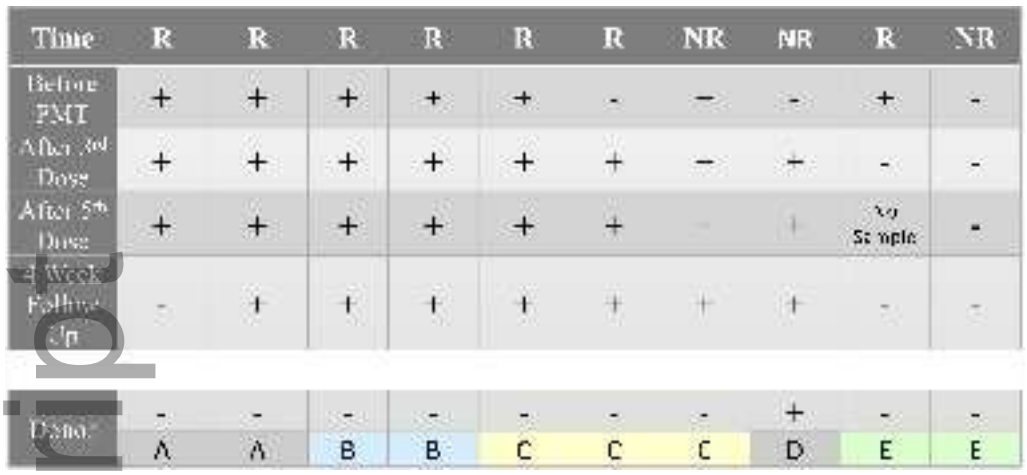


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