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The multi-omic basis for hepatic encephalopathy recurrence: Analysis of the THEMATIC trial

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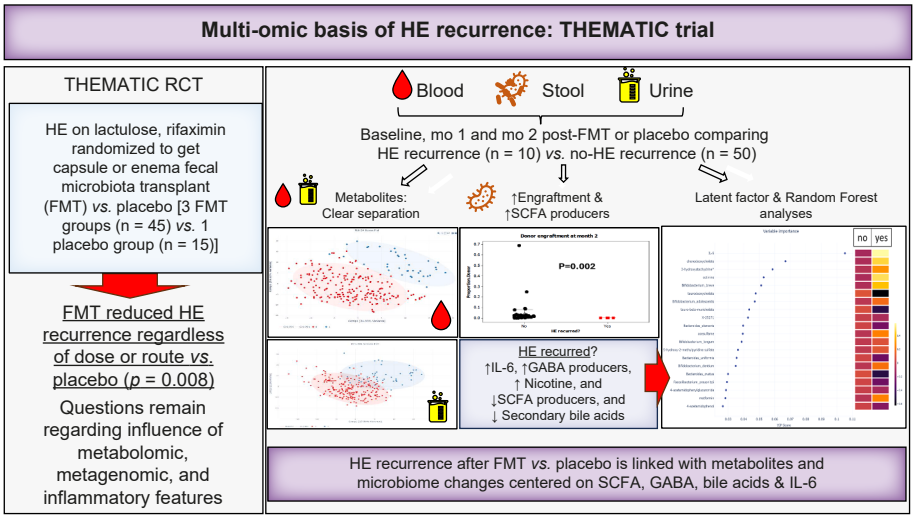
Graphical abstract



Highlights:

- FMT lowered HE recurrence *vs.* placebo in patients with cirrhosis and HE in the THEMATIC trial.
- Multi-omic analyses identified distinct metabolomic, microbiome, and inflammatory signatures predicting HE recurrence.
- HE recurrence was linked to SCFAs, secondary bile acids, dopamine, and GABA metabolites, particularly in serum.
- On RFA and latent factor analysis, lower SCFA producers, secondary bile acids, and acetaminophen, and higher IL-6 and nicotine predicted recurrence.

Impact and implications:

Fecal microbiota transplantation (FMT) reduced hepatic encephalopathy (HE) recurrence in patients receiving lactulose and rifaximin in the THEMATIC trial, but the multi-omic mechanisms underlying this effect were unclear. In this secondary analysis, we found that HE recurrence – regardless of FMT or placebo assignment – was associated with distinct multi-omic signatures, including reduced short-chain fatty acid-producing and increased pathobiont taxa, lower urinary and serum short-chain fatty acids, secondary bile acids, and acetaminophen derivatives, and higher GABA-related and nicotine metabolites, along with elevated IL-6 levels. Notably, patients with greater donor microbiota engraftment had lower rates of HE recurrence. These findings suggest that HE recurrence after FMT reflects a multifactorial process involving alterations in gut metagenomics, systemic metabolomics, inflammation, and donor engraftment.

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The multi-omic basis for hepatic encephalopathy recurrence: Analysis of the THEMATIC trial

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Background & Aims: The THEMATIC trial demonstrated that fecal microbiota transplantation (FMT) reduces recurrence of hepatic encephalopathy (HE) in patients already receiving lactulose and rifaximin. The aim of this analysis was to identify multi-omic predictors of HE recurrence among THEMATIC trial participants.

Methods: The THEMATIC trial enrolled patients with cirrhosis and HE who received oral or enema FMT *vs.* placebo (1–3 administrations) and were followed for 6 months. Outcomes included safety and HE recurrence. Serum, urine, and stool samples were collected at baseline and post-FMT for all participants. Stool metagenomics, serum and urine metabolomics, inflammatory cytokines, and clinical data were analyzed. Differences between patients with and without HE recurrence were assessed using pathway, random forest, and latent factor analyses.

Results: HE recurred in 10 of 60 patients (17%), with significantly higher recurrence in the placebo *vs*. the FMT groups (40% *vs.* 8%; $p = 0.005$). Due to the low recurrence rate in the FMT arms, all patients with recurrence were combined and compared with those without recurrence. Stool metagenomics showed that the abundance of short-chain fatty acid (SCFA) producers (*Faecalibacterium, Eubacterium, Bacteroides, Blautia* spp.) was lower, while that of GABA-producing taxa (*Lactobacillus, Bifidobacterium* spp.) was higher, in patients with recurrence. Urine and serum metabolomes separated HE recurrence groups on PLS-DA, with serum butyrate and isobutyrate being most significantly associated ($p = 0.008$). Pathway analyses revealed upregulation of GABA and neurotransmitter pathways in patients with HE recurrence. Random forest and latent factor analysis indicated that SCFA producers and secondary bile acids were protective, whereas IL-6, GABA producers, nicotine metabolites, and primary bile acids were associated with HE recurrence.

Conclusions: Secondary analysis of the THEMATIC randomized controlled trial indicates that HE recurrence in patients on lactulose and rifaximin is associated with distinct microbiome and metabolomic profiles, particularly involving SCFAs, GABA metabolism, bile acids, and IL-6.

Trial registration: www.clinicaltrials.gov: NCT03796598.

Introduction

Hepatic encephalopathy (HE) results from an altered gut-liver-brain axis and is associated with poor clinical, psychosocial, and financial outcomes.[1] Current therapies for HE predominantly target the gut microbiota and include lactulose and rifaximin.[1,2] However, despite these therapies, a large proportion of patients experience HE recurrence, which worsens cognitive function and is not adequately addressed by transplant priority.[3,4]

Over the last decade, fecal microbiota transplant (FMT) has shown promise for HE in initial phase I trials.[5–7] We recently completed a randomized, placebo-controlled phase IIA FMT clinical trial (THEMATIC) in HE.[8] This recent study showed that FMT using enema or oral capsule routes was safe and potentially effective in reducing HE recurrence in patients with cirrhosis already on lactulose and rifaximin. Notably, most clinical factors, demographics, and cirrhosis-related variables are poor predictors of HE recurrence. In this study we analyzed fecal shotgun metagenomics, urine and serum metabolomics, and circulating inflammatory cytokines from the biospecimens collected during the THEMATIC trial. These -omics data were then integrated using two complimentary methods, Random forest analysis and data integration analysis for biomarker discovery using latent components to identify potentially useful predictors of HE recurrence.

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Patients and methods

Study design

We completed a double-blind, placebo-controlled, randomized clinical trial (RCT) with two modes of administration and up to three FMT doses in cirrhosis patients with HE on lactulose and rifaximin (Fig. S1).

Study population

Our main inclusion criteria were age >21 years, confirmed cirrhosis,[9] on treatment for HE with lactulose and rifaximin for a confirmed grade ≥2 HE episode, and ability to consent, with the remaining criteria outlined in the supplementary methods.

Intervention

After consent and confirmation of eligibility, patients were randomized into four groups. Participants received three administrations (60 ml enema and five capsules at baseline and five more capsules at day 30). Group 1 received all active products at baseline and day 30, Group 2 received placebo enema and active capsules at baseline and day 30, Group 3 received active enema at baseline and placebo capsules at both timepoints, while Group 4 received placebo enema and capsules. Stopping points and safety review thresholds were outlined after FDA consultation (Protocol).

FMT products

Two donors from the University of Minnesota Microbiota Therapeutics Program were used (supplementary methods).[10,11] Individual patients received all FMT products from the same donor and both routes delivered 2.5 x 10^{12} bacteria. Both donors had a higher content of short-chain fatty acid (SCFA)-producing *Lachnospiraceae* and *Ruminococcaceae* relative to the recipients. FMT and placebo capsules were

Table 1. Baseline comparison of those who experienced HE recurrence (key secondary outcome) *vs.* those who did not in the entire cohort.

	Did not recur (n = 50)	Recurred (n = 10)	95% CI
Age (years)	62.8 ± 9.9	64.0 ± 4.5	-3.92, 1.52
Men	44 (88%)	6 (60%)	-0.66, 0.10
White	43 (86%)	8 (80%)	-0.39, 0.27
Charlson comorbidity index	5.6 ± 1.6	6.4 ± 2.0	-2.15, 0.55
Post-traumatic stress disorder	10 (20%)	0 (0%)	-0.37, -0.03
Depression	7 (14%)	3 (30%)	-0.2, 0.52
Active nicotine use	9 (18%)	3 (30%)	-0.24, 0.48
Hepatitis C etiology	4 (8%)	1 (10%)	-0.2, 0.24
Alcohol only etiology	11 (22%)	2 (20%)	-0.31, 0.27
Alcohol + hepatitis C etiology	4 (8%)	1 (10%)	-0.2, 0.24
MASLD etiology	29 (58%)	6 (60%)	-0.33, 0.37
Other etiologies	2 (4%)	0 (0%)	-0.13, 0.05
Proton pump inhibitors	28 (56%)	8 (80%)	-0.1, 0.58
Statins	17 (34%)	2 (20%)	-0.48, 0.2
Non-selective beta-blockers	29 (58%)	7 (70%)	-0.26, 0.5
Spironolactone	22 (44%)	5 (50%)	-0.34, 0.46
Furosemide	25 (50%)	6 (60%)	-0.29, 0.49
Opioids	9 (18%)	2 (20%)	-0.27, 0.31
Acetaminophen use	14 (28%)	3 (30%)	-0.31, 0.35
Prior TIPS	7 (14%)	1 (10%)	-0.29, 0.21
Last HE episode (mths) Median (Q1-Q3)	10.50 (5.00-25.25)	15.50 (3.5-15.50)	-15.17, 9.57
Laboratory values			
MELD score	11.0 ± 3.6	12.8 ± 3.5	-0.69, 4.29
INR	1.22 ± 0.17	1.29 ± 0.14	-0.17, 0.03
Bilirubin (mg/dl)	1.25 ± 0.77	1.97 ± 1.15	-1.48, 0.04
Creatinine (mg/dl)	1.09 ± 0.44	1.14 ± 0.29	-0.27, 0.17
Ammonia (μmol/L) Median (Q1-Q3)	44.0 (28.75-79.5)	66.0 (42.8-145.8)	-9.54, 53.74
AST (IU/L)	39.8 ± 15.9	42.0 ± 17.8	-14.35, 9.95
ALT (IU/L)	38.5 ± 17.5	32.2 ± 8.0	-0.69, 13.29
ALP (IU/L)	125.4 ± 65.1	119.4 ± 23.5	-9.08, 21.08
WBC count (10^3/μl)	5.4 ± 2.3	5.4 ± 1.7	-1.25, 1.25
Hemoglobin (g/dl)	12.9 ± 1.9	12.5 ± 2.3	-1.16, 1.96
Platelet count (10^3/μl)	111.7 ± 57.0	100.3 ± 28.6	-12.57, 35.37
Sodium (mmol/L)	139.5 ± 2.6	136.9 ± 2.2	**-4.36, -0.84**
Inflammatory biomarkers (serum)			
IL-1b (pg/ml)	0.08 (0.08–0.10)	0.08 (0.08–0.10)	-0.024, 0.078
IL-6 (pg/ml)	5.29 (3.34-12.91)	9.24 (5.42-29.61)	**0.12, 13.96**
IL-10 (pg/ml)	0.05 (0.05-0.54)	0.18 (0.05-0.31)	-0.48, -0.12
TNF (pg/ml)	2.65 (1.95-4.50)	2.92 (2.08-5.94)	-1.12, 1.96
LBP (ng/ml)	4,463 (3,261-5,586)	4,177 (3,518-5,575)	-1,616.56, 1,098.56
FMT and microbiome-related			
Randomized to FMT	41 (82%)	4 (40%)	-0.8, -0.038
Shannon diversity index	2.82 ± 0.64	2.73 ± 0.53	-0.29, 0.47
Evenness	0.63 ± 0.10	0.63 ± 0.06	-0.047, 0.047

Bold denotes statistical significance.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FMT, fecal microbiota transplant; HE, hepatic encephalopathy; MASLD, metabolic dysfunction-associated steatotic liver disease; TIPS, transjugular intrahepatic portosystemic shunt.

indistinguishable, and the liquid preparations were delivered in opaque containers to be delivered via enema by the investigational pharmacy. Enema was provided via opaque rectal tubes and was retained for 30 min.

Sample collection

Stool was collected for metagenomics, serum and urine for metabolomics, and inflammatory cytokines at each in-person visit. In addition, adverse events (AE) (supplementary methods)[1] were evaluated at each visit. Analysis was performed using samples collected at baseline, two months (one-month post-FMT/placebo administrations) and at study end.

All serious AEs were reported to the central data monitoring committee (DMC) of the Veterans Affairs. The trial was registered at clinicaltrials.gov (NCT03796598) before the first enrollment. The primary outcome was safety related to FMT, including outcomes directly related to FMT by the DMC (infections, FMT procedure-related events, or other AEs considered related by the DMC), which was adjudicated after review of hospitalizations or ER visits at the subsequent visit by the principal investigator who remained blinded to the assignment until the study was completed. A West-Haven grade ≥2 episode requiring a change in therapy or treatment under medical supervision was considered HE recurrence.[1] Other outcomes and statistical analyses/sample size details are provided in the supplementary methods.

Metagenomics

Stool DNA was extracted using published techniques and then analyzed for shotgun metagenomics at 20 million pair-end read depth at the University of Minnesota.[12] Details are provided in the supplementary methods. Ultimately, bacterial alpha diversity, and species-level variations throughout the study course were compared in patients whose HE recurred, and within/between groups. Analyses were performed using linear discriminant analysis effect size (LEfSe) with significance set at >2.0, and other validated techniques.[13] SourceTracker was used to determine engraftment of donor material into FMT recipients post-FMT.[14] Comparisons were made at months 1 and 2 between those whose HE recurred/not, between the individual donors, and between the three FMT recipient groups using Mann-Whitney and Kruskal-Wallis tests. Ammonia changes over time and correlation of ammonia with metagenomics and metabolomics in those with/without HE recurrence at baseline, month 1 and 2 were performed.[15]

Metabolomics

We collected serum and urine at baseline, and at 1 and 2 months, for metabolomic analysis, which was performed at Metabolon (Morrisville, NC) using validated, published liquid chromatography-mass spectrometry techniques.[16] Following normalization to osmolality (urine only), imputation of any

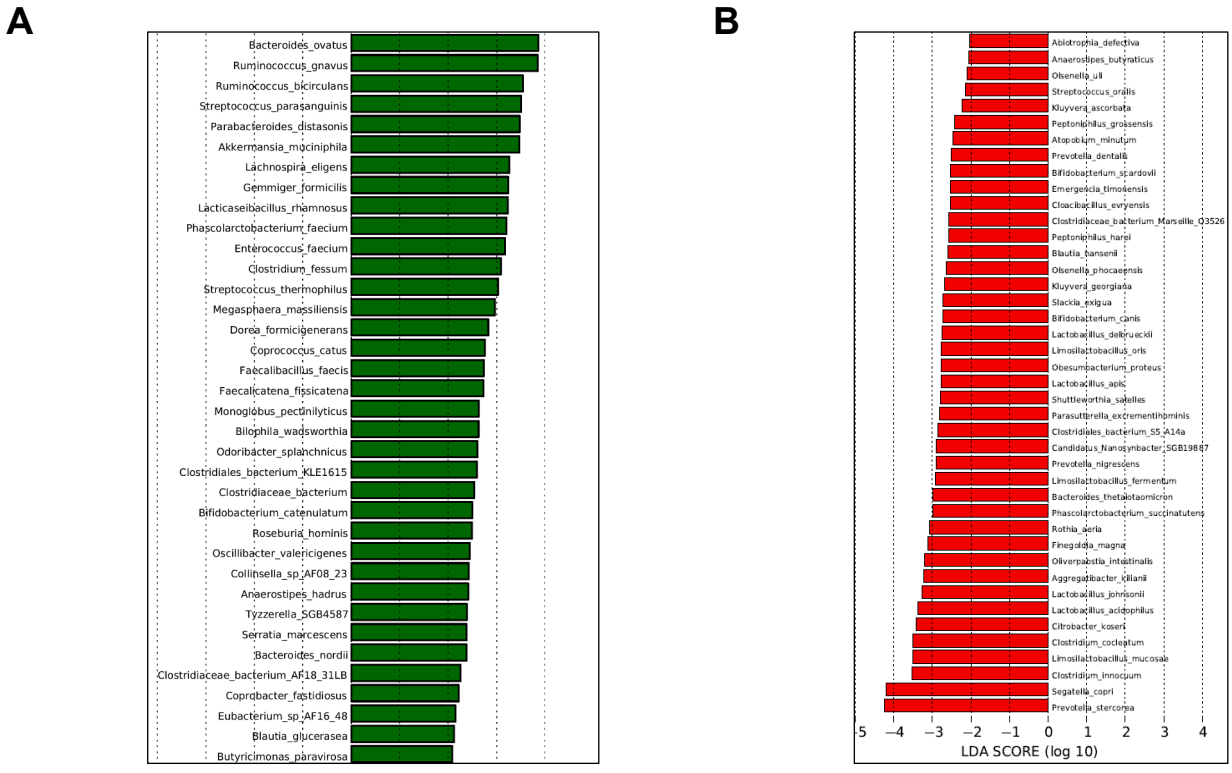

Fig. 1. Stool metagenomics compared using linear discriminant analysis effect size between patients with and without HE recurrence using samples collected at baseline, month 1 and month 2 (pre and post-FMT) for the entire cohort. 10 patients developed HE recurrence. (A) Higher relative abundance of species in those without recurrence (No = green); (B) higher relative abundance of species in those with recurrence (Yes = red). FMT, fecal microbiota transplant; HE, hepatic encephalopathy.

missing values with the minimum observed value for each compound, and log transformation, two-way repeated-measures ANOVA was used to identify biochemicals that differed significantly between experimental groups. Two-way repeated-measures ANOVA identified biochemicals exhibiting significant interaction and main effects for treatment and time point.

Bioinformatics analysis for metabolites was performed using partial least squares discriminant analysis (PLS-DA) for all groups and within those with/without HE recurrence. Pathway analysis was performed using clinical metadata and metabolomics, using the PathIntegrate algorithm followed by a classification approach to determine the importance of these pathways to HE recurrence *vs.* no HE recurrence.[17]

Combined bioinformatics analysis

Random forest analysis (RFA) was performed using microbiome, metabolome, clinical, demographic, and inflammatory cytokine data to identify features associated with HE recurrence. Analyses included values from all study visits. Finally, latent factor analysis using DIABLO was applied to integrate multi-omic variables associated with HE recurrence across the entire cohort using all time points[18].

Results

Patients were enrolled from August 2019 through June 2023 with a break for COVID-19 between March 2020 and January 2021 (Fig. S1). The first patient was enrolled on August 13th, 2019, and the last on June 2nd, 2023. The visits were a combination of in person or remote in part due to the pandemic-era restrictions. All patients consumed a typical Western diet. All initial interventions (enemas and capsules) for FMT *vs*. placebo were administered per protocol.

HE recurrence

As previously published, HE recurrence occurred in 10 of 60 patients (17%), with a significantly higher rate in the placebo-only group (6 of 15, 40%) *vs*. the FMT groups (4 of 45, 8%, $p = 0.005$; 95% CI -0.57 to -0.05.[8] The route or dose of FMT did not affect the rate of HE recurrence. Therefore, our analyses focused on participants who experienced HE recurrence compared with those who did not. Baseline comparisons showed a significantly lower serum sodium, and higher serum IL-6 in those whose HE recurred, while other clinical and demographic variables were statistically similar (Table 1). There was no significant difference in HE recurrence rates between patients who received material from either donor, or between

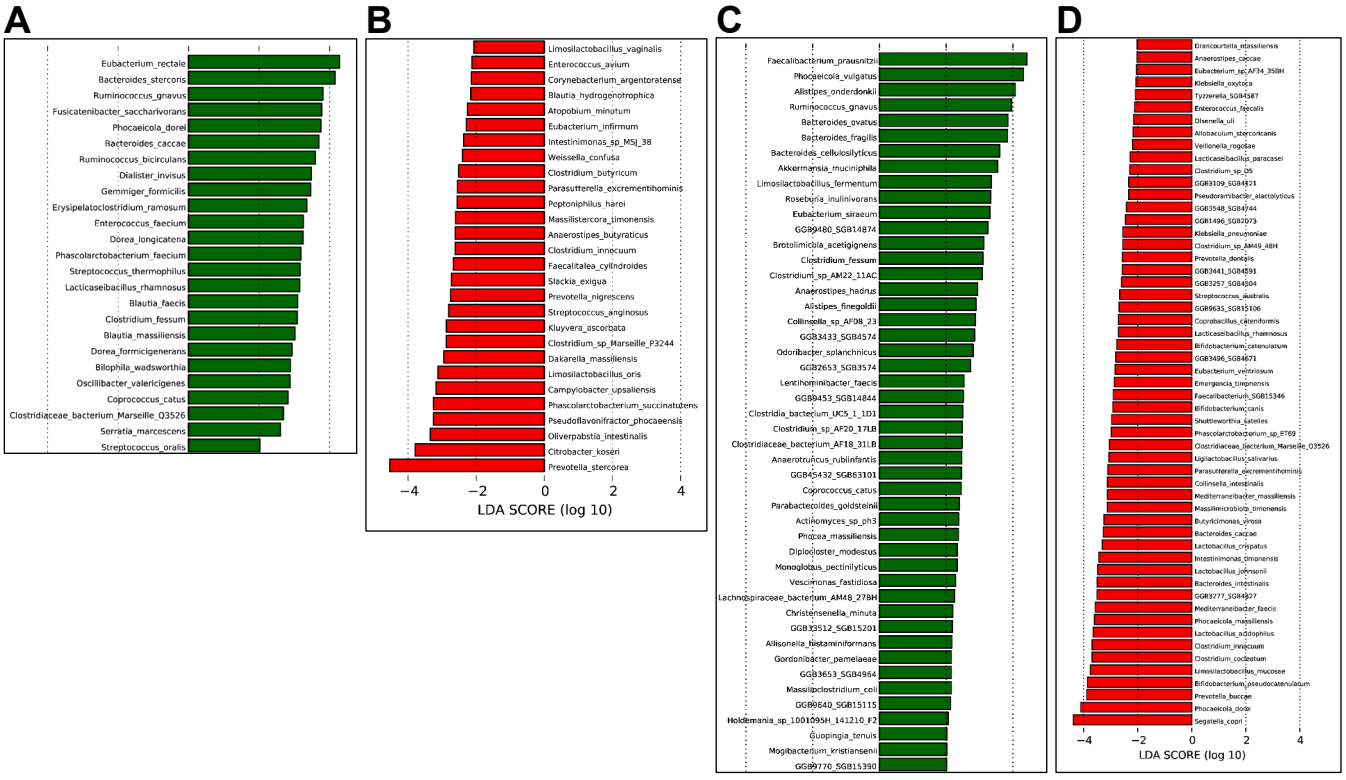

Fig. 2. Stool metagenomics of HE recurrence divided between FMT recipients or placebo recipients. (A,B) Stool metagenomics in FMT recipients compared using linear discriminant analysis effect size between patients with and without HE recurrence using samples collected at baseline, month 1 and month 2 (pre and post-FMT) for the 45 patients; 4 of these recurred while 41 remained free of recurrence. (A) Higher relative abundance of species in those without recurrence (No = green), (B) Higher relative abundance of species in those with recurrence (Yes = red). (C,D) Stool metagenomics in placebo recipients compared using linear discriminant analysis effect size between patients with and without HE recurrence using samples collected at baseline, month 1 and month 2 for the 15 patients (Group 4); 6 of these recurred while 9 remained free of recurrence. (A) Higher relative abundance of species in those without recurrence (No = green); (B) higher relative abundance of species in those with recurrence (Yes = red). FMT, fecal microbiota transplant; HE, hepatic encephalopathy.

those who received FMT by enema, capsules or both routes as reported previously.[8]

Metagenomics

HE recurrence including all samples

In the 45 patients who received at least one dose of active FMT, those who developed HE recurrence had a higher relative abundance of *Prevotella* spp, oral species (*Streptococcus anginosus, Limosilactobacillus oris*), and some members of Proteobacteria (*Citrobacter koseri*) in the merged analysis of baseline, 1-month and 2-month timepoints. In those who remained free of HE recurrence, again more SCFA producers (*Bacteroides ovatus*, *Eubacterium rectale, Bacteroides stercoris, Ruminoccocus gnavus, Dorea,* and *Blautia* spp) were seen along with some *Lactobacillus* and bilophillic species in the same comparisons (Fig. 2A,B). When only those who received placebo (Group 4) were considered, again *Lactobacillus* spp, *Bifidobacterium* spp, oral species (*Prevotella dentalis* and *buccae*), and potential pathobionts (*Enterococcus faecalis, Klebsiella pneumoniae*), were higher in those who experienced HE recurrence in the merged analysis of baseline, 1- and 2-month visits (Fig. 2). On the other hand, commensals belonging to Ruminococcaceae and Lachnospiraceae families among others (*Faecalibacterium prausnitzii, Akkermansia muciniphila*, *Ruminococcus gnavus,* and *Eubacterium* and *Odoribacter spp*), and other SCFA producers, were higher in those who did not experience HE recurrence (Fig. 2C,D). When the entire groups of HE recurrence *vs*. no recurrence were compared, similar changes were seen on LEfSe (Fig. 1). No changes in ammonia over time were found (Table S3). In patients who experienced HE recurrence, ammonia was positively correlated with two aromatic amino acid metabolites

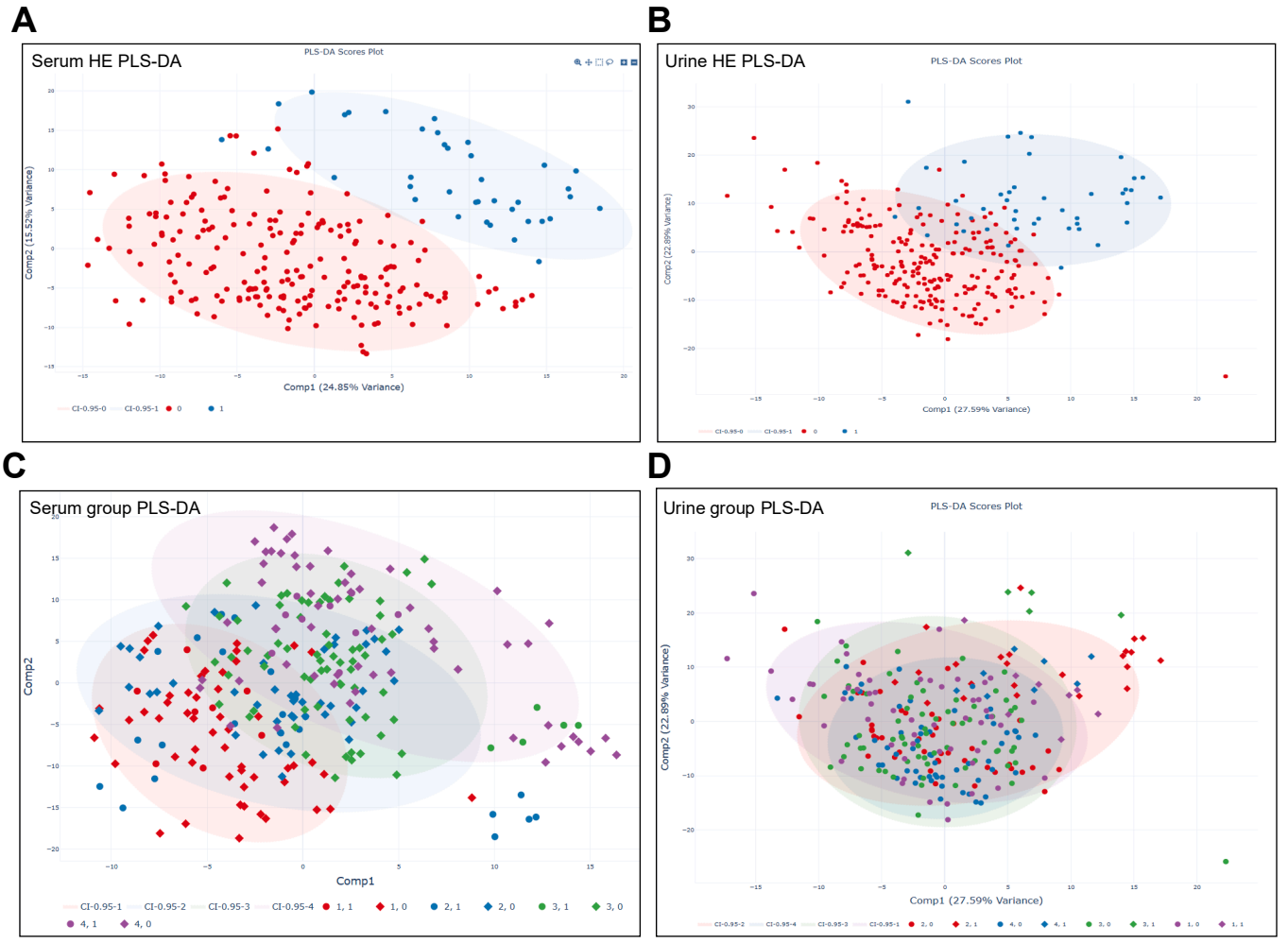

Fig. 3. Partial least squares discriminant analysis for serum and urine. Data are presented as individuals and the 95% CI; samples collected at baseline, month 1 and month 2. (A) Serum metabolomics for patients with and without HE recurrence: Good separation between those who recurred (blue) *vs.* those who did not recur (red). (B) Urine metabolomics for patients with and without HE recurrence: Separation between those who recurred (blue) *vs.* those who did not recur (red) not as pronounced as in serum. (C) Serum metabolomics for all four groups (All FMTs: red, only oral FMTs: blue, only enema FMT: green, only placebo: purple). Circles indicate those who remained free of recurrence while diamonds indicate those who experienced HE recurrence. The group with all FMTs and the group with only placebo were the farthest in separation. (D) Urine metabolomics for all four groups (All FMTs: red, only oral FMTs: blue, only enema FMT: green, only placebo: purple). Circles indicate those who remained free of recurrence while diamonds indicate those who experienced HE recurrence. The group with all FMTs and the group with only placebo were the farthest in separation but there was a higher overlap. FMT, fecal microbiota transplant; HE, hepatic encephalopathy; PLS-DA, partial least squares discriminant analysis.

(homovanillate, 7-hydroxyindoxyl sulfate), and negatively linked with tobacco metabolites (cotinine) and negatively with acetaminophen metabolites and other xenobiotic and food compounds (Table S4). Two species (*Parasutterella excrementihominis* and *Alistipes shahii*) and vitamin compounds (alpha-CEHC sulfate, and retinal) were positively linked with ammonia. In those without HE recurrence, correlations were sparse and only two negative linkages were found between ammonia, cotinine and trazodone (Table S4).

Changes within groups

LEfSe was used to compare microbial changes within groups between baseline and month 2. No significant changes were observed in the placebo-only group. In the FMT groups (groups 1–3), relative abundances of SCFA-producing species and *Fusobacterium mortiferum* were higher post-FMT compared with baseline (Fig. S2A–C).

Engraftment

There was no donor or group-wise difference (Fig. S4A and B). Engraftment was lower in the four patients who received FMT and recurred at all timepoints (Fig. S4A), at month 1 (Fig. S4C) and month 2 (Fig. S4D) compared to FMT recipients who did not experience recurrence.

Metabolomics

The serum dataset comprised a total of 1,565 biochemicals, 1,265 named and 300 unnamed, while the urine dataset comprises a total of 1,636 biochemicals, 1,151 named and 485 unnamed.

HE vs. *no HE recurrence comparisons*

PLS-DA showed excellent separation between patients with and without HE recurrence in both serum and urine, with the separation being more pronounced in serum than in urine metabolites. Analyses included samples from baseline and 1- and 2-month visits (Fig. 3A,B).

Pathway analysis metabolomics

HE recurrence was associated with higher expression of metabolites affecting the gut-liver-brain axis, including GABA, dopamine and other neurotransmitters, urea cycle and intermediates, and aromatic amino acid metabolism using samples from baseline, 1 and 2-month visits. In addition, carbohydrate digestion, sulfated amino acid metabolism, as well as bile acid intermediates were differentially expressed in patients who experienced HE recurrence (Table S1). Over-represented pathways were related to neurotransmitter, GLP-1, GIP, fatty acid and cholesterol synthesis in serum, and pathways related to bioenergetics in urine (Table S2).

Time-group interaction and PLS-DA

Sixty-eight serum and 54 urine metabolites had a significant time and group interaction (Tables 2 and 3); groups corresponded to the randomized treatment arms, and the timepoints included baseline and 1- and 2-month visits. Twenty serum and 69 urine metabolites had only a group interaction, and 208 serum and 41 urine metabolites had only a time interaction on RMANOVA. The leading metabolites in serum with significant time/group interactions were related to SCFAs, secondary sulfated bile acids, dopamine and urea cycle metabolites, and microbial metabolites, which tended to be higher post-FMT, especially in group 1 (Fig. S3A–D). In urine, dopamine and carnitine metabolites, as well as 2,3 dihydroxypyridine, pregnanetriol sulfate, and 3-hydroxyhexanoate were higher in FMT recipients over time (Fig. S3E).

On PLS-DA, when all timepoints (baseline, month 1 and 2) were included, there was a greater separation between groups in the serum compared to urine for the three visits. Visually, group 1 showed the greatest separation from group 4 (Fig. 3C,D).

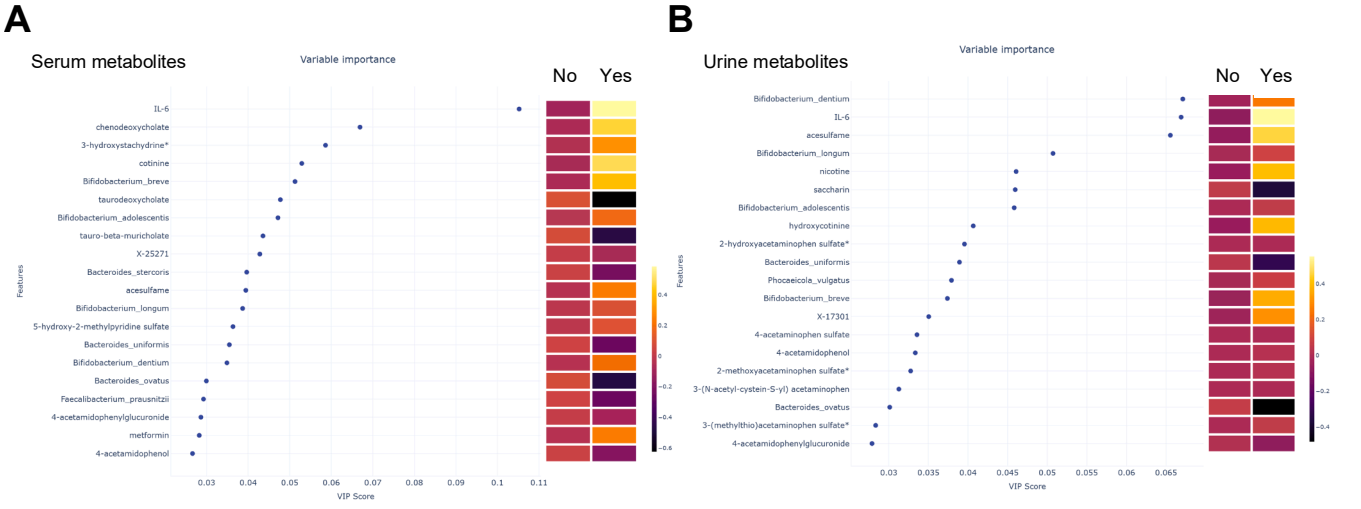

Fig. 4. Random forest analysis for serum and urine metabolites with metagenomics, inflammatory biomarkers and clinical variables. Performed on samples collected at baseline, month 1 and month 2. (A) Serum metabolites and other variables: top 20 variables associated with recurrence (yes) and no recurrence (no) according to VIP score. (B) Urine metabolites and other variables: top 20 variables associated with recurrence (yes) and no recurrence (no) according to VIP score. VIP, variable importance plot.

Table 2. Serum metabolites that showed a significant time and group interaction.

Metabolites	*p* value	Direction	Greatest change in group
Butyrate/isobutyrate (4:0)	0.0008	↑ post-FMT	Group 1 (3 FMTs)
Methylnaphthyl sulfate (2)	0.001	↑ post-FMT	Group 1 (3 FMTs)
N-acetylglutamine	0.0013	↑ post-FMT	Groups 1 and 3
4-methylcatechol sulfate	0.0018	↑ post-FMT	Group 1 (3 FMTs)
N4-acetylcytidine	0.002	↓ post-FMT	Group 1 (3 FMTs)
Gabapentin	0.0029	↑ post-FMT	Groups 2 & 3
3-methyl catechol sulfate (1)	0.005	↓ post-placebo	Group 4
3-(3-hydroxyphenyl) propionate	0.0076	↑ post-FMT	Group 1 (3 FMTs)
Indolebutyrate	0.0081	↑ post-FMT	Groups 2 & 3
2-amino-4-cyanobutanoate	0.0084	↓ post-FMT	Group 1 (3 FMTs)
(15:2)-anacardic acid	0.0087	↑ post-FMT	Groups 1 and 3
Stearoyl-arachidonoyl-glycerol (18:0/20:4)[2]	0.0091	↓ post-FMT	All 3 FMT groups
Beta-cryptoxanthin	0.0091	↑ post-FMT	Groups 1 and 3
Tauroursodeoxycholic acid sulfate (1)	0.0098	↑ post-FMT	Groups 1 and 3
3-hydroxyhippurate	0.0106	↑ post-FMT	All 3 FMT groups
Hydroxy-CMPF	0.0108	↑ post-FMT	Groups 1 & 2
Nicotinamide	0.011	↑ post-FMT	Group 3
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	0.0114	↓ post-FMT	All 3 FMT groups
Phenylacetylglutamate	0.0116	↑ post-FMT	All 3 FMT groups
Dopamine 4-sulfate	0.0118	↑ post-FMT	Group 1 (3 FMTs)
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)	0.0126	↓ post-FMT	All 3 FMT groups
N-acetylisoleucine	0.0129	↑ post-FMT	Group 3
Rosuvastatin	0.0146	↑ post-FMT	Group 1 (3 FMTs)
1-methyl-5-imidazoleacetate	0.0162	↑ post-FMT	Groups 1 & 2
(15:3)-anacardic acid	0.0172	↑ post-FMT	Groups 1 and 3
5,6-dihydrouracil	0.0206	↑ post-FMT	Groups 1 & 2
Methionine sulfone	0.021	↓ post-FMT	Groups 1 & 2
4-ethylcatechol sulfate	0.0213	↑ post-FMT	Groups 1 & 2
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)	0.0217	↓ post-FMT	All 3 FMT groups
Solanidine	0.0222	↓ post-FMT	All 3 FMT groups
Hydroxy-N6,N6,N6-trimethyllysine	0.0229	↓ post-FMT	Groups 1 & 2
1-(1-enyl-stearoyl)-2-oleoyl-GPC (P-18:0/18:1)	0.0248	↓ post-FMT	All 3 FMT groups
Acetylcarnitine (C2)	0.025	↓ post-FMT	All 3 FMT groups
3-ethylcatechol sulfate (1)	0.0258	↑ post-FMT	Groups 1 & 2
Dopamine 3-O-sulfate	0.0272	↑ post-FMT	Group 1 (3 FMTs)
Glutamate	0.0285	↑ post-FMT	Groups 1 and 3
Arabonate/xylonate	0.0292	↑ post-FMT	All 3 FMT groups
Gamma-CEHC	0.0323	↑ post-FMT	Groups 1 & 2
N-acetylthreonine	0.0329	↓ post-FMT	Group 1 (3 FMTs)
2-methylserine	0.0377	↑ post-FMT	All 3 FMT groups
1-stearoyl-2-adrenoyl-GPC (18:0/22:4)	0.0381	↓ post-FMT	All 3 FMT groups
3-hydroxyhippurate sulfate	0.0387	↑ post-FMT	All 3 FMT groups
Alpha-hydroxyisocaproate	0.039	↑ post-FMT	Group 1 (3 FMTs)
Delta-tocopherol	0.0396	↑ post-FMT	All 3 FMT groups
Sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	0.0417	↓ post-FMT	All 3 FMT groups
5-hydroxydecanoate	0.0445	↑ post-FMT	Groups 1 & 2
1-palmityl-2-stearoyl-GPC (O-16:0/18:0)	0.0453	↓ post-FMT	All 3 FMT groups
Syringol sulfate	0.0461	↑ post-FMT	All 3 FMT groups
Lactosyl-N-behenoyl-sphingosine (d18:1/22:0)	0.0466	↓ post-FMT	All 3 FMT groups
Trans-urocanate	0.0481	↑ post-FMT	All 3 FMT groups
Dihydroferulate	0.0481	↑ post-FMT	All 3 FMT groups
Behenoylcarnitine (C22)	0.049	↓ post-FMT	All 3 FMT groups
p-cresol glucuronide	0.0496	↑ post-FMT	Groups 1 & 2

FMT, fecal microbiota transplant.

Combined bioinformatics

RFA was performed using stool metagenomics, serum inflammatory cytokines and clinical/demographic variables, followed by analyses incorporating first serum and then urine metabolomics. Analysis with serum metabolites across all timepoints (baseline, 1- and 2-month visits) showed lower serum secondary BAs, stool SCFA producers ((*Faecalibacterium praunitzii, Eubacterium rectale, Bacteroides ovatus*), and acetaminophen/omeprazole derivatives, and higher inflammation (serum IL-6), *Bifidobacterium* spp, and drugs (metformin, trazodone) in those whose HE recurred. The training AUC was 1.00, the test AUC was 0.69 and the validation AUC was 0.76 (Fig. 4).

RFA with inclusion of urinary metabolites across all timepoints (baseline, 1- and 2-month visits) identified higher relative abundance of *Bifidobacterium* spp, and higher levels of urinary nicotine derivatives and serum IL-6 in patients with HE recurrence, while higher urinary acetaminophen derivatives and abundance of SCFA producers (*Bacteroides ovatus*) were associated with HE protection. The training AUC was 1.00, the test AUC was 0.61 and the validation AUC was 0.71.

Table 3. Urine metabolites that showed a significant time and group interaction.

Metabolites	*p* value	Direction	Greatest change in group
2,3-dihydroxypyridine	4.51E-05	↑ post-FMT	All 3 FMT groups
Dopamine 4-sulfate	0.0026	↑ post-FMT	Group 1 (3 FMTs)
3-hydroxyhexanoate	0.003	↑ post-FMT	Groups 1 & 2
Levulinoylcarnitine	0.0043	↓ post-FMT	Groups 1 & 3
1,5-anhydroglucitol (1,5-AG)	0.0088	↑ post-FMT	All 3 FMT groups
Deoxycarnitine	0.0091	↑ post-FMT	Groups 2 & 3
Gabapentin	0.0092	↑ post-FMT	Groups 2 & 3
Isobutyrylglycine	0.017	↑ post-FMT	Groups 1 & 2
Quinate	0.0174	↑ post-FMT	Group 1 (3 FMTs)
3-acetylphenol	0.021	↑ post-FMT	Groups 1 & 2
3-methyl catechol sulfate (1)	0.0211	↑ post-FMT	Groups 1 & 2
Cryptochlorogenic acid	0.0228	↑ post-FMT	Group 1 (3 FMTs)
Pyridoxal	0.023	↑ post-FMT	Group 3
4-allylphenol sulfate	0.0247	↓ post-FMT	Groups 1 & 3
cyclo(pro-tyr) (D,L)	0.0251	↑ post-FMT	Group 1 (3 FMTs)
pregnenetriol sulfate	0.0258	↑ post-FMT	Groups 1 & 3
Histidine	0.0271	↑ post-placebo	Group 4
Beta-hydroxyisovalerate	0.0272	↑ post-FMT	Group 3
Indoleacetate	0.0285	↑ post-FMT	Groups 1 & 2
2'-deoxyadenosine	0.0287	↑ post-FMT	Group 1 (3 FMTs)
feruloylquinate (2)	0.0306	↑ post-FMT	Group 1 (3 FMTs)
16alpha-hydroxy DHEA 3-sulfate	0.0321	↑ post-FMT	All 3 FMT groups
S-methylcysteine	0.0338	↓ post-FMT	Group 3
4-methylguaiacol sulfate	0.034	↑ post-FMT	Group 2
2-amino-2-methyl-1-propanol	0.034	↓ post-FMT	All 3 FMT groups
Azithromycin	0.0359	↑ post-placebo	Group 4
3-ethylcatechol sulfate (2)	0.0365	↑ post-FMT	Groups 1 & 2
3-hydroxyoctanoylcarnitine (2)	0.0368	↓ post-FMT	All 3 FMT groups
Dihydroferulate	0.0385	↑ post-FMT	Groups 1 & 3
Feruloylquinate (5)	0.0398	↑ post-placebo	Group 4
2-aminoheptanoate	0.0413	↑ post-FMT	Groups 1 & 2
gamma-glutamylphenylalanine	0.0451	↑ post-FMT	All 3 FMT groups
Citrate	0.0451	↓ post-FMT	All 3 FMT groups
Glucuronide of $C_{19}H_{28}O_4$ (1)	0.0455	↑ post-placebo	Group 4
Sedoheptulose	0.0464	↑ post-FMT	Groups 1 & 3
Citraconate/glutaconate	0.0475	↑ post-FMT	All 3 FMT groups
Cyclo(leu-pro)	0.0483	↑ post-FMT	Groups 1 & 3
Syringol sulfate	0.0483	↑ post-FMT	All 3 FMT groups
Tetrahydrocortisol	0.0488	↓ post-FMT	All 3 FMT groups
3-dehydrocholate	0.0499	↑ post-FMT	Groups 1 & 3

FMT, fecal microbiota transplant.

Latent factor analysis: Using DIABLO, linking serum metabolomics with other variables, IL-6, dietary/exposure variables (cotinine, acesulfame), *Bifidobacterium* spp, and primary bile acids were associated with HE recurrence, while secondary bile acids, and SCFA-producing species (*Faecalibacterium praunitzii, Eubacterium rectale, Bacteroides ovatus, Bacteroides uniformis*) were associated with lower recurrence across all timepoints (baseline, 1- and 2-month visits) (Fig. 5A; Table 4). Drugs and xenobiotics (acesulfame, cotinine, omeprazole, trazodone, acetaminophen derivatives) also affected recurrence.

When urine metabolites were studied with other variables similarly to the serum, HE recurrence was associated with higher serum IL-6, xenobiotics (nicotine and derivatives, acesulfame), and *Bifidobacterium* spp (Fig. 5B; Table 3). Again, SCFA producers (*Faecalibacterium praunitzii, Eubacterium rectale, Bacteroides ovatus, Bacteroides stercoris*) and acetaminophen derivatives were associated with protection against recurrence.

Discussion

The trial results showed a lower recurrence of HE in patients on lactulose and rifaximin with FMT compared to placebo, regardless of dose or mode of administration. The analysis of the metagenomics, metabolomics, and inflammatory cytokines collected during the trial presented an opportunity to identify factors predictive of HE. This analysis was needed to determine a holistic view of clinical, demographic, and cirrhosis-related variables that go beyond the baseline visit for HE recurrence. Patient samples were collected in the entire cohort, comparing those who experienced HE recurrence *vs*. those who did not over a 6-month period. We used two complimentary methods for high-dimensional data analysis, RFA and DIABLO (latent factor). RFA is designed for prediction and evaluation of features, while DIABLO analysis is especially useful for integration of multiple omics datasets and identifying correlations between them and relationships to clinical outcomes. Both types of analysis highlighted similar variables that are associated with the risk of HE.

Higher relative abundances of SCFA-producing bacteria correlated with reduced HE recurrence. Specifically, bacterial species belonging to Ruminococcaceae, Lachnospiraceae, and *Bacteroides* spp were consistently higher in patients protected from HE recurrence. This result is consistent with our prior 16S rRNA gene analysis. It is likely that FMT

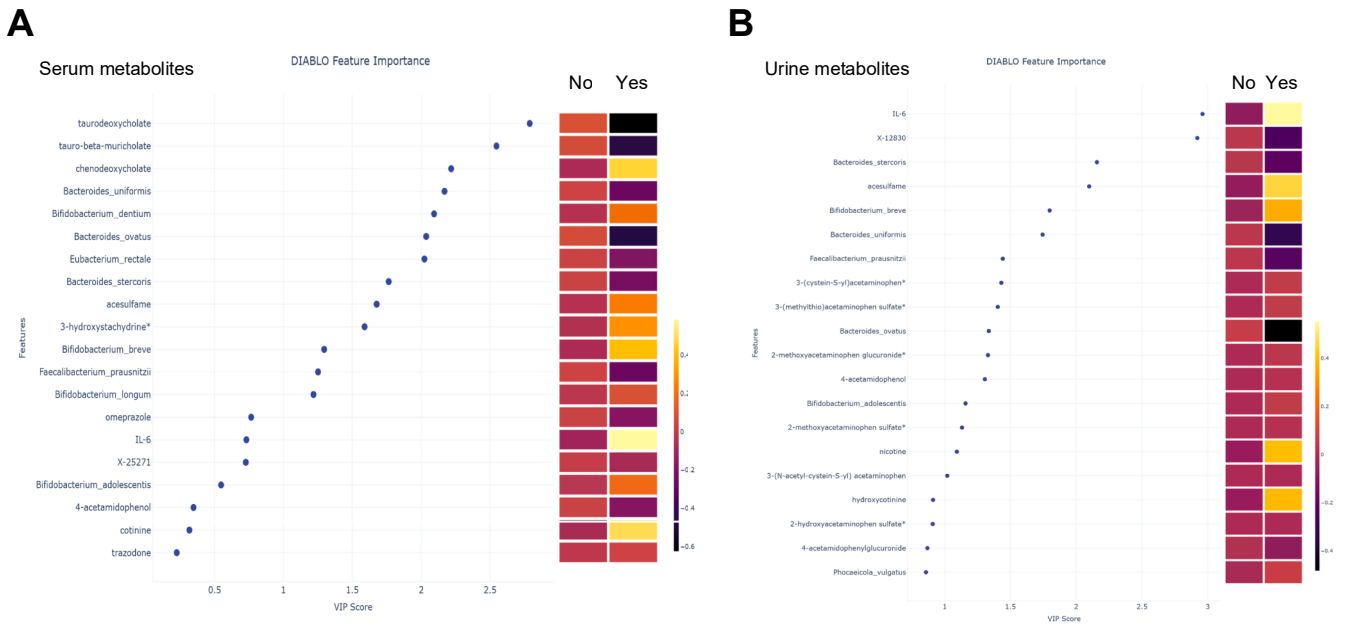

Fig. 5. Latent factor analysis for serum and urine metabolites with metagenomics, inflammatory biomarkers and clinical variables. Performed on samples collected at baseline, month 1 and month 2. (A) Serum metabolites and other variables: top 20 variables associated with recurrence (yes) and no recurrence (no) according to VIP score. (B) Urine metabolites and other variables: top 20 variables associated with recurrence (yes) and no recurrence (no) according to VIP score. VIP, variable importance plot.

increased the presence of these bacterial taxa. Serum butyrate and isobutyrate were the most changed between groups and times and tended to be the highest in the group that received the highest dose of FMT (three administrations). Pathway analyses showed fatty acid receptors that include butyrate and medium chain fatty acids as contributors towards reduced HE recurrence. The higher representation of these SCFA-producing taxa in those who had protection from recurrence on latent factor analysis, even when clinical, inflammatory, and metabolomic parameters were included, defines these compounds as major contributors towards this outcome. The results are striking because it demonstrates that even in this selected population on lactulose and rifaximin, there is still room to further improve microbial functionality with FMT.

In addition to SCFA, we found that several other pathways and metabolites belonging to neuro-active compounds were differentially associated with HE recurrence. Dopamine, GABA, glutamate derivatives, and other neurotransmitter pathways (acetylcholine, serotonin, etc.) were most affected in pathway analysis. Dopamine sulfation, which typically inactivates dopamine neurotransmitter activities, was higher in the FMT groups. Dopamine 4 and 3 sulfate levels increased with greater FMT dose on time/group interaction in both urine and serum metabolomics analysis. Since active dopamine is associated with worsening HE, it is likely that sulfation is protective.[19,20] Most dopamine sulfation occurs in the gut and the change post-FMT is likely due to lower microbial de-sulfation.[20] In addition, GABA pathways, which were the most altered and higher in those with HE recurrence, are important in the pathogenesis of gut-brain axis alterations in HE, as well as other liver-unrelated mental health disorders.[21,22] Interestingly, the relative abundances of bacterial taxa associated with GABA production, including *Bacteroides* spp, *Bifidobacterium* and *Lactobacillus* spp,[21] were higher in patients who suffered HE recurrence. Notably, *Lactobacillus* and *Bifidobacterium* spp abundance could modulate GABA production despite being higher after lactulose.[23,24] Therefore, GABA may partially explain why the relative abundance of these typically beneficial taxa and their metabolites was increased in patients who experienced HE recurrence.

Higher levels of secondary bile acids were associated with reduced HE recurrence, while higher levels of primary bile acids showed the opposite pattern. Deconjugation and 7-alpha dehydroxylation are important bacterial functions, and the lack of bacteria able to carry out these functions suggests a greater degree of dysbiosis and depletion of important members of healthy microbiota. Increased levels of primary BAs are associated with advancing cirrhosis, and the data here underlines their importance.[25] Recovery of secondary bile acid metabolism is a key feature of FMT in the treatment of recurrent *C. difficile* infection; in our trial, higher levels of secondary bile acids in FMT recipients may indicate similar microbiome restoration.[26,27]

We also assessed inflammation and endotoxemia; at baseline, only IL-6 was elevated in patients who later experienced HE recurrence. Importantly, IL-6 both in interaction with urinary and serum metabolites was significantly associated with HE recurrence on latent factor analysis. It is interesting that LBP, IL-1β, and TNF were not associated with recurrence; this could be because all our patients had decompensated cirrhosis and due to the presence of rifaximin.[28]

An interesting group of metabolites associated with HE recurrence were xenobiotics such as nicotine, acesulfame and drugs such as acetaminophen, omeprazole, gabapentin, trazodone etc. Nicotine and acetaminophen intake was similar between groups with and without HE recurrence. On latent factor analysis, urinary and serum nicotine and cotinine were associated with greater HE recurrence. Nicotine and smoking

Table 4. DIABLO feature importance metabolomics, metagenomics, inflammatory cytokines, and clinical features.

Features	Score	No recurrence	Recurrence
With serum metabolites			
taurodeoxycholate	2.78814387	0.096419063	-0.62442822
tauro-beta-muricholate	2.546450345	0.071004239	-0.459836978
chenodeoxycholate	2.217563587	-0.071964708	0.466057155
Bacteroides_uniformis	2.169122901	0.040065913	-0.259474487
Bifidobacterium_dentium	2.09314751	-0.03058314	0.198062237
Bacteroides_ovatus	2.036038492	0.073031698	-0.472967185
Eubacterium_rectale	2.023290943	0.030215313	-0.195680123
Bacteroides_stercoris	1.76375085	0.036531681	-0.236586125
Acesulfame	1.676557616	-0.036982508	0.239505763
3-hydroxystachydrine	1.589364382	-0.044937934	0.291026621
Bifidobacterium_breve	1.295660858	-0.062897864	0.40733855
Faecalibacterium_prausnitzii	1.251299389	0.039477493	-0.255663762
Bifidobacterium_longum	1.218155762	-0.014008926	0.090724476
Omeprazole	0.765362829	0.028612647	-0.185300951
Serum IL-6	0.730689496	-0.090172667	0.583975365
X-25271	0.726610281	0.0120217	-0.077854821
Bifidobacterium_adolescentis	0.547124794	-0.027980537	0.181207284
4-acetamidophenol	0.346733327	0.029615943	-0.191798488
Cotinine	0.316649112	-0.076060393	0.49258159
Trazodone	0.224866761	-0.006973428	0.045161246
With urine metabolites			
Serum IL-6	2.960248035	-0.077757002	0.552483964
X-12830	2.920757277	0.03451003	-0.245202844
Bacteroides_stercoris	2.157962101	0.028348385	-0.201422736
Acesulfame	2.099245579	-0.064108165	0.455505382
Bifidobacterium_breve	1.798907969	-0.050163539	0.356425147
Bacteroides_uniformis	1.744867984	0.043992462	-0.312578018
Faecalibacterium_prausnitzii	1.442451913	0.03099231	-0.220208517
3-(cystein-S-yl)acetaminophen	1.431020377	-0.00779218	0.055365486
3-(methylthio)acetaminophen sulfate	1.404000385	-0.008073748	0.057366107
Bacteroides_ovatus	1.335930788	0.06791788	-0.482574407
2-methoxyacetaminophen glucuronide	1.329695405	-0.004529172	0.032180959
4-acetamidophenol	1.305793104	-0.002861682	0.020333006
Bifidobacterium_adolescentis	1.15874199	-0.007547731	0.053628617
2-methoxyacetaminophen sulfate	1.132241613	-0.003479	0.024719208
Nicotine	1.092750855	-0.055839275	0.396752742
3-(N-acetyl-cystein-S-yl) acetaminophen	1.020004721	6.2922E-06	-4.47075E-05
Hydroxycotinine	0.91192475	-0.055377338	0.393470563
2-hydroxyacetaminophen sulfate	0.909326674	0.000172765	-0.001227538
4-acetamidophenylglucuronide	0.868796685	0.010632966	-0.075550019
Phocaeicola_vulgatus	0.85840438	-0.010593688	0.075270943

have been linked with progression of steatotic liver disease, and alcohol-related liver injury, which is partly linked with gut microbial changes.[29–31] Our data extends this to HE recurrence as well. Acesulfame is an artificial sweetener which has been associated with virulence in pathobionts and worsening resilience in the microbiome.[32,33] Interestingly, acetaminophen is linked with liver injury in larger quantities, but our results showed acetaminophen and its serum and urinary metabolites were associated with lower recurrence rates.[34] The reasons behind these observations are unclear, but it could be that acetaminophen use could signify reduced use of other drugs that predispose to HE such as opioids and gabapentin.[35] Most of these were independently associated with HE recurrence despite microbiome and other clinical characteristics, which underlines the multifactorial nature of HE recurrence.

While we studied both urinary and serum metabolites, the separation on PLS-DA for recurrence was greater with serum compared to urine with respect to HE recurrence. While the reasons are unclear, the findings suggest that serum biomarkers may be more useful in predicting HE recurrence. We did not find significant changes in ammonia at baseline or over time, and there were few correlations between ammonia and aromatic amino acid derivatives, xenobiotics and two potentially commensal species that were greater in those with recurrence. Further analyses in larger samples may be needed to elucidate this impact but, in our dataset, ammonia did not seem to contribute significantly. This could be partly due to the use of HE medications in all participants.

Our data are limited due to the small sample size and even lower proportion with recurrent HE (n = 10), but the consistent patient population characteristics, relatively uniform HE characteristics, and reliance on a clinically relevant outcome are strengths. We combined cases of HE recurrence because most of the patients with HE recurrence were in the placebo group (40% of placebo recipients) and only 8% of the FMT recipients developed HE recurrence. Moreover, the route or dose of FMT was not associated with protection from HE recurrence so these were merged.[8] Regardless, the results are exploratory due to the low number of recurrences overall and specifically within the FMT recipients.

We conclude that HE recurrence is associated with multifaceted alterations in serum and urinary metabolites, bacterial

metagenomics, and inflammatory cytokines in patients with cirrhosis receiving rifaximin and lactulose. Changes in metabolites and the microbiome – particularly involving SCFAs, GABA, bile acids, and IL-6 – along with key xenobiotics, including nicotine and acetaminophen, were especially predictive of recurrence, with serum proving more informative than urine. These findings highlight the potential of a multi-omic approach to better understand HE recurrence and to individualize risk prediction in patients already on rifaximin and lactulose.

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Abbreviations

AEs, adverse events; DMC, data monitoring committee; FMT, fecal microbiota transplant; HE, hepatic encephalopathy; LEfSe, linear discriminant analysis effect size; PLS-DA, partial least squares discriminant analysis; RCT, randomized clinical trial; RFA, random forest analysis; SCFAs, short-chain fatty acids.

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Conflict of interest

None for any author pertaining to this trial apart from JPM who is employed by Metabolon who were contracted to perform metabolomics and assist with bioinformatics.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

JSB was involved in all aspects including conceptualization, funding, data curation, formal analysis, and clinical conduct and along with PMG and LRT had access to the data, AF, MLG, TM, RKS, HL, SCM, AB, BCD, PP, MF were involved in study conduct, MLG, TM, and AF were involved in project administration. LRT was involved in formal statistical analysis, MS and PMG were involved in microbiome analysis and data curation, MS, PMG and JPM were involved in formal bioinformatics analysis, AK was involved in product manufacturing, quality control, research conduct, research conceptualization, visualization, and validation. JSB wrote the initial draft, and all authors were involved in the revision.

Data availability

Due to IRB restrictions individual level data cannot be shared.

Previous presentation

Portions of this manuscript were a plenary presentation at the EASL Congress in Milan in June 2024 and poster of distinction at the Digestive Disease Week 2025 in San Diego.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2025.101634.

References

[1] Vilstrup H, Amodio P, Bajaj J, et al. Hepatic encephalopathy in chronic liver disease: 2014 practice guideline by the American association for the study of liver diseases and the European association for the study of the liver. Hepatology 2014;60:715–735.

[2] European Association for the Study of the Liver. EASL Clinical Practice Guidelines on the management of hepatic encephalopathy. J Hepatol 2022;77:807–824.

[3] Lucidi C, Ginanni Corradini S, Abraldes JG, et al. Hepatic encephalopathy expands the predictivity of model for end-stage liver disease in liver transplant setting: evidence by means of 2 independent cohorts. Liver Transpl 2016;22:1333–1342.

[4] Silvey S, Patel N, O'Leary JG, et al. Enhancement of cirrhosis mortality prediction by including hepatic encephalopathy to Meld3.0 in a National veteran cohort. Am J Gastroenterol 2025;120(7):1649–1652. https://doi.org/10.14309/ajg.0000000000003317.

[5] Bloom PP, Donlan J, Torres Soto M, et al. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. Hepatol Commun 2022;6:2079–2089.

[6] Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology 2017;66(6):1727–1738. https://doi.org/10.1002/hep.29306.

[7] Bajaj JS, Salzman NH, Acharya C, et al. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. Hepatology 2019;70(5):1690–1703. https://doi.org/10.1002/hep.3069.

[8] Bajaj JS, Fagan A, Gavis EA, et al. Microbiota transplant for hepatic encephalopathy in cirrhosis: the THEMATIC trial. J Hepatol 2025;83:81–91.

[9] Tapper EB, Goldberg D, Parikh ND, et al. The liver cirrhosis network cohort study: cirrhosis definition, study population, and endpoints. Am J Gastroenterol 2024;120(3):570–575. https://doi.org/10.14309/ajg.0000000000002953.

[10] Hamilton MJ, Weingarden AR, Sadowsky MJ, et al. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol 2012;107:761–767.

[11] Staley C, Hamilton MJ, Vaughn BP, et al. Successful resolution of recurrent Clostridium difficile infection using freeze-dried, encapsulated fecal microbiota; pragmatic cohort study. Am J Gastroenterol 2017;112:940–947.

[12] Gohl DM, Vangay P, Garbe J, et al. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat Biotechnol 2016;34:942–949.

[13] Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.

[14] Knights D, Kuczynski J, Charlson ES, et al. Bayesian community-wide culture-independent microbial source tracking. Nat Methods 2011;8:761–763.

[15] Naqvi A, Rangwala H, Keshavarzian A, et al. Network-based modeling of the human gut microbiome. Chem Biodivers 2010;7:1040–1050.

[16] Bajaj JS, Tandon P, O'Leary JG, et al. Admission serum metabolites and thyroxine predict advanced hepatic encephalopathy in a multicenter inpatient cirrhosis cohort. Clin Gastroenterol Hepatol 2023;21:1031–1040 e1033.

[17] Wieder C, Cooke J, Frainay C, et al. PathIntegrate: multivariate modelling approaches for pathway-based multi-omics data integration. Plos Comput Biol 2024;20:e1011814.

[18] Singh A, Shannon CP, Gautier B, et al. DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. Bioinformatics 2019;35:3055–3062.

[19] Ding S, Wang W, Wang X, et al. Dopamine burden triggers neurodegeneration via production and release of TNF-alpha from astrocytes in minimal hepatic encephalopathy. Mol Neurobiol 2016;53:5324–5343.

[20] Goldstein DS, Swoboda KJ, Miles JM, et al. Sources and physiological significance of plasma dopamine sulfate. J Clin Endocrinol Metab 1999;84:2523–2531.

[21] Braga JD, Thongngam M, Kumrungsee T. Gamma-aminobutyric acid as a potential postbiotic mediator in the gut-brain axis. NPJ Sci Food 2024;8:16.

[22] Sorensen M, Andersen JV, Bjerring PN, et al. Hepatic encephalopathy as a result of ammonia-induced increase in GABAergic tone with secondary reduced brain energy metabolism. Metab Brain Dis 2024;40:19.

[23] Bloom PP, Tapper EB, Young VB, et al. Microbiome therapeutics for hepatic encephalopathy. J Hepatol 2021;75:1452–1464.

[24] Odenwald MA, Lin H, Lehmann C, et al. Bifidobacteria metabolize lactulose to optimize gut metabolites and prevent systemic infection in patients with liver disease. Nat Microbiol 2023;8:2033–2049.

[25] Kakiyama G, Pandak WM, Gillevet PM, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. J Hepatol 2013;58:949–955.
[26] Bajaj JS, Salzman N, Acharya C, et al. Microbial functional change is linked with clinical outcomes after capsular fecal transplant in cirrhosis. JCI Insight 2019;4(24):e133410. https://doi.org/10.1172/jci.insight.133410.
[27] Weingarden AR, Chen C, Bobr A, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol 2014;306:G310–G319.
[28] Patel VC, Lee S, McPhail MJW, et al. Rifaximin-alpha reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. J Hepatol 2022;76:332–342.
[29] Chen B, Sun L, Zeng G, et al. Gut bacteria alleviate smoking-related NASH by degrading gut nicotine. Nature 2022;610:562–568.
[30] Savin Z, Kivity S, Yonath H, et al. Smoking and the intestinal microbiome. Arch Microbiol 2018;200:677–684.
[31] Lin HM, Zhang JR, Li MX, et al. Cigarette smoking and alcohol-related liver disease. Liver Res 2024;8:237–245.
[32] Kidangathazhe A, Amponsah T, Maji A, et al. Synthetic vs. non-synthetic sweeteners: their differential effects on gut microbiome diversity and function. Front Microbiol 2025;16:1531131.
[33] Shahriar S, Ahsan T, Khan A, et al. Aspartame, acesulfame K and sucralose-influence on the metabolism of Escherichia coli. Metabol Open 2020;8:100072.
[34] Ma J, Bjornsson ES, Chalasani N. The safe use of analgesics in patients with cirrhosis: a narrative review. Am J Med 2024;137:99–106.
[35] Williams S, Louissaint J, Nikirk S, et al. Deprescribing medications that may increase the risk of hepatic encephalopathy: a qualitative study of patients with cirrhosis and their doctors. United Eur Gastroenterol J 2021;9:193–202.

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