Clinical and Translational Report

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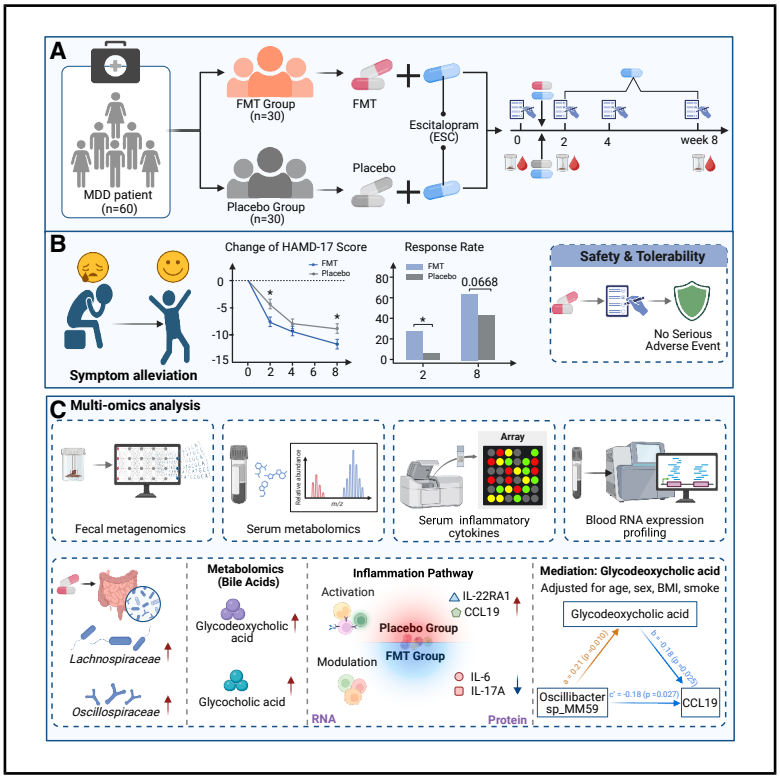
Adjunctive fecal microbiota transplantation for major depressive disorder: A randomized, double-blind, placebo-controlled trial

Graphical abstract



Highlights

- Adjunctive oral FMT accelerates antidepressant response as early as week 2
- Superior efficacy persists through 8 weeks with no additional side effects
- FMT reshapes gut microbes, with donor strains coexisting in recipients
- Benefits are mediated by elevated bile acids and reduced systemic inflammation

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In brief

Li et al. report that adjunctive oral fecal microbiota transplantation provides a rapid and sustained antidepressant response in patients with major depressive disorder. Multi-omics integration reveals that this clinical benefit is linked to successful donor strain engraftment, which subsequently modulates bile-acid metabolism and suppresses systemic inflammatory signaling.

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Adjunctive fecal microbiota transplantation for major depressive disorder: A randomized, double-blind, placebo-controlled trial

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SUMMARY

Gut microbiota may influence antidepressant treatment outcomes, yet whether targeted modulation can enhance efficacy remains unclear. We conducted a randomized, double-blind, placebo-controlled trial in patients with major depressive disorder, administering a 2-week course of fecal microbiota transplantation (FMT) capsules or placebo as an adjunct to escitalopram (ChiCTR2300071421). Remission rates at week 8 did not differ significantly between groups, but FMT produced greater reductions in Hamilton Depression Rating Scale, 17-item version (HAMD-17) scores at weeks 2 and 8. FMT was well-tolerated with a safety profile comparable to placebo. Multi-omics analyses show durable donor microbial engraftment and enrichment of beneficial Lachnospiraceae and Oscillospiraceae taxa. Microbial remodeling is accompanied by an increase in serum bile acids that correlate with the alleviation of depressive symptoms. Mediation analysis supports a bile-acid-mediated suppression of inflammatory pathways linking microbial changes to antidepressant effects. Overall, FMT may provide a safe avenue to enhance escitalopram efficacy through microbiota-directed regulation of bile-acid metabolism and inflammation.

INTRODUCTION

Major depressive disorder (MDD), a debilitating psychiatric disorder, affects more than 300 million individuals globally and is characterized by high prevalence, misdiagnosis, and disability rates.[1–3] Selective serotonin reuptake inhibitors (SSRIs) are regarded as the first-line treatment modality for patients with MDD.[4] Nevertheless, it is crucial to emphasize that the remission rate related to SSRIs can be as low as one-third, and approximately 35% of patients develop treatment-resistant depression (TRD).[5,6] These low remission rates connect directly to poor treatment compliance, high relapse rates, and high risks of suicide and functional impairment, thus increasing societal and economic burdens. Consequently, improving the efficacy of antidepressant therapies remains a critical unmet clinical need.

A multitude of factors influence the efficacy of antidepressants, including genetic predisposition, gender disparities, childhood trauma, substance misuse, and situational stressors.[7,8] An increasing body of evidence has underscored the role of the gut microbiota in the pathophysiology of depression,[9–11] thereby fueling a burgeoning interest in its potential impact on the efficacy of antidepressants.[12,13] On the one hand, antidepressants have been demonstrated to exert antimicrobial effects against diverse bacterial strains.[14] On the other hand, the gut microbiome can regulate an individual's response to antidepressant treatment by influencing the bioavailability, bioactivity, and toxicity of these drugs through mechanisms such as biotransformation and bioaccumulation.[15,16] For example, the antidepressant duloxetine can be sequestered within bacterial cells via bioaccumulation, which reduces its bioavailability and, in turn, its therapeutic efficacy. Moreover, intracellular drug accumulation alters bacterial metabolism, which may trigger a cascade of events in interspecies interactions, ultimately reshaping the structure of the gut microbial

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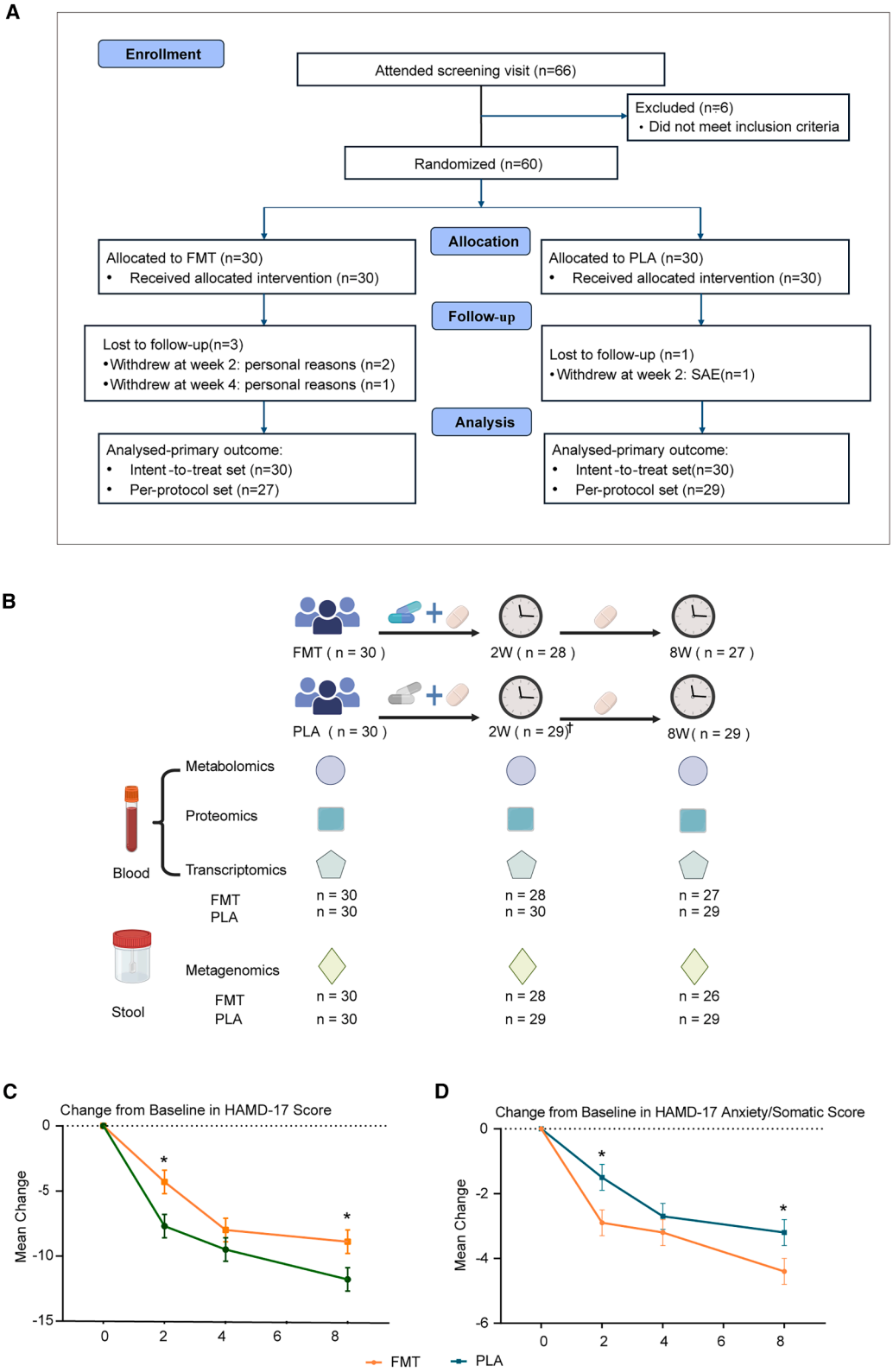

Figure 1. Overview of the study cohorts and clinical outcomes

(A) CONSORT diagram of participant flow for the study.

(B) Sample collection overview. † indicates the modified ITT (mITT) dataset.

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community.[16] Additionally, the composition of the gut microbiota and its metabolites can modulate an individual's response to antidepressants and may even serve as a predictive biomarker for treatment efficacy.[17–20] In a recent longitudinal depression cohort study, individuals who responded to escitalopram exhibited a significantly greater baseline gut microbial diversity than non-responders did. Furthermore, baseline microbial gene profiles can accurately predict the therapeutic response to escitalopram.[21] These findings collectively suggest that the therapeutic modulation of gut microbial ecosystems may be a promising strategy for enhancing the efficacy of antidepressants.

Fecal microbiota transplantation (FMT) is an efficacious and expeditious method for reconstructing the patient's gut microbiota through the administration of microbiota from healthy donors. Consequently, it has emerged as a promising therapeutic strategy for chronic diseases linked to dysbiosis. Common administration routes include oral and colonoscopy-based delivery; notably, oral capsules have demonstrated a therapeutic efficacy comparable to colonoscopy while offering a more practical and less invasive approach.[22–24] In recent years, increasing evidence from clinical investigations has substantiated the therapeutic potential of FMT for treating MDD. Nevertheless, the extant clinical data predominantly pertain to cases of depression comorbid with gastrointestinal conditions.[25–27] The application of FMT in primary depressive disorders remains significantly underexplored, with the current evidence restricted to isolated case reports[28,29] and a single exploratory study.[30] Considering the limitations of prior studies and the dearth of rigorously designed, well-controlled trials, we conducted a randomized, double-blind, placebo (PLA)-controlled trial to assess the efficacy and safety of oral FMT as an adjunctive therapy for patients with depressive disorders. Furthermore, multi-omics technologies were used to explore the potential mechanisms of action.

RESULTS

Participant enrollment and characteristics

From May 2023 through March 2024, 66 patients were assessed for eligibility, of whom 60 were successfully recruited and were included in the intent-to-treat (ITT) analyses. As detailed in the CONSORT flow diagram (Figure 1A), 56 of the 60 randomized participants completed the 8-week study, yielding a highly controlled overall attrition rate of 6.7%. In the FMT group, three patients withdrew for personal reasons (two at week 2, one at week 4). In the PLA group, one patient was withdrawn at week 2 due to a serious adverse event (SAE), which was later confirmed to be unrelated to the study intervention. This high retention rate minimizes attrition bias and robustly supports the internal validity of the clinical outcomes. The participants' characteristics are summarized in Table S1. The groups were matched at baseline, as expected. The average age of the participants was 29.6 years old (range 19.7 to 60.7), and 63.3% (38/60) were female. Forty percent of the participants were cases of first-episode MDD. Both patient groups were administered escitalopram at doses ranging from 5 to 20 mg. The precise distribution of patient numbers for each administered dose over the 8-week period is presented in Table S2. The overall workflow of sample collection is shown in Figure 1B. No significant within-group changes in dietary structure or habits were observed from baseline to week 8 in either the FMT or the PLA group (all $p > 0.05$; Table S3).

Clinical efficacy

The primary endpoint was the difference in remission rate between the FMT and PLA groups by week 8. The remission rate (Hamilton Depression Rating Scale, 17-item version [HAMD-17] total score ≤ 7) was 43.3% in the FMT group and 26.7% in the PLA group, with a between-group difference of 16.7 percentage points (95% confidence interval [CI], −9.4 to 40.1; $p = 0.1760$) at week 8. The response rate (HAMD-17 reduction $\geq$ 50%) was 30% in the FMT group and 6.7% in the PLA group at week 2, with a between-group difference of 23.3 percentage points (95% CI, 1.1–43.7; $p = 0.0195$) (Table 1). After adjusting for age, sex, first vs. recurrent episode, baseline symptom severity, and the duration of the current episode, the results remained aligned with the initial models (Tables S4 and S5). This discrepancy was more pronounced in the per-protocol set (PPS) analysis (Table S6). The changes in clinical scale scores from baseline to week 8 are shown in Figures 1C and 1D. Depressive symptoms improved in both arms, with greater reductions in the FMT group. More specifically, the mean (± SE) changes in the HAMD-17 score from baseline to week 8 were 11.8 ± 0.9 points in the FMT group and 8.9 ± 0.9 points in the PLA group, for a between-group difference of 2.9 points (95% CI, −5.5 to −0.3; $p = 0.0303$). Consistent with the change in the HAMD-17 score, the HAMD-17 anxiety/somatic subscale score differed significantly between the two groups (95% CI, −2.4 to −0.1; $p = 0.0356$). No significant differences were observed in the changes in the total 7-item Generalized Anxiety Disorder scale (GAD-7) or Gastrointestinal Symptom Rating Scale (GSRS) scores. There were no notable differences between the full analysis set (FAS) and the PPS datasets (Table S7). In parallel, a comparison of symptoms between the two groups across multiple time points revealed a significantly lower HAMD-17, HAMD-17 anxiety/somatic factor, and GAD-7 scores in the FMT group at week 2 (all $p < 0.05$) (Table S8).

Safety and blinding

The safety analysis set included 30 subjects in the FMT group and 30 in the PLA group. Following randomization, 9 participants (30.0%) in the FMT group and 8 (26.7%) in the PLA group reported 23 adverse events (AEs), with no statistically significant difference between the groups ($\chi^2 = 0.08$, $p = 0.7745$). Two SAEs were reported during the study (one attempted suicide and one myocardial infarction). Upon blinded clinical adjudication based on alternative etiologies and biological plausibility, both were deemed unrelated to the study intervention.

(C and D) Changes in HAMD-17 scores (C) and HAMD-17 anxiety/somatic scores (D) from baseline to week 8 between the FMT group and the PLA group. Statistical analyses were performed using the LMMs, $^*p < 0.05$.

Abbreviations; HAMD-17, 17-item Hamilton Depression Rating Scale; PLA, placebo; FMT, fecal microbiota transplantation.

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Table 1. Primary (HAMD-17 score ≤ 7; remission) and secondary (HAMD-17 scores reduced by at least 50% between baseline and each follow-up assessment; response) outcomes at the 2-week and 8-week follow-ups FAS

Improvement from and reduction in HAMD-17	PLA group (n = 30) n of total n (%)	FMT group (n = 30) n of total n (%)	Risk difference[c]	Odds ratio for PLA vs. FMT (95% CI)[b]	p[a]
Primary outcome					
Remission at 8 weeks[d]	8 (26.7)	13 (43.3)	16.7 (−9.4 to 40.1)	2.1 (0.7–6.2)	0.1760
Secondary outcome[e]					
Response at 2 weeks	2 (6.7)	9 (30.0)	23.3 (1.1–43.7)	6.0 (1.2–30.7)	0.0195
Response at 8 weeks	14 (46.7)	21 (70.0)	23.3 (−3.7 to 46.4)	2.7 (0.9–7.7)	0.0668

Abbreviations; HAMD-17, 17-item Hamilton Depression Rating Scale; FAS, full analysis set; FMT, fecal microbiota transplantation.
[a]The p values of primary outcome reported are based on one-sided Wald tests and the p values of secondary outcome reported are based on two-sided Wald tests, with no adjustments for multiple tests.
[b]Univariate logistic regression model.
[c]The difference between the groups is expressed as percentage points.
[d]The primary outcomes of improvement from depressive symptomatology were defined as HAMD-17 scores less than 7.
[e]The secondary outcome of reduction in depressive symptomatology was defined as a reduction in HAMD-17 scores by at least 50% between baseline and each visit assessment (yes/no).

Subsequent unblinding confirmed that both SAEs occurred in patients assigned to the PLA group. The most frequently reported AEs were nausea and fatigue (Table S9). Masking was successful, as indicated by a nonsignificant difference in the correct guess rate between the groups (8 [32%] in the FMT group and 13 [44.8%] in the PLA group; Table S10). This low correct guess rate is likely due to the effective concealment and the low AE profile of the intervention.

Donor microbiota engraftment after FMT

To investigate the gut microbiota dynamics following FMT, we applied a predefined conceptual model of ecological engraftment (Figure 2A)[31] and sequentially analyzed the microbial changes from the species to the strain level. We first evaluated the compositional overlap among the donor, baseline, and post-FMT groups at a species level (Figure 2B). At week 2, 16% (446 species) of the detected species were newly acquired from donor microbiota (colonization), while 43% (1,209 species) were present across all three groups (coexistence). By week 8, the colonization species decreased to 7% (186 species), whereas the shared species remained stable at 47% (1,166 species). This pattern suggests an initial phase of broad species acquisition followed by ecological stabilization. To further dissect the shared species, we employed SNP-based tracking to distinguish donor engraftment from recipient persistence. Based on the presence or absence of strains across the donor, pre-FMT, and post-FMT communities, we utilized a conceptual model to classify the strain-level dynamics into four distinct ecological categories: colonizing-conspecific, persistence-conspecific, coexistence-conspecific, and novel-conspecific (see STAR Methods for details). At the individual level, this SNP-based analysis revealed that coexistence was the predominant outcome (~40% at both time points). The donor strains typically integrated into the resident microbial community alongside the pre-existing recipient strains, rather than completely replacing them (Figure 2C). Notably, no instances of complete donor rejection or total loss of recipient strains were observed. This coexistence-dominant engraftment pattern was clearly demonstrated in the specific representative taxa selected for detailed tracing, such as *Lachnospira eligens*, *Dysosmobacter welbionis*, *Anaerotruncus colihominis*, and *Lawsonibacter asaccharolyticus* (Figures 2D and S1). Collectively, these findings indicate that FMT establishes a new ecological equilibrium marked by the long-term coexistence of donor- and recipient-derived strains, which is characterized by an initial phase of broad engraftment followed by ecological consolidation.

Microbiota dynamics following FMT

We next evaluated the baseline differences in gut microbial diversity and the longitudinal effects of FMT. Cross-sectional analysis confirmed significantly lower species richness in patients relative to healthy donors at baseline. To characterize the longitudinal trajectories, we employed linear mixed-effects models (LMMs) adjusting for sex, age, and the clinical covariates. LMM revealed significant group-by-time interactions at week 2 for α-diversity (Shannon index, p = 0.006; observed species, p = 0.009). The FMT group demonstrated significant increases from baseline to week 2 in both the Shannon index (p = 0.027) and the species richness (p = 0.002), whereas the PLA group showed a continuous decline over the 8-week period (Figures 3A and 3B), which is consistent with the prior evidence that antidepressant treatment reduces microbial diversity.[21] For β-diversity (Bray-Curtis distance to donor), the LMM likewise identified a significant group-by-time interaction (p = 0.006): the FMT group shifted significantly toward a healthy donor-like composition by week 2, a trajectory that was absent in the PLA group (Figure 3C). Collectively, these findings indicate that FMT robustly enhances microbial diversity after the adjustment for multiple clinical covariates.

Further analysis of microbial alterations following FMT was conducted at the individual taxon level. At the family level, Enterobacteriaceae abundance significantly decreased in both the FMT and the PLA groups, which indicated a potential association with antidepressant treatment (Figures 3D and S2A). In the FMT group, an early increasing trend by week 2 was a key feature for specific taxa. This rapid increase was statistically significant for Oscillospiraceae (p = 0.047 at week 2) (Figures 3D and 3E), whereas the concurrent early upward trend observed in Lachnospiraceae

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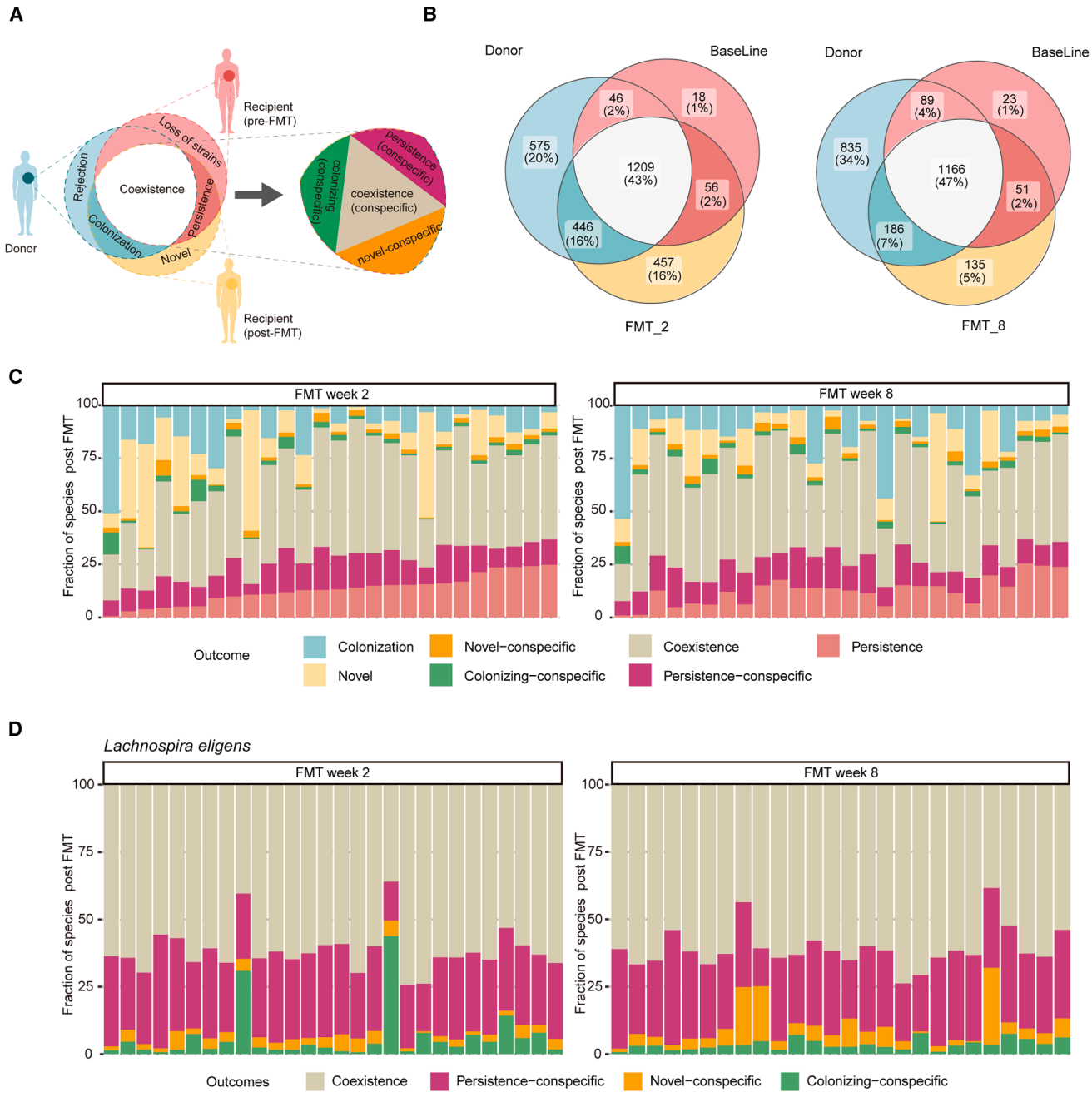

Figure 2. Strain dynamics and post-FMT engraftment outcomes across recipients and time points

(A) Conceptual framework depicting major possible outcomes of microbiota dynamics after FMT, including colonization, persistence, coexistence, the emergence of novel strains, rejection, and the loss of resident lineages. A refined categorization further partitions coexistence into four subtypes (novel-conspecific, colonizing-conspecific, persistence-conspecific, and coexistence).

(B) Venn diagrams showing species-level overlap between the donor, baseline (week 0), and post-FMT microbiota at weeks 2 (left) and 8 (right). Numbers indicate species counts with percentages representing the proportion of total detected species (relative abundance > 0.01%). Each Venn diagram region represents a distinct microbial source composition that is conceptually aligned with the engraftment categories defined in (A).

(C) Distribution of outcomes at the individual level across recipients by week 2 (left) and week 8 (right).

(D) Strain-level tracking of *L. eligens*, illustrating persistence of donor-derived lineages across both time points.

Abbreviations; FMT, fecal microbiota transplantation; PLA, placebo.

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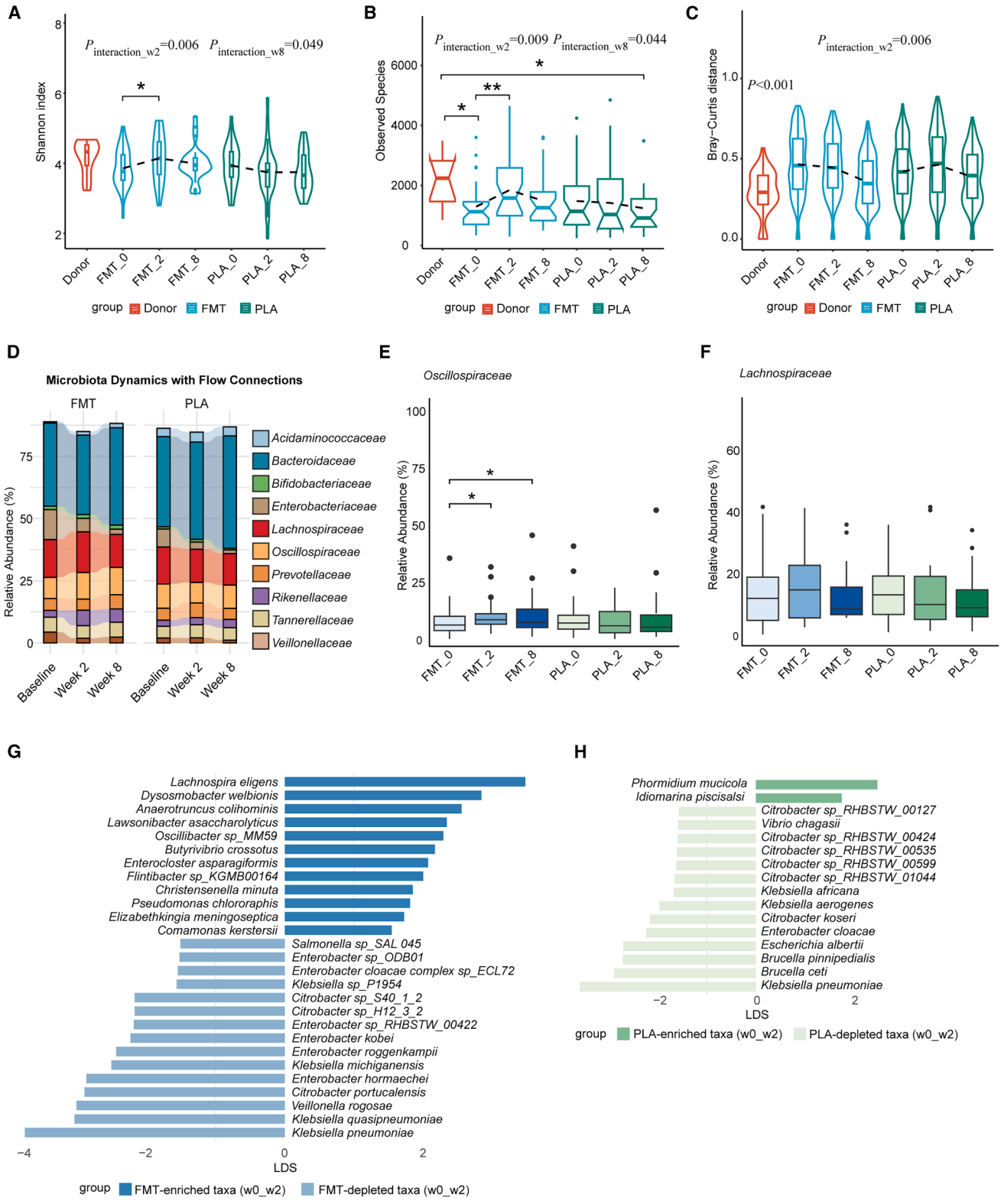

Figure 3. Alterations in the gut microbiota composition after FMT

(A–C) The Shannon index (A), the observed species richness (B), and the Bray-Curtis distance (C) were calculated for fecal samples from the donors (donor), the FMT recipients at baseline, week 2, and week 8 (FMT_0, FMT_2, and FMT_8), and the PLA recipients at the corresponding time points (PLA_0, PLA_2, and PLA_8). Pairwise comparisons between baseline and post-treatment time points within groups were derived from the LMM to account for repeated measures.

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(Figures 3D and 3F) did not reach statistical significance. Linear discriminant analysis effect size (LEfSe) combined with linear discriminant analysis (LDA) was subsequently performed to identify the differentially abundant bacterial species (LDA score > 1.5, $p < 0.05$). At week 2 and week 8 after FMT, 12 and 10 bacterial species were enriched compared with baseline, respectively, most of which belonged to Lachnospiraceae or Oscillospiraceae (7/12 at week 2 and 5/10 at week 8). Notably, four of the twelve enriched taxa at week 2, namely, *L. eligens*, *D. welbionis*, *A. colihominis*, and *L. asaccharolyticus*, were also identified in strain-level engraftment analyses. By week 8, three of these four initially engrafted taxa remained enriched, which indicated their sustained engraftment and potential long-term contribution to FMT-mediated microbiota remodeling. Among these persisting taxa, *L. eligens* has been consistently reported to be depleted in individuals with MDD across multiple independent cohorts.[32,33] In our study, *L. eligens* also showed a negative correlation with HAMD-17 scores (Figure S2B). Concurrently, 15 and 30 bacterial species were depleted by weeks 2 and 8, respectively, with the majority belonging to the Enterobacteriaceae (Figures 3G and S2C), a group previously widely implicated in inflammation.[34] In contrast, the PLA group exhibited a pronounced reduction in most taxa by weeks 2 and 8, nearly all of which were Enterobacteriaceae, with only two taxa enriched at each time point (Figures 3H and S2D). To ensure that the key differentially abundant taxa identified above were robust against the compositional nature of microbiome data, we cross-validated our findings using ALDEx2 and DESeq2. Although strict statistical significance was not uniformly reached across all platforms, largely due to the highly conservative nature of ALDEx2, the relative effect sizes and directions of change remained highly consistent (Figure S2E). Collectively, these findings indicate that FMT leads to a persistent and favorable remodeling of the gut microbiota composition.

FMT alters serum metabolites, especially bile acids

To investigate the impact of FMT-induced gut microbiota alterations on host metabolism, a targeted serum metabolomic analysis covering 342 metabolites was conducted at baseline, week 2, and week 8. According to linear model analysis, relative to baseline, 62 and 42 metabolites had increased in the FMT group at weeks 2 and 8, respectively, compared with 38 and 36 in the PLA group. Notably, a substantial proportion of these changes comprised bile acids, which was most prominent at week 2 (23/62 in FMT and 9/36 in PLA) and underscores their significant involvement ($|\log FC| > 0$ and $p < 0.05$) (Figure 4A). Moreover, the increase in bile acids was more pronounced in the FMT group. Specifically, under a more stringent cutoff ($|\log FC| > 0.5$ and adjusted $p < 0.1$), only 13 metabolites remained significant in the FMT group, of which 12 were bile acids. In contrast, in the PLA group, only five metabolites reached significance, including just one bile acid (Figure 4A). Among the 12 significant bile acids in the FMT group, three—glycocholic acid (GCA), glycodeoxycholic acid (GDCA), and taurochenodeoxycholic acid (TCDCA)—showed significantly greater increases at week 2 compared with the PLA group (GCA, $p = 0.011$; GDCA, $p = 0.033$; and TCDCA, $p = 0.04$) (Figures 4B, 4C, and S3A). Additionally, several other bile acids, such as glycochenodeoxycholic acid (GCDCA) and taurodeoxycholic acid (TDCA) also exhibited consistent upward trends (Figures S3B–S3F). This suggests a synergistic effect of FMT on escitalopram-induced increases in bile acids. Furthermore, correlation analysis revealed that the seven bile acids enriched in the FMT group were negatively associated with HAMD-17 scores (Spearman correlation, $|r| > 0.1$, $p_{adj} < 0.01$) (Figure 4D), thus indicating that bile-acid elevation may represent a key mechanism through which FMT enhances the antidepressant efficacy of escitalopram.

To explore the potential crosstalk between the gut microbiota and host metabolism, we further evaluated the associations linking the altered taxa to specific serum bile-acid profiles. FMT-enriched taxa identified at 0–2 weeks, including the four core engrafting species *L. eligens*, *D. welbionis*, *A. colihominis*, and *L. asaccharolyticus*, showed positive correlations with multiple bile acids. In contrast, the FMT-depleted taxa, predominantly Enterobacteriaceae members (e.g., *Enterobacter cloacae complex*, *Klebsiella quasipneumoniae*, and *Citrobacter portucalensis*), exhibited negative correlations ($|\text{rho}| > 0.3$, $p < 0.05$) (Figure 4E). We also performed canonical correspondence analysis (CCA), in which the first two canonical axes explained 51.9% of the total variance (CCA1, 38.7%; CCA2, 13.2%). Most of the differential bile acids were projected in the upper-central region of the ordination plot, in close proximity to the majority of the FMT-enriched taxa. In contrast, the FMT-depleted taxa, particularly Enterobacteriaceae members, were distributed on the right side of the plot, opposite to the bile-acid-associated cluster (Figure 4F). This spatial separation suggests that the FMT-enriched taxa may be functionally involved in bile-acid alterations. To further validate these associations, we performed data integration analysis for biomarker discovery using latent components (DIABLO) integrative modeling, which demonstrated a high concordance with the CCA findings and reinforced the robust microbiome-metabolome interactome reshaped by FMT (Figure S3G).

Comparisons between donors and other subgroups were performed using the Kruskal-Wallis test followed by Dunn's post hoc test with Benjamini-Hochberg correction. Only significant differences ($p < 0.05$) are highlighted.

(D) Stacked bar plots display the relative abundance of the top-10 bacterial families in fecal samples from the FMT and PLA groups at baseline, week 2, and week 8.

(E) Relative abundance of Oscillospiraceae. A significant group-by-time interaction was observed ($p = 0.0061$). Asterisks indicate significant differences using LMM followed by post hoc emmeans comparisons (*$p < 0.05$).

(F) Relative abundance of Lachnospiraceae. No significant group-by-time interaction was observed based on the LMM analysis ($p = 0.2176$).

(G and H) LEfSe revealed the bacterial taxa that were significantly enriched or depleted between baseline and week 2 in the FMT group (G) and the PLA group (H). Positive LDA scores represent enriched taxa at week 2, whereas negative scores represent depleted taxa at week 2. Only taxa with an |LDA score| > 1.5 and $p < 0.05$ are shown.

Abbreviations; FMT, fecal microbiota transplantation; PLA, placebo.

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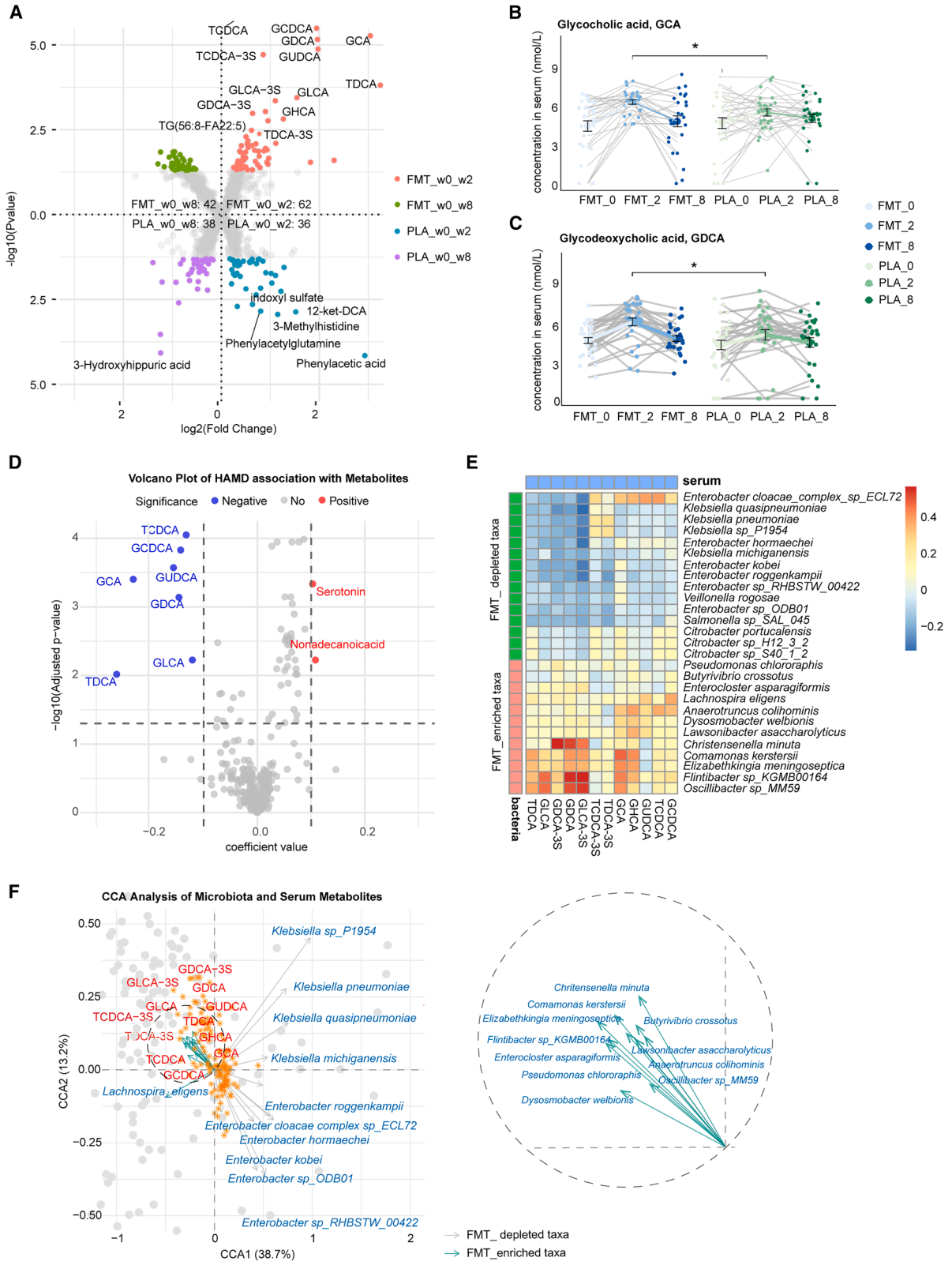

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Proteomic and transcriptomic analyses support a potential anti-inflammatory role of FMT

Next, we performed whole-blood RNA sequencing to explore the downstream molecular pathways through which the gut microbiota-bile acid axis mediates the antidepressant effects of FMT. In the PLA group, an enrichment analysis of differentially expressed genes (DEGs) from baseline to week 2 revealed prominent enrichment in the pathways involved in small GTPase-mediated signaling, lymphocyte differentiation, leukocyte adhesion, and T cell activation, thus indicating a state of enhanced immune activation (Figure 5A). In contrast, the FMT group exhibited enrichment in multiple immune regulatory pathways, which indicated the effective modulation and potential restoration of immune homeostasis (Figure 5B). By week 8, the DEGs from both groups were predominantly enriched in inflammation-related responses (Figure S4), which suggested that the enhanced antidepressant efficacy observed in the FMT group was likely driven by the modulation of inflammatory pathways.

An Olink inflammation panel was used to further examine alterations in inflammation-related immune proteins. Notably, the levels of pro-inflammatory cytokines such as interleukin (IL)-6 and IL-17A markedly decreased after FMT at week 2 compared with baseline, whereas their levels remained relatively stable in the PLA group. By week 8, these differences were no longer significant within either the FMT or the PLA groups (Figures 5C and 5D). At week 2, the level of IL-22 receptor subunit alpha 1 (IL-22RA1) significantly increased in the PLA group but exhibited a nonsignificant decreasing trend in the FMT group (Figure 5C). Another noteworthy finding was the alteration of chemotaxis-related factors. The C-C motif chemokine ligand 20 (CCL20) exhibited pronounced decreases in the FMT group at both weeks 2 and 8, while remaining relatively stable in the PLA group (Figures 5C and 5E). Similarly, other chemokines, including C-C motif chemokine ligand 19 (CCL19) and C-C motif chemokine ligand 11 (CCL11), together with monocyte chemoattractant protein-1(MCP-1), monocyte chemotactic protein-2 (MCP-2), and matrix metalloproteinase 1 (MMP-1) showed marked elevations in the PLA group but remained stable in the FMT group at week 2 (Figure 5C). The pathway enrichment of the differentially expressed proteins indicated a significant involvement of cytokine-mediated signaling, chemokine activity, and leukocyte migration (Figure 5F), which corroborated the transcriptomic findings by consistently highlighting the engagement of inflammation-related signaling. Collectively, these findings suggest a potential role of FMT in attenuating inflammation.

Spearman correlation analysis was performed to evaluate the associations between the microbial taxa that significantly changed during the first 2 weeks after FMT and inflammatory factors. The pro-inflammatory cytokine IL-17A was positively correlated with 9/15 FMT-depleted taxa, and IL-6 was positively correlated with 4/15 FMT-depleted taxa. In contrast, IL-22RA1 showed negative correlations with 3/12 FMT-enriched taxa (Figure 5G). These findings suggest that FMT-induced changes in the gut microbiota are closely linked to alterations in inflammatory profiles. The reduced inflammation in the FMT group may be tightly associated with FMT-mediated gut microbial remodeling.

Mediation analysis reveals a microbial-bile acid-inflammation pathway underlying the antidepressant effect of FMT

Having established that FMT induces coordinated shifts in gut microbial composition, bile-acid metabolism, and inflammatory signaling, we next sought to determine how these interconnected changes contribute to clinical improvement. Using mediation analysis in a randomized FMT vs. PLA intervention, we integrated multi-omics data, including the microbiome, metabolome, inflammatory markers, and clinical outcomes, to investigate how FMT reshapes host physiology, including depressive symptoms (HAMD-17). FMT increased the abundance of beneficial taxa such as *L. eligens* and *Butyrivibrio crossotus*, which was accompanied by higher levels of bile-acid metabolites, particularly GCA and GDCA. These metabolites were associated with the suppression of inflammatory mediators, including caspase-8 (CASP-8), CCL19, and CCL20. In particular, GCA mediated 27% of the association between *L. eligens* and CASP-8 (average causal mediation effect [ACME] = −0.09; 95% CI, −0.19 to −0.01; $p = 0.022$) (Figures 6A–6D).

Clinically, FMT resulted in a greater reduction in HAMD-17 scores (ΔHAMD) compared with the PLA. Moreover, changes in IL-22RA1 at week 2 partially mediated the beneficial effect of FMT on depressive symptoms (ACME = 0.93; 95% CI, 0.11–2.18; $p = 0.012$) (Figure 6E). Together, these results delineate a mechanistic cascade whereby FMT-induced microbial shifts modulate bile-acid metabolism and inflammatory signaling, ultimately contributing to the alleviation of depressive symptoms.

Figure 4. Gut microbiota remodeling is associated with serum bile-acid elevation following FMT

(A) Dual volcano plot showing the significantly increased metabolites relative to baseline (week 0) identified by linear models implemented in limma across four within-group comparisons: FMT_week 0→week 2 (red), FMT_week 0→week 8 (green), PLA_week 0→week 2 (cyan), and PLA_week 0→week 8 (purple). Metabolites with |logFC| > 0 and $p < 0.05$ were considered significant and are shown in color; nonsignificant metabolites are shown in gray.

(B and C) Longitudinal concentration changes of representative bile acids, namely, GCA (B) and GDCA (C), in the FMT and PLA groups. Statistical significance was assessed using the Wilcoxon rank-sum test.

(D) Volcano plot of the associations between serum metabolites and HAMD scores. Red indicates positive associations, and blue indicates negative associations. Significant associations were defined as |Spearman's correlation coefficient| > 0.05 and p adj < 0.01.

(E) Heatmap illustrating the correlations between the differential bacterial species and serum bile-acid metabolites. The rows correspond to bacterial taxa, with pink indicating the FMT-enriched taxa (0–2 weeks) and green indicating the FMT-depleted taxa (0–2 weeks). Columns represent differential bile-acid metabolites, highlighted in blue. The scale denotes Spearman's correlation coefficients, with positive correlations in red and negative correlations in blue.

(F) CCA of the differential bacterial species identified by LEfSe (0–2 weeks) and serum metabolites. Green vectors indicate enriched taxa, while gray vectors indicate depleted taxa. Orange asterisks indicate serum metabolites. The FMT-increased bile acids are labeled in red, and the individual subjects are shown as gray points. The first two CCA axes (CCA1 38.7%, CCA2 13.2%) are displayed. The arrows oriented along an axis indicate a strong correlation with that axis.

Abbreviations; FMT, fecal microbiota transplantation; PLA, placebo.

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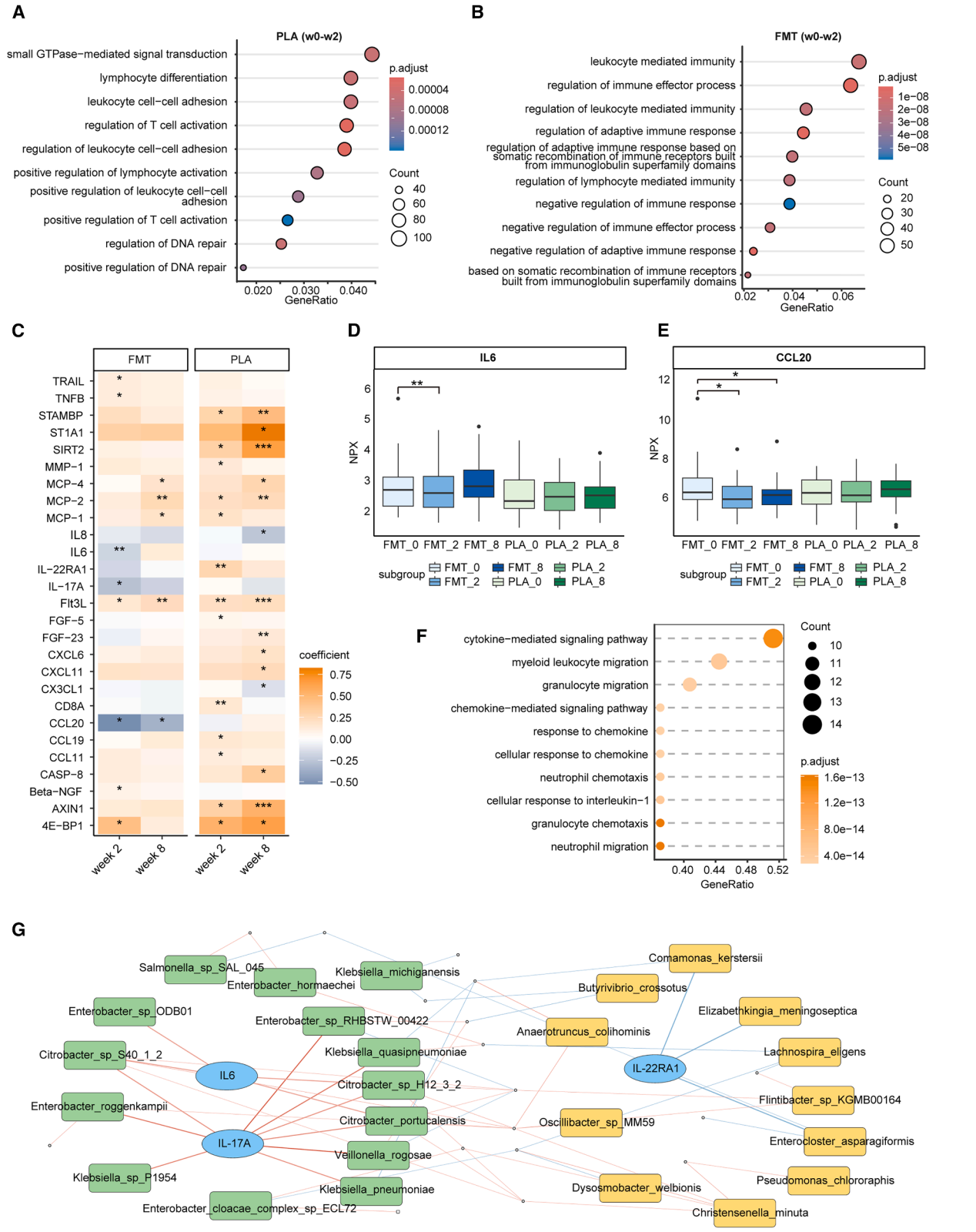

Figure 5. Transcriptomic and proteomic analyses suggest reduced inflammatory signaling in the FMT group relative to the PLA group

(A and B) Functional enrichment analysis of the DEGs identified from whole-blood RNA sequencing in the PLA group (A) and in the FMT group at weeks 0–2. (B) Bubble size indicates the number of genes involved, and the bubble color represents the adjusted *p* value.

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DISCUSSION

This randomized controlled trial evaluated the antidepressant efficacy and safety of adjunctive oral capsule FMT in a clinical setting. We observed a higher remission rate at week 8 in the FMT group (43.3%) than in the PLA group (26.7%), although this difference did not reach statistical significance. However, as early as week 2, the response rate based on HAMD-17 scores was significantly higher in the FMT group than in the PLA group (30.0% vs. 6.7%). Furthermore, adjunctive FMT demonstrated a favorable safety profile, with no statistically significant differences in the incidence of AEs between the two groups. These results suggest that oral FMT may be a feasible, effective, and safe adjunctive therapeutic strategy for patients with MDD.

Our trial differs from previously reported studies, which have focused primarily on patients with depression comorbid with irritable bowel syndrome.[27,35] In those studies, FMT was applied mainly to alleviate gastrointestinal symptoms, and the improvement in depressive symptoms was likely a secondary outcome resulting from the amelioration of gastrointestinal conditions. In contrast, all the patients included in our study were diagnosed with MDD without comorbid gastrointestinal diseases. To date, two case reports have suggested that adjunctive FMT therapy can improve depressive symptoms in MDD patients within 2 weeks,[28,29] which is similar to our observations. Additionally, a pilot randomized, double-blinded, PLA-controlled trial exploring the feasibility, acceptability, and safety of FMT as an adjunct therapy for MDD established that enema-delivered FMT was feasible, acceptable, well-tolerated, and safe in patients with MDD. However, this study included only 10 participants in the FMT group and was not designed to evaluate clinical outcomes.[30] Our study uses a randomized controlled trial to demonstrate that FMT can increase the acute-phase efficacy of antidepressants and significantly accelerate the onset of their therapeutic action. These results indirectly corroborate our earlier hypothesis that modulation of the gut microbiota may increase the efficacy of antidepressants.[21] A recently published preclinical study further supports our findings, showing that adjunctive FMT therapy can improve depression-like behaviors in chronic restraint stress mice that do not respond to SSRIs.[36] Notably, the primary efficacy endpoint of our clinical study, the difference in remission rates between the FMT and PLA groups at week 8, was not statistically significant. This result may be due to the limited sample size and the short duration of FMT intervention. Additionally, biological sex was balanced between the treatment groups at baseline and was adjusted as a covariate in our statistical models. We observed no significant association between biological sex and treatment outcomes or sex-by-treatment interaction. Future studies should aim to enroll larger cohorts and consider extending the FMT treatment period.

We performed an in-depth analysis of the metagenomic data and identified Lachnospiraceae and Oscillospiraceae as the key microbial taxa that mediate the antidepressant-enhancing effects of FMT. Both Lachnospiraceae[32,37,38] and Oscillospiraceae[39] have been consistently reported to be depleted in depression, which suggests that restoring their ecological niches may be a primary therapeutic target of FMT. To ensure the robustness of these findings, we integrated multiple analytical tools (DESeq2 and ALDEx2). While these methods differ in their statistical conservatism, the biological directionality of our key candidates remained consistent across both platforms. This methodological consensus reinforces the reliability of our taxonomic identifications beyond simple p value thresholds, thus highlighting the importance of incorporating robust, multi-tool approaches in future microbiome research.

We also found that the FMT-induced microbial remodeling was accompanied by shifts in bile-acid profiles, with the taxa enriched after FMT generally correlating positively with multiple bile acids. This pattern is consistent with the concept that gut microbes shape the bile-acid pool. Microbial deconjugation, which prevents the active reabsorption of bile acids in the small intestine via the apical sodium-dependent bile-acid transporter (ASBT), further influences bile-acid metabolism.[40] Furthermore, hepatic bile-acid synthesis (via Cyp7a1) is tightly controlled by negative feedback from the ileal farnesoid X receptor (FXR),[41,42] which is a signaling axis known to be disrupted in germ-free models.[3] Our findings further highlight bile-acid metabolism as a potential pathway, offering a different perspective for understanding the mechanisms of SSRIs within the framework of "microbiota-lipid-host" interactions.

Building upon these microbial and metabolic foundations, our host transcriptomic and proteomic data further nominate systemic immune regulation as a potential downstream effector pathway. We observed that adjunctive FMT induced a marked attenuation of inflammatory and chemotaxis-related signals. Notably, this immune regulation was most pronounced during the early phase of treatment (week 2), which perfectly coincided with the window of maximal clinical separation between the FMT and PLA groups. Bile acids are well-recognized as potent signaling molecules that can suppress nuclear factor κB (NF-κB)-dependent pathways and exert systemic anti-inflammatory effects through the activation of host receptors such as FXR, Takeda G protein–coupled receptor 5 (TGR5), and pregnane X receptor (PXR).[43,44] Our multi-omics readouts converge with

(C) Heatmap of differential inflammation-related proteins measured by the Olink inflammation panel in the FMT and PLA groups at weeks 2 and 8 relative to baseline. The color scale represents the regression coefficients estimated by linear models, with orange indicating increases and blue indicating decreases. Asterisks denote significance relative to baseline (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; Benjamini-Hochberg correction).

(D and E) Longitudinal changes in the Olink NPX (log_2-scaled normalized protein expression) levels of representative inflammation-related proteins, IL-6 (D) and CCL20 (E), in the FMT and PLA groups.

(F) Bubble plot of pathway enrichment based on the differentially expressed proteins, with the bubble size representing the number of proteins and the color indicating the adjusted p value.

(G) Correlations between significantly altered gut microbial taxa during weeks 0–2 following FMT (yellow, FMT-enriched taxa; green, FMT-depleted taxa) and inflammatory factors (blue nodes). The edge colors represent the direction of correlation: red indicates a negative correlation, and blue indicates a positive correlation. Correlations were determined using Spearman's analysis with thresholds of $|rho| > 0.15$ and $p < 0.05$.

Abbreviations; FMT, fecal microbiota transplantation; PLA, placebo.

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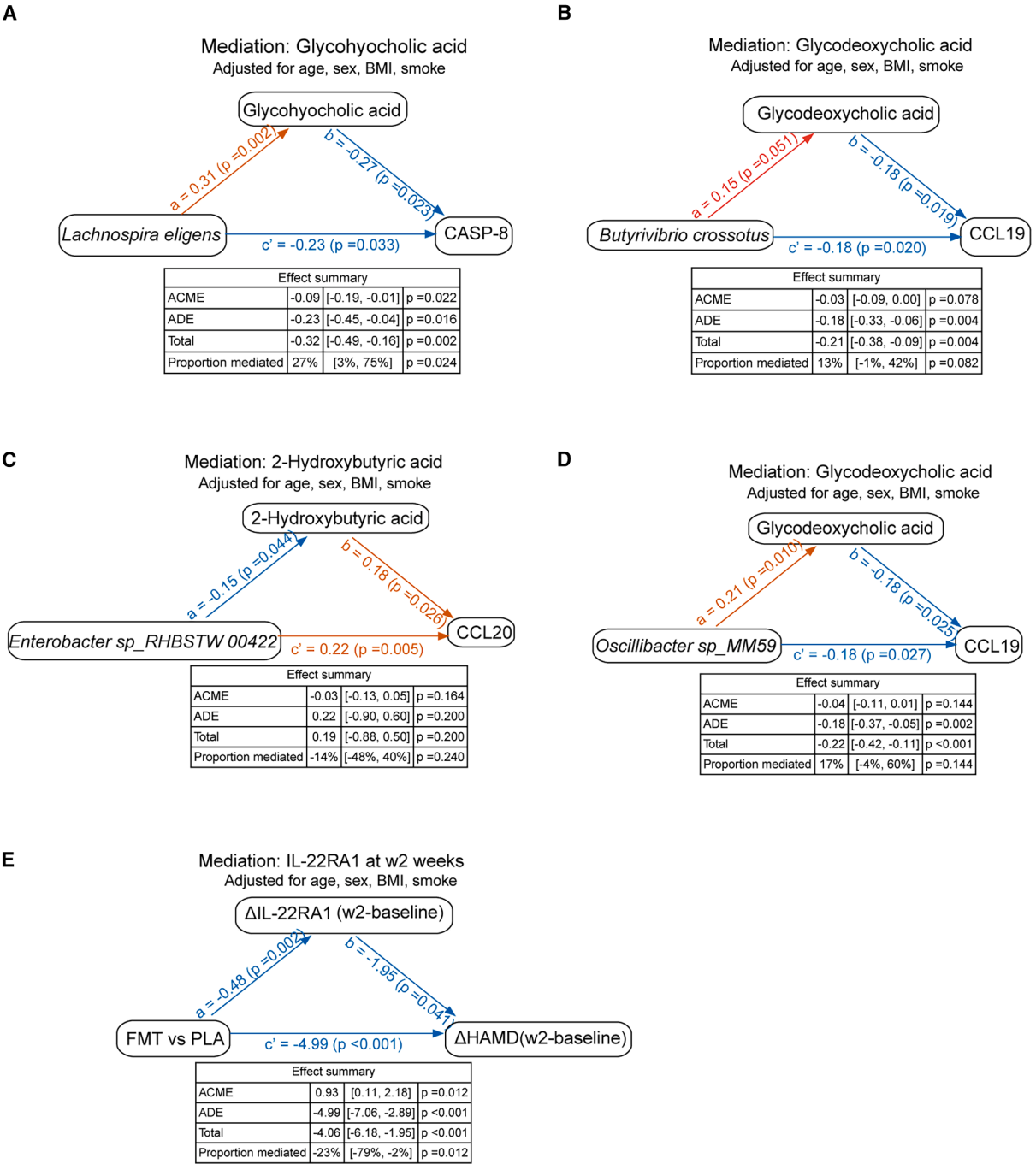

Figure 6. Mediation analysis reveals microbial-metabolite-immune-clinical pathways of FMT efficacy

(A–D) Beneficial taxa enriched by FMT (*L. eligens*, *Butyrivibrio crossotus*, and *Oscillibacter* spp.) were linked to reduced pro-inflammatory mediators through bile-acid metabolites, including GCA and GCDCA.

(E) Clinically, FMT reduced depressive symptoms (ΔHAMD) compared with PLA, partly mediated by changes in IL-22RA1 at week 2.

Abbreviations; FMT, fecal microbiota transplantation; PLA, placebo; HAMD, Hamilton Depression Rating Scale.

this framework. In inflammatory proteomics, the FMT arm showed an early shift toward a lower inflammatory tone and reduced chemotaxis signatures, in contrast to the PLA arm, which exhibited early increases in several chemokines and migration-related proteins. At the transcriptomic level, early gene expression changes in PLA participants were enriched for pathways related to immune activation and cell trafficking, whereas FMT recipients showed a profile more compatible with immune regulation and the restoration of homeostasis. While causality cannot be established from these observational

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layers alone, the consistency across independent platforms strengthens the inference that bile-acid-linked immune modulation is a plausible contributor to the enhanced antidepressant response observed with adjunctive FMT.

To quantitatively evaluate this hypothesis, we employed mediation analysis. Although mediation modeling does not prove a causal direction, it provides a rigorous statistical framework to assess whether these multi-omics shifts position logically along a mechanistic chain linking the intervention to symptom improvement. Within this framework, we identified a coherent, statistically significant cascade linking FMT-associated microbial changes, specific bile-acid elevations, and the downstream modulation of inflammatory mediators. Specifically, mediation analyses identified a microbiota-bile acid-inflammation axis (GCA linking *L. eligens* to CASP-8) and IL-22RA1 as plausible intermediates of FMT-associated symptom improvement. CASP-8 serves as a pivotal hub linking apoptotic programs with inflammatory signaling,[45] whereas IL-22RA1 points to a core, immune-relevant receptor axis in the intestinal epithelium.[46,47] Together, these nodes provide biological plausibility for the mediation structure, which links the FMT-driven microbial and bile-acid remodeling to host immune regulation.

In addition to bile-acid metabolism, we observed significant alterations in nucleotide metabolism (e.g., uridine, 5′-methylthioadenosine, and xanthine), aromatic amino acid derivatives (e.g., serotonin and 3-hydroxyhippuric acid), and lipid metabolism intermediates (notably multiple acylcarnitines). These changes highlight that FMT not only modulates lipid-host signaling but also broadly reshapes host energy metabolism, neurotransmitter pathways, and oxidative stress responses. Although the mechanistic relevance of these findings requires further investigation, they provide additional clues for understanding the systemic impact of microbiota-targeted interventions in depression.

Limitations of the study

This study has several limitations. First, given the single-center design, small sample size, and limited follow-up of this study, further validation through multi-center, large-scale research is required to confirm the present findings and establish the long-term efficacy and safety of FMT as an adjunct therapy. Second, since escitalopram is the most widely used SSRI in clinical practice,[48] it was combined with FMT as a representative regimen. However, it remains unclear whether the observed effects are specific to the interaction with SSRIs or can be generalized to other antidepressant treatments. Third, although a 2-week course of FMT intervention temporarily increased the abundance of certain bacterial species, their levels returned to baseline after discontinuation. This indicates that a longer duration of FMT administration may be required to promote the sustained colonization of these bacterial strains. Fourth, the lack of fecal metabolomics data precluded the direct characterization of luminal metabolic changes, thereby limiting a more granular mechanistic interpretation of how microbial shifts affect local metabolite dynamics. Finally, the mechanisms through which FMT enhances antidepressant efficacy are highly complex. Although we have proposed potential pathways from a multi-omics perspective, the specific underlying mechanisms still require thorough validation by preclinical studies.

In conclusion, this randomized, double-blind, PLA-controlled trial demonstrates that adjunctive FMT enhances the antidepressant efficacy of escitalopram in patients with MDD, accompanied by transient engraftment of donor-derived taxa, bile-acid-centered metabolic remodeling, and attenuation of systemic inflammation, with multi-omics analysis identifying a gut microbiota-bile acid-inflammation axis as the possible underlying mechanism. Our findings underscore the significant role of gut microbiota in modulating therapeutic responses to antidepressant treatment, which suggests that microbiome-targeted interventions may represent a viable strategy for optimizing therapeutic outcomes in MDD.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jian Yang (yangjian@ccmu.edu.cn).

Materials availability

This study did not generate any new, unique reagents.

Data and code availability

- The data on whole-blood gene expression, fecal metagenomics, and serum metabolomics generated during this study have been deposited in the National Genomics Data Center (https://ngdc.cncb.ac.cn/). The accession number is PRJCA051109.
- The source code supporting the findings of this study has been archived at Zenodo (https://doi.org/10.5281/zenodo.20045745) and is also available at GitHub (https://github.com/ADYYomics/FMT_omics).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

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AUTHOR CONTRIBUTIONS

Study design and supervision, G.W., J.Y., and J.Z.; patient recruitment and clinical assessments, J.Z., R.L., X. Zhu, and L.Z.; sample collection and data curation, R.L., X. Zhu, J. Li, Y.W., J. Liu, X. Zhang, S.R., H.W., and M.L.; statistical analysis, H.H., J.Z., X. Zhu, J. Li, and Y.W.; manuscript drafting, J. Li, Y.W., H.H., X. Zhu, and J.Z.; revised the paper for intellectual content, G.W. and J.Y.; administrative, technical, or material support, X.L., P.Z., Z.S., Y.H., F.W., and Y.L.; and all authors critically revised the manuscript for important intellectual content.

DECLARATION OF INTERESTS

J.Y. has received research funding from Shenzhen JCY Biotech Ltd., the source involved in the manufacture and marketing of FMT capsules. Shenzhen

JCY Biotech Ltd. had no role in the study design, data collection, data analysis, data interpretation, or in the manuscript writing.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human fecal samples from study participants	This paper	N/A
Human peripheral blood samples from study participants	This paper	N/A
Chemicals, peptides, and recombinant proteins		
TRIzol reagent	Invitrogen	15596026
Critical commercial assays		
MagPure Soil DNA LQ Kit	Magen	D6356-02
VAHTS Universal DNA Library Prep Kit for Illumina V3	Vazyme,	ND607
AMPure XP Beads	Beckman Coulter	A63880
VAHTS Universal V6 RNA-seq Library Prep Kit	Vazyme	NR604
Deposited data		
Raw sequencing and metabolomics data	This paper	NGDC: PRJCA051109
Software and algorithms		
R	R Core Team	V4.5.2 https://cloud.r-project.org/
Cytoscape	Cytoscape Consortium	v3.9.1 https://github.com/cytoscape/cytoscape
AB SCIEX Analyst	AB SCIEX	v1.7.3 https://sciex.com/products/software/analyst-tf-software
fastp	Chen et al.[49]	v0.23.2 https://github.com/OpenGene/fastp
Trimmomatic	Bolger et al.[50]	v0.36 https://github.com/usadellab/Trimmomatic
Bowtie2	Langmead and Salzberg[51]	v2.2.9 https://bowtie-bio.sourceforge.net/bowtie2/index.shtml
BWA	Li Lab (Broad Institute)	v 0.7.19-r1273 https://github.com/lh3/BWA
Samtools	Li Lab (Broad Institute)	v1.10 https://github.com/samtools/samtools
vegan	CRAN	v2.7-2 https://github.com/vegandevs/vegan
ggplot2	CRAN	v4.0.1 https://github.com/tidyverse/ggplot2
Limma	Bioconductor	v3.66.0 https://bioconductor.org/packages/3.22/bioc/html/limma.html
clusterProfiler	Bioconductor	v4.18.4 https://bioconductor.org/packages/3.22/bioc/html/clusterProfiler.html
Olink® Analyze	Olink	V3.0.0 https://cran.r-project.org/src/contrib/Archive/OlinkAnalyze/
mediation	CRAN	v4.5.1 https://cran.r-project.org/web/packages/mediation/index.html
tidyverse	CRAN	v2.0.0 https://github.com/tidyverse/tidyverse
ALDEx2	Bioconductor	v1.42.0 https://bioconductor.org/packages/3.22/bioc/html/ALDEx2.html
DESeq2	Bioconductor	v1.50.2 https://bioconductor.org/packages/3.22/bioc/html/DESeq2.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design

This single-center, double-blind, randomized, parallel-controlled clinical trial was designed to compare the efficacy and safety of escitalopram combined with FMT in the treatment of MDD. The study was carried out at Beijing Anding Hospital in Beijing, China. The clinical protocol and informed consent forms were reviewed and formally approved by the Ethics Committee of Beijing Anding Hospital, Capital Medical University (Approval No. (2023) Keyan Di (29)). The study was registered on the Chictr.org.cn website before

enrollment (ChiCTR2300071421, https://www.chictr.org.cn/index.html). There was no change in the protocol during the study. The trial was carried out at Beijing Anding Hospital in Beijing, China. A total of 60 eligible participants of Han Chinese ancestry (22 males and 38 females) were recruited and randomly assigned in a 1:1 ratio to either the experimental group (FMT group) or the control group (PLA group). The trial spanned 8 weeks, encompassing the screening phase, baseline assessment phase, 2 weeks of intervention phase, and 6 weeks of follow-up phase. All patients provided written informed consent prior to enrollment. The trial was completed on reaching predetermined target enrollment numbers. Efficacy outcomes included assessments of depressive symptoms and social functioning, while safety was evaluated through the monitoring of AEs and scale-based assessments.

Participants

An individual must satisfy all the following inclusion criteria to be included in the study: (1) Conforms to the diagnostic criteria for MDD as stipulated in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5); (2) Outpatients or inpatients aged between 18 and 65 years (inclusive of 18 and 65 years), without gender limitations; (3) body mass index (BMI) ranging from 18 to 24 kg/m^2; (4) Patients who have refrained from taking antidepressant medication for a minimum of 14 days prior to enrollment (for fluoxetine, at least 28 days); (5) A total score of 17 or above on the 17-Item Hamilton Rating Scale for Depression (HAMD-17); (6) Patients who possess the ability to comprehend and adhere to the research requirements and sign an informed consent form.

Individuals meeting any of the following exclusion criteria shall be excluded from the study: (1) Current or past diagnosis of mental disorders other than MDD as diagnosed in accordance with the Mini International Neuropsychiatric Interview (MINI) diagnostic criteria; (2) History of current or past substance abuse; (3) Score of Item 3 of HAMD-17 (the suicide risk item) being $\geq$3, indicating patients with severe suicidal tendencies; (4) Presence of notable psychotic symptoms (delusions, hallucinations); (5) Current severe or unstable conditions necessitating medication or surgical treatment; (6) Current medical conditions such as acute infectious diseases; (7) Patients with gastrointestinal organic diseases caused by infection, neoplasm, or other structural abnormalities, including but not limited to irritable bowel syndrome, Crohn's disease, ulcerative colitis, and celiac disease; (8) Patients with a history of gastrointestinal surgery; (9) Current or recent (within 3 months before inclusion) medical use of antibiotics, probiotics, or prebiotics; (10) Individuals with a history of allergy or ineffectiveness to escitalopram intake; (11) Patients allergic to capsule ingredients or contents; (12) Patients using antipsychotics, antidepressants, and mood stabilizers with a pre-baseline wash-out period of less than 5 half-lives; (13) Pregnancy or lactation; (14) Inability (e.g., dysphagia) or unwillingness to swallow capsules; (15) Patients who have received modified electroconvulsive therapy (MECT) in the past six months.

METHOD DETAILS

Trial sample size

Based on the primary hypothesis of this study, the FMT group was expected to demonstrate a higher remission rate than the placebo group after 8 weeks of treatment. As an exploratory interventional trial designed to evaluate the efficacy of the investigational treatment, and in accordance with the statistical design conventions commonly adopted in similar phase II clinical studies conducted both domestically and internationally,[52] a target sample size of 30 participants per group was planned. Because this study was designed as a superiority trial to test the unidirectional hypothesis that FMT would provide superior add-on efficacy compared with placebo, the sample size calculation and primary efficacy analyses were pre-specified using a one-sided significance level. To ensure that this exploratory study would provide reliable parameter estimates for subsequent confirmatory trials, sample size estimation was performed using PASS software based on the expected effect size (h), reflecting the anticipated difference in remission rates between the two groups. Assuming a moderate effect size (h = 0.5), 80% power, and a one-sided alpha level of 0.05, at least 29 participants per group were required. Therefore, a total of 60 participants were planned for enrollment.

Although the study sample size was determined based on the primary clinical endpoint (week-8 remission), we retrospectively evaluated the statistical power for the secondary multi-omics analyses using the framework proposed by Ferdous et al. (2022).[53] This framework posits that hypothesis-driven comparisons of specific microbial taxa (Node 5) require smaller sample sizes than broad community-level explorations. Given the observed effect sizes of our key discriminatory taxa (e.g., Lachnospiraceae), our sample size of 30 participants per group was estimated to provide adequate statistical power (>80%) to detect these specific biological signatures. Furthermore, to control for the high dimensionality of the data, all differential analyses were corrected for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method.

FMT and PLA capsule preparation

The FMT capsules containing freeze-dried fecal microbiomes were prepared by Shenzhen JCY Biotech Ltd. Healthy donors were pre-screened in accordance with international guidance.[54,55] To rule out underlying psychopathology, donors were further evaluated using the Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS), with all scores falling well below the clinical cut-off of 50. Fresh fecal samples collected in sterile sealed bags were transported to the laboratory within two hours of production and processed immediately upon receipt. The fecal sample was first diluted with sterile 0.9% w/v NaCl solution at a ratio of 1 g of sample to 2 ml of solution. Trehalose (GMP Grade) was then added to achieve a concentration of 0.1 g per gram of original sample. The mixture was homogenized in a sterile sealed bag using a bag mixer. The suspension was then filtered using 100 and 400 mesh sterile, single use poly-propylene coarse sieves. The filtrate was further centrifuged at 400 g for 10 min to remove unwanted smaller matter. The pellet was removed and the supernatant was collected into sterile ice cube trays. The trays containing processed fecal microbiota

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suspension were frozen at -80°C for 2 h before lyophilization. The lyophilization was carried out using a freeze dryer at -65°C and 0.06 Pa for 48 to 72 h. Freeze-dried FMT powder was filled into acid-resistant size 0 capsules (Huangshan Capsule) and saved at -80°C before use. The PLA capsules were prepared by filling the same capsules with food-grade starch. The FMT and PLA capsules were transported to the recipients in insulation boxes with blue ice.

Interventions

Eligible patients were randomly assigned to either a FMT group or a PLA group for a two-week intervention period. Both groups were administered escitalopram, accompanied by either active or PLA capsules that were identical in terms of appearance, packaging, and administration protocol. All capsules (whether containing active FMT substances or PLA) were to be ingested orally during breakfast on an empty stomach, swallowed with warm water, once daily (two capsules per intake) for 14 consecutive days. The active capsules contained lyophilized microbiota powder, whereas the PLA capsules contained starch. Both types of capsules were supplied in vacuum-sealed boxes, with each box containing 14 capsules. Each individual capsule had a length of 22.5 mm, a weight of 0.42 g, a blue outer shell, and a brown, odorless solid filling.

Both groups initiated treatment with a daily dosage of 5 mg of escitalopram. On the fifth day of treatment, the dosage was escalated to 10 mg per day, and this dosage was sustained until the conclusion of the two-week period. Subsequently, the follow-up treatment persisted with escitalopram monotherapy until the end of the eighth week. The treating physician adjusted the treatment dosage as necessary, while keeping the daily dosage within the range of 10 mg to 20 mg.

Measures

This study employs a comprehensive set of research tools to facilitate in-depth data collection. Diagnostic evaluations are conducted using the Mini-International Neuropsychiatric Interview, version 7.0.2 (MINI 7.0.2). Demographic information, such as date of birth, gender, height, weight, educational level, and hand preference, is systematically recorded. Medical histories are obtained to capture essential details, including the duration of the current depressive episode. Physical examinations are performed across multiple domains: head, face, skin, lymph nodes, eyes, ears-nose-throat, oral cavity, respiratory, abdominal, cardiovascular, musculoskeletal, and neurological systems. A battery of standardized scales is utilized for comprehensive evaluation at baseline, week 2, week 4, and week 8, comprising the HAMD-17[56]; the GAD-7[57] and the GSRS.[58] The HAMD-17 is a clinician-rated measure of depressive symptom severity over the past week; item ratings (scored on either 0–2 or 0–4 scales depending on the item) were summed to yield a total score (generally 0–52), with higher scores indicating more severe depression. Clinical remission was defined as a HAMD-17 total score $\leq$7, a commonly used threshold in clinical trials,[5,59] and clinical response was defined as a $\geq$50% reduction in the HAMD-17 total score from baseline, reflecting substantial symptomatic improvement.[60] The GAD-7 is a 7-item self-report measure assessing anxiety symptom frequency over the past two weeks; each item is scored from 0 to 3, and total scores range from 0 to 21, with higher scores indicating greater anxiety severity. The GSRS is a 15-item measure of gastrointestinal symptom severity over the past week; each item is originally rated on a 7-point Likert scale and was coded from 0 to 6 in the present study, with total scores calculated by summing item responses, where higher scores indicate more severe gastrointestinal symptoms.

Additionally, the Eating Behavior Questionnaire (EBQ), adapted from prior research,[61] was administered at baseline and week 8 to assess dietary habits, with particular attention to major dietary patterns relevant to gut microbiota composition, including high-fat, high-salt, and high-protein intake. Safety and tolerability were assessed through the monitoring of AEs, vital signs, clinical laboratory tests, and electrocardiogram parameters. SAEs were defined as any untoward medical occurrence that resulted in death, was life-threatening, required prolonged inpatient hospitalization, or resulted in persistent or significant disability/incapacity. The relatedness of AEs/SAEs to the study intervention was assessed by the investigators under blinded conditions, based on temporal association, alternative etiologies, dechallenge information when applicable, and biological plausibility/known safety profile. Together, these rigorous and multifaceted instruments ensure a thorough and precise assessment of the study outcomes.

Outcome Measures

The primary clinical endpoint was the difference in remission rates between the two groups at week 8. Secondary endpoints included the difference in response rates between the two groups at weeks 2 and 8, as well as changes from baseline to week 8 in HAMD-17, GAD-7, GSRS, and HAMD anxiety/somatic subscale scores. The HAMD-17 anxiety/somatic subscale score was calculated by summing six HAMD-17 items (items #10, #11, #12, #13, #15, and #17), representing anxiety and somatic symptom domains, based on the factor structure of the HAMD identified in previous studies.[62] Higher scores indicate greater severity of anxiety and somatic symptoms. AEs were recorded at every study visit over the 8-week trial period.

Metagenomics of fecal samples

DNA extraction, library construction, and metagenomic sequencing of fecal samples were conducted as previously reported.[21] Genomic DNA was extracted from fecal samples using the MagPure Soil DNA LQ Kit (Magen, D6356-02, China) in accordance with the manufacturer's instructions. After extraction, the concentration and purity of the DNA were evaluated by NanoDrop 2000 and agarose gel electrophoresis. The DNA was fragmented to an average length of approximately 300 base pairs using the Covaris S220 ultrasonicator. End repair, A-tailing, and adapter ligation were executed using the VAHTS Universal DNA Library Prep Kit for Illumina V3 (Vazyme, China). PCR amplification was followed by purification with AMPure XP beads (Beckman Coulter, USA). The size distribution of the library was assessed with a Bioanalyzer 2100 (Agilent, USA), and the concentration was measured using a

Qubit fluorometer (Thermo Scientific, USA). Qualified libraries were subjected to 150 bp paired-end sequencing on the Illumina NovaSeq platform, with each sample generating no less than 6 Gb of data.

Randomization and blinding

A randomization plan was developed by an independent statistician according to the study protocol. Using the block randomization method, a random number table was generated with the PLAN procedure in SAS 9.4 software (Windows system). Each enrollment number was linked to an emergency envelope, which was retained by the research staff. To ensure blinding, a study technician prepared sequentially numbered packages that concealed all information regarding the study medication. Both FMT capsules and matching PLA capsules, identical in size and color, were packaged identically across all trial groups. The success of blinding was evaluated by asking participants to guess their assigned group at the end of the study.

Sample collection

Blood and stool specimens were collected from enrolled subjects at baseline, week 2, and week 8. Serum samples were obtained using clot-activator tubes, while whole blood for RNA analysis was collected in PAXgene® Blood RNA Tubes (762165, BD Biosciences, USA). Fecal specimens were collected using specialized containers with integrated sampling spoons (SARSTEDT, Australia).

In the PLA group, one participant did not provide a blood sample at week 8, while two participants failed to provide stool samples at week 2 and week 8, respectively. In the FMT group, two participants did not provide either blood or stool specimens at both week 2 and week 8. Additionally, one participant missed the blood collection and two participants missed the stool collection at week 8. All remaining participants completed all scheduled collections of both fecal and blood samples. In total, 174 serum and whole blood RNA samples and 172 fecal specimens were collected.

Targeted metabolomics of serum samples

Metabolite quantification was quantified using the High-throughput targeted metabolomics platform, which integrates four complementary LC-MS/MS methods to quantify approximately 600 metabolites in biological fluids and tissues.[63,64] Metabolites were categorized into assay panels according to their chemical properties and physiological concentration ranges. All samples underwent four rounds of extraction, and pooled quality control (QC) samples were prepared in parallel to monitor and control batch effects. For each extraction, 20–50 μL of serum was mixed with pre-cooled methanol or methanol/acetonitrile solution containing isotope-labeled internal standards for protein precipitation. After vortexing and centrifugation, the supernatant was collected for LC-MS/MS analysis. For Assay 3, metabolites containing carboxyl or aldehyde groups were further derivatized with 3-nitrophenylhydrazine prior to analysis.

Specifically, Assay 1 quantified 133 polar endogenous metabolites, including amino acids, nucleotide bases, oligopeptides, vitamins, as well as choline and carnitine derivatives, using hydrophilic interaction chromatography (HILIC). Assay 2 analyzed 57 bile acids and 54 fatty acids, which were separated via reversed-phase chromatography and detected in negative electrospray ionization (ESI) mode. Assay 3 encompassed 86 highly polar metabolites, such as short-chain fatty acids, intermediates of the tricarboxylic acid (TCA) cycle and glycolysis, sugars, and organic acids, which were derivatized with 3-nitrophenylhydrazine before analysis. Assay 4 targeted 108 microbial-associated metabolites, including polyamines and catabolites of tryptophan, tyrosine, and phenylalanine.

Quantitative data obtained from Panels 1, 3, and 4 were analyzed using Analyst 1.7.3 and OS-MQ software (AB SCIEX, Singapore), and metabolite quantification was performed based on stable isotope internal standard curves. Data from Panel 2 were analyzed using TraceFinder (Thermo Scientific, USA), with metabolites quantified via internal standard calibration. The m/z extraction window for product ions was set at 10 ppm. Metabolic features exhibiting a coefficient of variation greater than 30% in QC samples were excluded from further analysis. Metabolites with missing values exceeding 50% in any group were removed, and the remaining missing values were imputed using the K-nearest neighbor (KNN) algorithm (sample-wise).

Transcriptomic analysis of whole blood by RNA-Seq

Total RNA was extracted from whole blood using the TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's instructions. RNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Subsequently, libraries were constructed with the VAHTS Universal V6 RNA-seq Library Prep Kit (NR604, Vazyme, China) according to the manufacturer's protocol. Transcriptome sequencing and analysis were performed by BioMiao Biological Technology Co., Ltd (Beijing, China). The pooled libraries were sequenced on an Illumina Novaseq 6000 platform, generating approximately 49 million paired-end reads (150 bp) per sample.

Inflammatory proteomic profiling of serum

The proximity extension analysis (PEA) technology of Olink® proteomics[65] (Uppsala, Sweden) was employed to detect 96 inflammation-associated protein biomarkers in serum. Experiments were conducted in accordance with the manufacturer's guidelines. Samples were initially thawed at 4°C, followed by vortexing and centrifugation at 400 × g for one minute. One microliter of the supernatant was mixed with 3 μL of the incubation mixture, which comprised probes, incubation solution, and stabilizer, and then incubated overnight at 4°C. On the following day, 96 μL of the extension mixture, containing PEA solution, PEA enzyme, and PCR polymerase, was introduced to generate a unique PCR target sequence. The obtained DNA sequence was detected and quantified using a microfluidic

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real-time PCR instrument (Signature Q100, Olink®). The acquired Ct data are subjected to quality control and normalization through the utilization of both internal and external controls. The ultimate results of the assay are presented as Normalized Protein Expression (NPX) values. This metric, which encompasses normalization and log2 scaling, is a specialized unit developed by Olink®. Higher values of NPX signify elevated protein expression.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical Analysis of Clinical Data

Statistical analyses were performed using SAS version 9.4. Randomization groups were unblinded only after completion of all statistical analyses. The primary and secondary endpoints were analyzed using the FAS and PPS, with the primary conclusions based mainly on the FAS. The definitions of the analysis sets were as follows:

ITT Set

All randomized participants analyzed according to their original group assignment, regardless of treatment adherence or subsequent withdrawal, thereby preserving the integrity of randomization.

FAS

Based on the modified intention-to-treat (mITT) principle, all randomized participants who received at least one dose of the study treatment and completed the baseline HAMD-17 assessment.

PPS

A subset of the FAS comprising participants who completed the study without major protocol violations (e.g., deviations from eligibility criteria, unapproved concurrent interventions, or failure to complete the primary outcome follow-up).

Categorical clinical outcomes, including the proportion of remission at week 8 (defined as a HAMD-17 total score $\leq$7) and the proportion of response at weeks 2 and 8 (defined as a $\geq$50% reduction in HAMD-17 total score from baseline), were compared using the Chi-square test or Fisher's exact test, as appropriate. For the primary efficacy outcome, statistical significance was assessed using a one-sided test. Changes in paired categorical dietary variables between baseline and week 8 were assessed using Bowker's test of symmetry. For continuous longitudinal outcomes (e.g., HAMD-17 total scores), changes over time were assessed using linear mixed-effects models (LMMs). In these models, treatment group (FMT vs. placebo), time (week 2 to week 8), and the group-by-time interaction were included as fixed effects. To account for within-subject correlations across repeated measures, each participant was modeled as a random intercept, and an unstructured covariance matrix was specified for the within-subject residuals. Furthermore, to ensure the robustness of our findings, sensitivity analyses were conducted by incorporating predefined baseline covariates into the LMMs, including age, sex, illness onset pattern (first episode vs. recurrence), and duration of the current depressive episode.

Statistical Analysis of Metagenomics

Raw metagenomic sequencing reads in FASTQ format were first subjected to quality control. Adapter and low-quality sequence removal was performed using Trimmomatic v0.36,[50] with default parameters for paired-end Illumina data. To avoid host-derived contamination, reads were aligned to the host reference genome using Bowtie2 v2.2.9[51] and matching reads were discarded. Only non-host, high-quality reads were retained for downstream taxonomic profiling. Microbial taxonomic assignment was conducted using Centrifuge[66] against a custom-built abv reference index (include *Bacteria, Archaea, Viruses* genome reference). Classification results were further processed with kraken-mpa-report to obtain human-readable microbial abundance profiles. Individual sample reports were merged into a single abundance matrix using the merge_metaphlan_tables.py script (from MetaPhlAn package). From the merged taxonomic table, relative abundance was calculated at different taxonomic levels. Taxonomic tables were normalized by column sums to yield relative abundances.

Alpha diversity metrics were calculated using the vegan R package. Specifically, the Shannon index and Observed species richness were computed to assess within-sample diversity, and results were visualized across groups and timepoints. For beta diversity, pairwise distances were estimated using both Bray-Curtis dissimilarity and Euclidean distance. Principal Coordinate Analysis (PCoA) and non-metric multidimensional scaling (NMDS) were performed to visualize sample clustering patterns. To identify differentially enriched microbial taxa among groups, relative abundance tables were reformatted and subjected to LEfSe (Linear Discriminant Analysis Effect Size).[67] LEfSe was executed within a BioBakery Docker container using default settings, with logarithmic LDA score threshold set to 1.5. To further validate the robustness of these findings against the compositional nature of microbiome data, supplementary cross-validations were performed using DESeq2 and ALDEx2 in R. DESeq2[68] analysis was conducted on un-normalized, rounded count data, adjusting for patient covariates including sex and scaled age (design = $\sim$ age + sex + subgroup). The dispersion estimates were fitted using a local regression model (fitType = 'local'), and statistical significance was evaluated at an FDR threshold of 0.05. Concurrently, strict compositional data analysis was executed using the ALDEx2[69] package. The analysis generated 128 Monte Carlo Dirichlet instances (mc.samples = 128) using all features as the reference denominator (denom = "all"). For ALDEx2, effect sizes and expected p-values were calculated using paired tests to evaluate the consistency of the biological effect direction across methodologies.

Statistical Analysis of Targeted metabolomics

Differential abundance analysis was performed using Linear Models for Microarray Data (limma).[70] Briefly, data were log2-transformed and normalized across samples with normalizeBetweenArrays. For each time-series comparison (e.g., baseline vs. week

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2 or week 8, within FMT or PLA groups), linear models were fitted including subject IDs as blocking factors to account for repeated measures. Contrasts were set to evaluate post-treatment vs. pre-treatment differences. *P* values were corrected using the Benjamini–Hochberg method, and significantly altered metabolites (adjusted $P < 0.1$, absolute logFC > 0.5) were retained.

Statistical Analysis of Transcriptomic and Inflammatory Proteomic Data

Raw reads in FASTQ format were preprocessed with fastp[49] to remove low-quality reads, resulting in about 46 million clean reads per sample for subsequent analysis. Clean reads were aligned to the reference genome using HISAT2 with default parameters. Gene expression levels were quantified as FPKM using Cufflinks, and read counts per gene were obtained by HTSeq-count. Prior to differential expression analysis with DESeq2, principal component analysis (PCA) was conducted to assess the similarity and reproducibility among biological replicates. To functionally characterize the differentially expressed genes (DEGs), Gene Ontology (GO) enrichment analysis was carried out. This analysis was implemented with the clusterProfiler R package (v 4.18.4) to identify GO terms that were significantly over-represented among the DEGs. The enrichment results were subsequently visualized using the ggplot2. Quality control, data normalization, and quantification of protein levels for the analyzed samples were carried out using the Olink® Analyze R package.

Microbial strain-level genomic origin analysis after FMT

To trace the bacterial origin after FMT, we constructed a strain-level genomic reference and performed single nucleotide polymorphism (SNP)-based genotyping of microbial populations. We first obtained the assembly summary file from NCBI RefSeq and extracted the assembly accessions and species names. For each bacterial species that was commonly detected in both donors and recipients, one representative genome assembly was downloaded from NCBI Datasets. Genome sequences were combined into a multi-species reference, and a Burrows–Wheeler Aligner (BWA v 0.7.19-r1273) index was constructed. High-quality sequencing reads from donors and recipients were aligned against the reference genome using BWA mem with default parameters. Alignments were filtered using Samtools (v1.10) to retain mapping quality $\geq$20. Species-specific genomic regions were defined according to NCBI metadata, and SNP calling was performed using bcftools mpileup and bcftools call with minimum base quality $\geq$30 and read depth $\leq$50,000 to reduce false positives. Resulting VCF files were parsed using a custom Python script, which re-genotyped each sample at high-confidence SNP positions based on allelic depth (AD) and depth of coverage (DP). Samples were grouped according to their source: donors, recipients at baseline (pre-FMT), and recipients at 2 and 8 weeks post-FMT. For each SNP site, we assigned genotypes to one of four origin categories:

a) Colonizing−conspecific: present in the donor but not in recipient baseline.
b) Persistence−conspecific: present in the recipient baseline but not in donor.
c) Coexistence-conspecific: shared by both donor and recipient baseline.
d) Novel−conspecific: absent in both donor and baseline recipient.

This classification scheme allowed us to quantify, for each post-FMT sample, the relative proportions of bacterial strains that were donor-derived, recipient-derived, co-existing, or newly emerged.

Canonical correspondence analysis (CCA)

Gut microbiota taxonomic profiles were preprocessed by Hellinger transformation using the vegan R package.[71] Only significantly discriminant bacterial taxa identified by LEfSe (using LDA score > 1.5) were retained to reduce dimensionality. To enable multi-omics integration, metabolite and bacterial profiles were aligned at the level of matched sample IDs across serum metabolomics and microbiome datasets. Metabolite abundances were log-transformed with log1p before modeling. Associations between microbial communities and significantly altered serum metabolites were modeled using canonical correspondence analysis (CCA) implemented in vegan. Selected bacterial species (from LEfSe) were related to differentially abundant metabolites (from limma, *P*adj < 0.1). CCA models were tested for overall significance using permutation-based ANOVA. Loadings for bacteria (species), metabolites, and samples were extracted for visualization.

Mediation analysis of microbiota, metabolites, inflammation, and clinical outcomes

To examine whether metabolites mediate the effect of microbiota on host inflammatory responses, causal mediation analysis was performed with the mediation R package.[72] For each bacterium–metabolite–inflammatory protein triplet, the following models were fitted: Mediator model: metabolite ~ bacterium + covariates (BMI, age, sex, smoking status); Outcome model: inflammatory protein ~ bacterium + metabolite + covariates.

Bootstrapping (1,000 iterations) was used to estimate direct effects, indirect (mediated) effects, and the proportion mediated. Results were visualized with mediation triangle plots, denoting the pathways X→M→Y (bacteria→metabolite→inflammation). Additionally, longitudinal mediation models (baseline vs. week 2/8) were implemented by computing Δ (change) values for bacterial abundance, metabolites, inflammation markers, and clinical scores (e.g., HAMD-17). This allowed investigating whether metabolite changes mediate the impact of microbiota shifts on inflammatory and clinical outcomes over time.

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DIABLO Analysis

To integrate microbiome and metabolome data and validate findings from canonical correlation analysis, we performed Data Integration Analysis for Biomarker discovery using Latent components (DIABLO) using the mixOmics package (version 6.34.0) in R (version 4.5.1). The analysis integrated species-level microbial relative abundance data and metabolite abundance data from paired pre- and post-FMT samples. We applied block sparse partial least squares discriminant analysis (block.splsda) with a design matrix specifying full correlation between the two omics blocks (microbiome-metabolome correlation = 1, all other correlations = 0). The optimal number of components and features to retain was determined through 5-fold cross-validation repeated 20 times using centroid distance as the classification metric. An initial screening retained up to 10 features per component for each omics block across 5 components. Based on cross-validation performance, 2 components were selected for the final model. Correlations between features selected by the final DIABLO model were calculated using Pearson correlation. Associations with absolute correlation coefficients $|r| \geq 0.5$ were visualized using a circos plot. Given the paired study design where each individual serves as their own control, time-invariant individual characteristics (age, sex) were inherently controlled through the within-subject comparison and were not explicitly included as covariates in the model.