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ORIGINAL RESEARCH

Multi-Omics Reveal That Gut Microbial Dysbiosis Drives Lipid Metabolic Disturbances and Inflammation in Gestational Hypertension

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Background: Gestational hypertension (GH) is a common complication during pregnancy that poses serious health risks to both mother and fetus. Recent studies have underscored the potential roles of gut microbiota, lipid metabolism, and inflammatory response in GH's development and progression. However, the exact mechanisms behind these interactions are still unclear. Understanding how gut microbial composition impacts lipid metabolism and inflammation could offer valuable insights into GH's pathogenesis and may lead to new prevention and treatment methods.

Methods: In this study, we conducted ELISA experiments to detect inflammatory cytokines in the serum of GH patients. Additionally, we performed 16S-rDNA sequencing analysis on the feces of GH patients to investigate the characteristics of their intestinal microbial communities; GH mouse model was constructed to assess the impact of intestinal flora on offspring. Furthermore, we utilized non-targeted lipid metabolomics to analyze lipid metabolic characteristics in the feces and blood of GH patients and established connections between the microbiome and lipidome through correlation analysis.

Results: ELISA tests suggested the levels of inflammatory factors in the serum of GH patients increased significantly, including IL-6, IL-8, IL-17, IL-18, and IFN-γ. In comparison to the normal group, the GH group exhibited a marked reduction in microbial richness. LEfSe analysis found 16 distinct bacterial communities between the two groups. Animal models suggested that fecal microbiota transplantation from the GH group's intestinal flora resulted in a significant decrease in the birth weight of the offspring. Furthermore, comparative analysis of fecal and blood metabolic profiles suggested that TG (54:5/FA22:5) may serve as a key metabolite. Correlation analysis demonstrated that *f-Oxalobacteraceae* exhibited a significant negative correlation with the inflammatory factor IL-17 and TG (54:5/FA22:5) in the blood, while showing a significant positive correlation with *g-Oxalobacter* and *s-formigenes*.

Conclusion: Our results establish a connection between gut microbiota, lipid metabolism, and the inflammatory response in patients with GH. This understanding may enhance our comprehension of the underlying mechanisms associated with GH.

Keywords: gestational hypertension, lipid metabolism, gut microbes, inflammation

Introduction

Gestational hypertension (GH), defined as two episodes of blood pressure ≥140/90 mmHg occurring after 20 weeks of gestation, is a condition that arises during pregnancy.[1] If left uncontrolled, this condition may progress to preeclampsia. Preeclampsia can further escalate into eclampsia, characterized by seizure-like convulsions associated with hypertensive disorders during pregnancy. This condition represents the most severe complication of hypertensive disorders during pregnancy and poses significant risk to both maternal and fetal health.[2] Relevant statistics show that GH affects

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approximately 10% of pregnant women, with its incidence rate rising in recent years.[3] There is compelling evidence linking GH to fetal cardiovascular diseases and impaired nervous system development.[4] Furthermore, GH is recognized as one of the contributing factors to fetal maldevelopment.[5] Although various factors, including genetics, immunity, metabolism, and gut microbiota, have been investigated and reported to influence the progression of GH,[6–9] the precise pathogenesis of GH remains unclear.

The study of intestinal flora has provided insight into the identification of biomarkers and has introduced novel targets for disease treatment. The intestine, hosting over 2,000 species of microorganisms, represents the largest micro-ecosystem in the human body, interacting intricately with immune system, reproductive system, and both cardiovascular and cerebrovascular systems.[10–13] Intestinal flora can generate a variety of metabolites, including corticosterone, hydrogen sulfide (H_2S) and short-chain fatty acids (SCFAs), which have been demonstrated to be closely associated with the onset of hypertension.[14,15] SCFAs exert their physiological effects by activating specific G protein-coupled receptors. These receptors are widely expressed in the vascular, renal, and nervous systems, regulating vascular tone and inflammatory responses, thereby influencing blood pressure.[16,17] Profiling of gut microbiota in a rat model of preeclampsia revealed a significant reduction in α diversity and firm-walled flora during late pregnancy. Annotation via the Kyoto Encyclopedia of Genes and Genomes (KEGG) identified abnormal metabolic signaling pathways involving SCFAs, suggesting a connection between pregnancy-induced hypertension and gut microbiota dysbiosis.[18] Furthermore, evidence indicates that decreased intestinal flora diversity in patients with preeclampsia correlates with diminished butyrate production, and butyrate supplementation in a rat model has been shown to effectively alleviate hypertension symptoms.[19] Notably, a nested case–control study, observed that microbial dysbiosis in GH patients commenced during early pregnancy.[20] Collectively, these findings underscore the critical role of intestinal micro-ecological stability and its metabolites in GH.

Patients with GH exhibit lipid metabolism disorders. Lipids play crucial structural and metabolic roles in the body; disturbances in lipid processing are associated with the onset of various diseases.[21,22] A study has demonstrated that the lipid metabolism of the placenta in pregnant women with hypertension experiences significant alterations, characterized by increased levels of total cholesterol and triglycerides. Concurrently, the expression of genes with fatty acid oxidation and transport, including PPARα, CPT-1, CD36, and LPL, is diminished.[23] This abnormality may lead to impaired placental function,[24] subsequently affecting fetal growth and development. Research has indicated that patients with gestational hypertension frequently exhibit insulin resistance. The Homeostasis Model Assessment (HOMA) index, a measure of insulin resistance, shows a positive correlation with lipid metabolism indicators such as triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), while demonstrating a negative correlation with high-density lipoprotein cholesterol (HDL-C).[24] This relationship implies that disorders in lipid metabolism may further aggravate the pathological processes associated with gestational hypertension by influencing insulin sensitivity.

Excessive inflammatory responses during pregnancy are a pathological feature of GH.[25] Inflammation, which involves immune cells, blood vessels, and various molecules, is a complex resistance response initiated against harmful stimuli, mediated by a network of cytokines. Previous studies have reported that elevated serum levels of interleukin-6 (IL-6), IL-8, and tumor necrosis factor (TNF)-α in patients with GH.[25–27] Gut ecology may contribute to increased inflammation and hypertension; however, the relationship between these factors remains poorly understood. This study integrates 16S-rDNA sequencing with macro-target lipid sequencing and ELISA experiments to identify differential bacterial flora and metabolites, and to explore their interactions with inflammatory cytokines.

Materials and Methods

Patient Sample Acquisition

The inclusion criteria for patients with GH are as follows: Both standard blood pressure measurements show that the blood pressure is ≥ 140/90 mmHg. All pregnant women do not have other complications, such as hypertension, diabetes, heart disease, chronic nephritis or lupus. The assessment is conducted by the hospital's designated nurse during two or more separate visits throughout the pregnancy. During the 30 to 40th weeks of pregnancy, and serum specimens were collected through standard venipuncture methods. The normal group samples consisted of pregnant women with normal blood pressure during the same period and without the above medical history. Covariates were chosen a priori based on

previous studies, and included age, early pregnancy body mass index (BMI), pregnancy blood pressure, smoking history, and aspirin use during pregnancy. See Supplementary Table 1 for details.

Animal Model

Establishment of a mouse model of gestational hypertension by subcutaneous injection of Nω-nitro-L-arginine methyl ester hydrochloride.[28] During the research process, each group prepared 7 mice, randomly selected 5 mice for subsequent related experiments, and kept an additional 2 as backups to prevent mouse mortality during the experiments. The blood pressure of female mice was monitored according to the following method: blood pressure 7 days before cage placement, 3 days before cage placement, and at 8:00 a.m. on the day of gestation; blood pressure 2, 6, 10, 14, and 17 days after gestation. Those with vaginal plugs were recorded as 0.5 days of gestation. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of mice were measured using CODATM2 blood pressure measuring instrument (Shanghai Zande Medical Instrument Co., Ltd., Shanghai, China). In addition to measuring blood pressure, we also measured the expression levels of inflammatory factors in the blood of mice via ELISA.

After the model is successfully prepared, the feces of different groups were taken to prepare bacterial liquid for fecal microbiota transplantation (FMT). Collect 5 g of donor feces and mix with 50 mL of sterile PBS. Emulsify the mixture in an anaerobic chamber. Filter the supernatant using sterile gauze, ensuring thorough filtration 2–3 times. After mixing, aliquot into 15 mL sterile centrifuge tubes. The phenotypic information of different FMT groups was examined. Briefly, the intestinal microbiota of mice was depleted by antibiotic solution (neomycin 1 g/L, ampicillin 1 g/L, vancomycin 0.5 g/L, metronidazole 1 g/L, sterile saline) for one week, the daily dosage for each mouse is 200 μL. Then, Each gavage of FMT for gut microbiota reconstruction was administered at 5 mL, three times a week, for a duration of 4 weeks. After another two weeks, the mice were caged together. We collected newborn pups and measured their body weight using a small electronic balance (Beyotime, E0284, Shanghai, China).

Cytokines Assay by the Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA experiments were conducted on the obtained serum samples (including human serum and mouse serum).After centrifugation of the blood plasma, the cytokines were detected according to the instructions of the kit, including IL-6 (Fankew, F10316-A, Shanghai, China), IL-8 (Fankew, F10927-A, Shanghai, China), IL-17 (Fankew, F9770-A, Shanghai, China), IL-18 (Fankew, F0138-A, Shanghai, China), TNF-α (Fankew, F9770-A, Shanghai, China) and IFN-γ (Fankew, F0121-A, Shanghai, China). For mouse model, IL-6 (Fankew, F2163-A, Shanghai, China), IL-17 (Fankew, F2170-A, Shanghai, China), sENG (Fankew, F2005-A, Shanghai, China) and TGF-β (Fankew, F2686-A, Shanghai, China) were tested. Finally, the absorbance was measured at 450 nm using an enzyme reader and Graphpad Prism software (v8.0) was used for data statistics and difference detection.

16S rDNA Sequencing and Analysis Workflow

Conduct 16S rDNA sequencing analysis on fecal samples to assess the gut microbiota status. First, use 1% agarose gel electrophoresis to detect DNA sample degradation and contamination; use NanoPhotometer spectrophotometer to test sample purity; use Qubit 2.0 Fluorometer to measure DNA sample concentration. Take 10ng of DNA template and amplify the target region: select the corresponding amplification primers based on the sequencing region: V3 region primers are 338F-533R, V3+V4 region primers are 341F-805R, V6 region primers are 967F-1046R; use TaKaRa's EXtaq enzyme to ensure amplification efficiency and accuracy. Subsequently, the amplified target fragments were enriched, and specific index sequences were added. After library construction, preliminary quantification was performed using Qubit 2.0, and the library was diluted to 1 ng/μL. Then, the insert size of the library was detected using Agilent 2100. Upon confirming that the insert size met expectations, QPCR was conducted using the Bio-RAD CFX 96 Fluorescence Quantitative PCR Instrument and Bio-RAD KIT iQ SYBR GRN. Libraries that passed the Agilent 2100 detection were sequenced using MiSeq, with a sequencing mode of PE250.

For the sequences obtained from sequencing, we perform data filtering by removing low-quality bases, Ns, adapter contamination sequences, and other processes to obtain reliable target sequences for subsequent analysis. The filtered sequences are referred to as Clean Reads.;[29] First, the corresponding Read1 and Read2 of paired-end sequencing (Read1

and Read2 refer to the sequence fragments obtained from the 5′ and 3′ ends, respectively) are assembled using the sequence assembly method PEAR. Then, the assembled sequences are analyzed using the software QIIME version 1.8.0, including OTUs extraction, Linear Discriminant Analysis Effect Size (LEfSe) analysis, and α and β diversity analyses.[30]

Untargeted Liquid Chromatography-Mass Spectrometry (LC-MS)-Based Lipidomics

In this work, non-targeted lipidomics based on LC-MS was used. Of note, blood samples were collected using sodium heparin anticoagulant tubes and immediately subjected to plasma separation: centrifugation at 3000 rpm and 4°C for 10 min, the upper layer was taken, and then 0.2 mL/tube was dispensed into 1.5 mL centrifuge tubes. For stool samples, fresh stool was collected and immediately packaged into 250 mg/tube, and then immediately frozen in liquid nitrogen for 15 min. The subsequent LC-MS steps were handed over to VeryGeneme company (Guangzhou, China) to be carried out according to standard procedures.

SCIEX OS was used to perform upstream analysis, including aligned, retention time corrected, and peak area extracted. The lipid structure was accurately matched with the primary and secondary spectra, and the self-built database (VGDB, VeryGenome, Inc., Guangzhou, China) and public databases were searched.

Identification of Differential Lipid Metabolites

Through orthogonal partial least squares discriminant analysis (OPLS-DA) analysis, a variable importance in projection (VIP) value can be calculated for each metabolite, and the difference fold analysis can be used to screen metabolites with significant differences with the screening criteria of |log2FoldChange|≥1, $P < 0.05$ and VIP value ≥ 1.

Correlation Analysis

Due to the non-normal distribution of the data (including cytokine concentrations, relative abundance of bacterial communities, and relative expression values of metabolites), we used the Pearson correlation analysis method via Hmisc (R package, v5.2–2, R version 4.2.0) to calculate the correlation coefficients between bacterial communities, metabolites, and cytokines. And we corrected the *p*-values by using the Benjamini & Hochberg method.

Statistical Analysis

All biological experiments were repeated more than five times (n≥5), and the difference test was performed using the Student's unpaired *T* test. All data is managed and visualized by Graph Prism (v9.5.0). $P < 0.05$ indicated a statistically significant difference. $^{*}P < 0.05$ and $^{**}P < 0.01$.

Results

Changes of Plasma Inflammatory Cytokines in Pregnant Women with GH

There were significant differences in the expression levels of inflammatory factors between pregnant women with GH and normal pregnant women. GH pregnant women suggested increased levels of proinflammatory cytokines, including IL-6, IL-8, IL-17, IL-18, and IFN-γ (Figure 1A–E). Although the expression level of TNF-α demonstrated an increasing trend in the GH group, the difference was not significant (Figure 1F). These results suggested that the inflammatory level of GH pregnant women has significant proinflammatory characteristics compared with normal pregnant women. Above ELISA results demonstrated that the expression levels of blood inflammatory factors in the GH group increased significantly, indicating an enhanced inflammatory response.

Alterations in Intestinal Flora in GH Patients

In total, intestinal flora information was obtained from 14 patients. First, we conducted strict data quality filtering, and only clear data that met the requirements were processed later (Figure 2A). Further OTUs abundance ranking analysis demonstrated the differences in bacterial communities between different samples (Figure 2B). The Venn diagram suggested the distribution of detected OTUs in different groups (Figure 2C). Next, we observed the bacterial flora characteristics of each sample under different grades. At the class level, we displayed 18 bacterial communities, the top two in abundance were *Clostridia* and

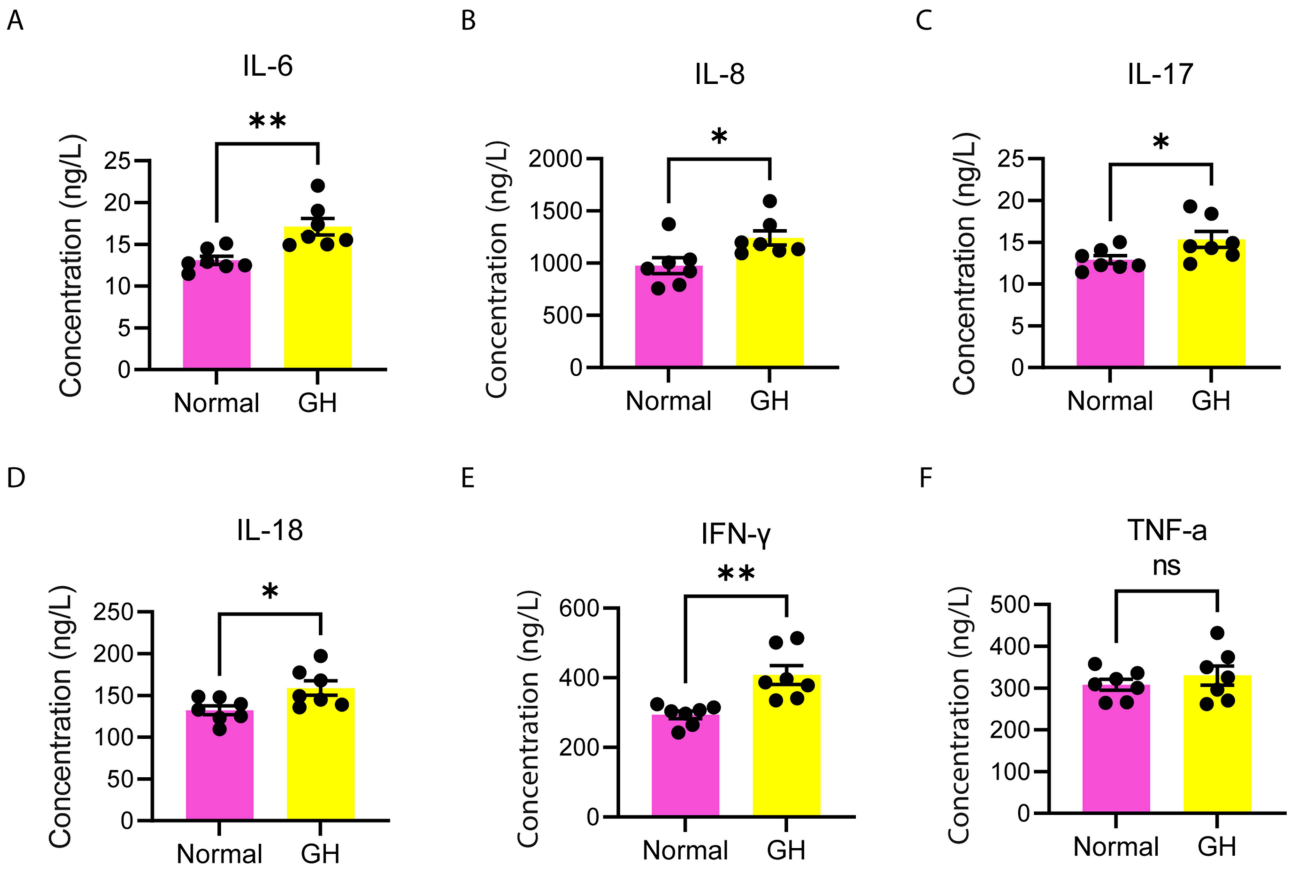

Figure 1 Expression levels of inflammatory factors. **(A–F)** The expression levels of inflammatory factors were detected by ELISA, sequentially IL-6 (**A**) IL-8 (**B**) IL-17 (**C**) IL-18 (**D**) IFN-γ (**E**) and TNF-α (**F**). The expression levels of inflammatory factors in the GH group were higher than those in the control group (n = 7, two-tailed Student's *T*-test). Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ns indicates no significant difference.

Bacteroidia, among which *Clostridia* was dominant in most samples (Figure 2D). At the family level, we showed 25 bacterial communities, and the top two with the highest abundance were *Lachnospiraceae* and *Ruminococcaceae* (Figure 2E). At the genus level, we displayed 25 bacterial species and found that they varied greatly among the samples, but *Bacteroides* seemed to be dominant in the GH group (Figure 2F). The above results suggested the differences in intestinal flora between each sample.

The Intestinal Flora of GH Model Mice Affects the Development of Mouse Fetuses

The previous results suggested that GH patients have disordered intestinal microecology. We speculated that the intestinal microecology may play a key role. To this end, we constructed a GH mouse model and collected their feces to make bacterial liquid for FMT experiments. GH model mice demonstrated typical inflammatory responses, including increased expression of IL-6 and IL-17 (Figure 3A and B). In addition, sENG was significantly downregulated and TGF-β was significantly increased, which also confirmed the success of our GH model (Figure 3C and D). We further used FMT experiments to study the impact of intestinal flora on the fetus. We found that intestinal flora had no significant effect on the number of fetuses produced, but significantly reduced birth weight (Figure 3E and F). Taken together, these results suggested that the intestinal flora of GH patients might play a key role, ultimately resulting in adverse effects on the fetus. The above results suggested that intestinal flora may have adverse effects on GH offspring.

Identification of Taxonomic Biomarkers in Gut Microbiota of GH Patients

We first used Alpha diversity index, including Chao1, shannon, and observed_species, to evaluate the differences in gut microbiota between normal pregnant women and GH patients. The Chao1 index was used to evaluate the richness of the intestinal flora, and we found that Chao1 index of the GH group was significantly lower than that of the normal

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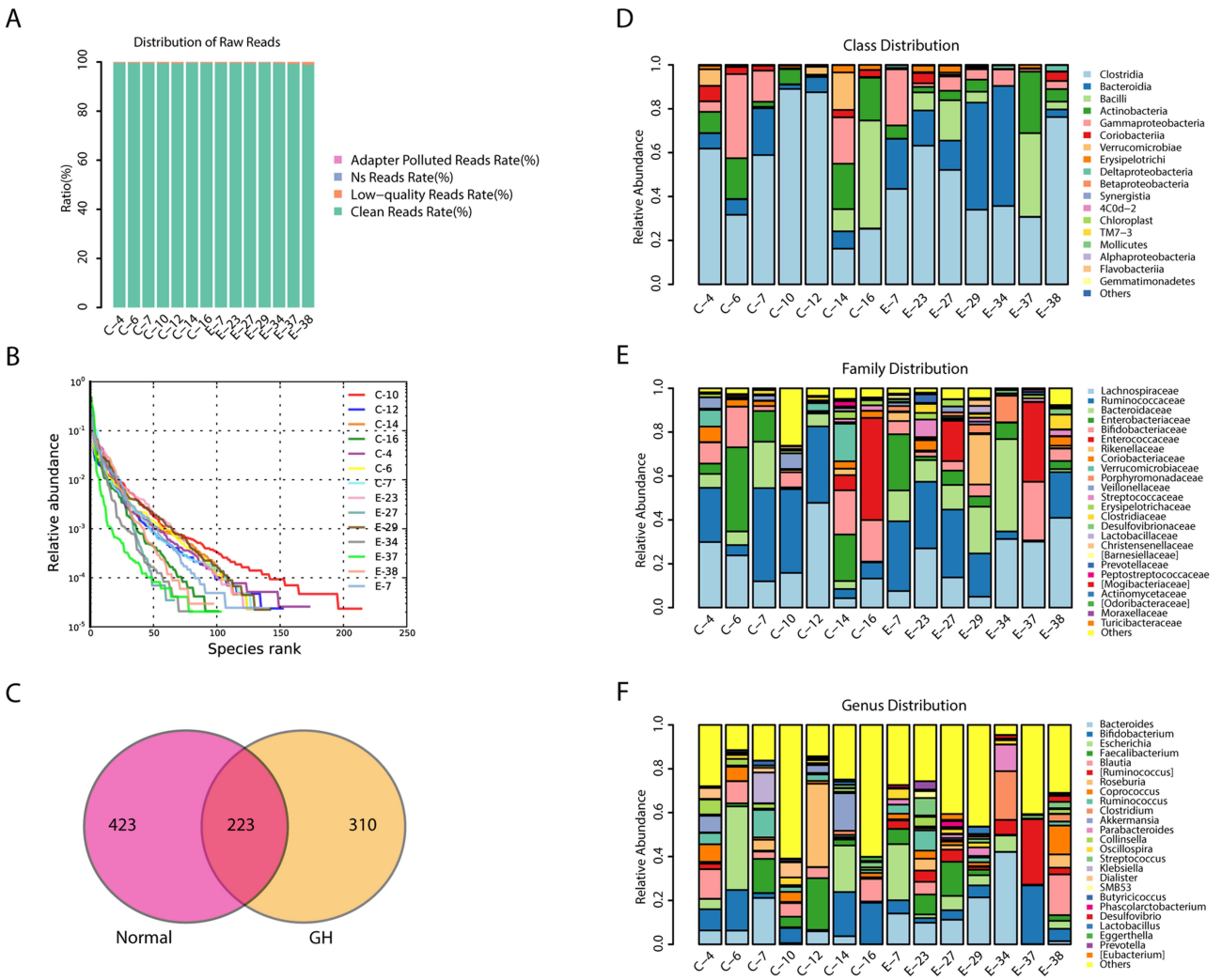

Figure 2 Differences in relative abundance of intestinal flora in different samples. (**A**) Stacked bar chart showing the data quality of different samples. (**B**) The line graph shows the OTU distribution of different samples. (**C**) Venn diagram showing the detected OTUs among different groups. (**D**) The relative abundance of bacterial communities in each sample is displayed at the class level. (**E**) The relative abundance of bacterial communities in each sample is displayed at the family level. (**F**) The relative abundance of bacterial communities in each sample is displayed at the genus level.

pregnancy group, indicating that the richness of the intestinal flora in GH patients was decreased (Figure 4A). The Shannon index was used to measure species diversity, and the results indicated no difference between the normal group and the GH group (Figure 4B). The observed_species is another index to measure species richness, which is consistent with the Chao1 index. In comparison between the two groups, the GH group demonstrated a reduction. (Figure 4C). PCoA analysis suggested that the distribution of the GH group and the normal group presented two significantly separated groups, which indicated the heterogeneity of the intestinal flora between the GH group and the normal group (Figure 4D). The LEfSe algorithm was used to identify taxonomic biomarkers between the GH group and the normal group. The results suggested that a total of 26 bacterial species were differentially expressed between the two groups, and we visualized the top 15 values of Linear Discriminant Analysis Effect Size (LDA), a comprehensive metric that measures both the significance and magnitude of differences in microbial species or functional unit abundance between distinct groups, such as healthy versus diseased populations. A larger LDA value indicates a more significant difference in species abundance between the groups. The normal group had higher levels of *g_Klebsiella, g_Oxalobacter, s_obeum*, while the GH group had higher levels of g_*Ruminococcus, s_gnavus, s_torques, p_Synergistetes* (Figure 4E). The cladogram results suggested that *f_Oxalobacteraceae, o_Synergistales, c_Synergistia, and p_Synergistetes* were significantly different between the two groups and could be used as taxonomic biomarkers (Figure 4F). The above results

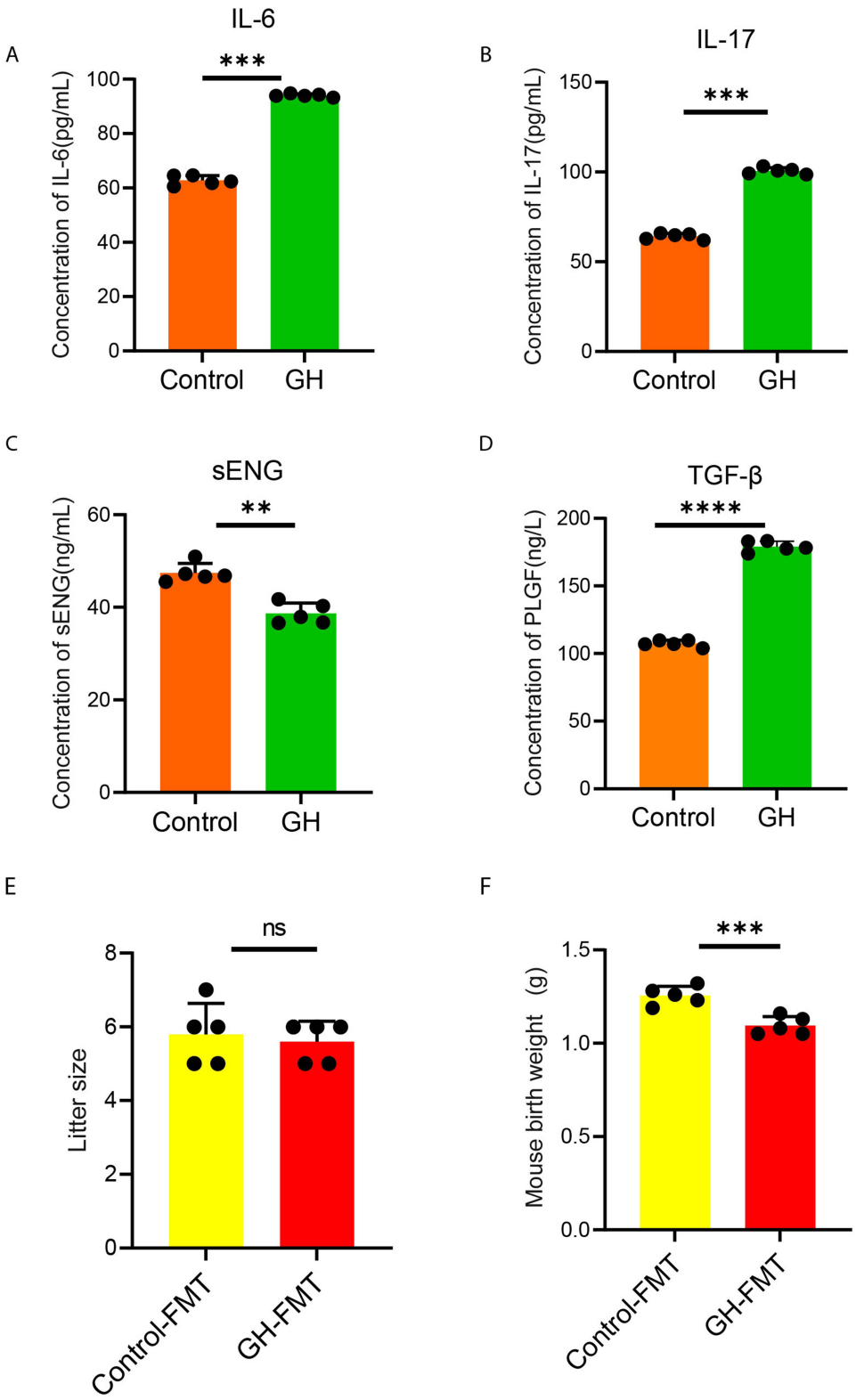

Figure 3 Gut flora plays a key role in GH. (**A**–**D**) The expression levels of inflammatory factors were detected by ELISA, sequentially IL-6 (**A**) IL-17 (**B**) sENG (**C**) TGF-β (**D**). (**E**) The number of offspring born after the FMT experiment. (**F**) The birth weight of offspring after FMT experiment. (n = 5, two-tailed Student's *T*-test), Data represent mean ± SD, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 and ns means no significance.

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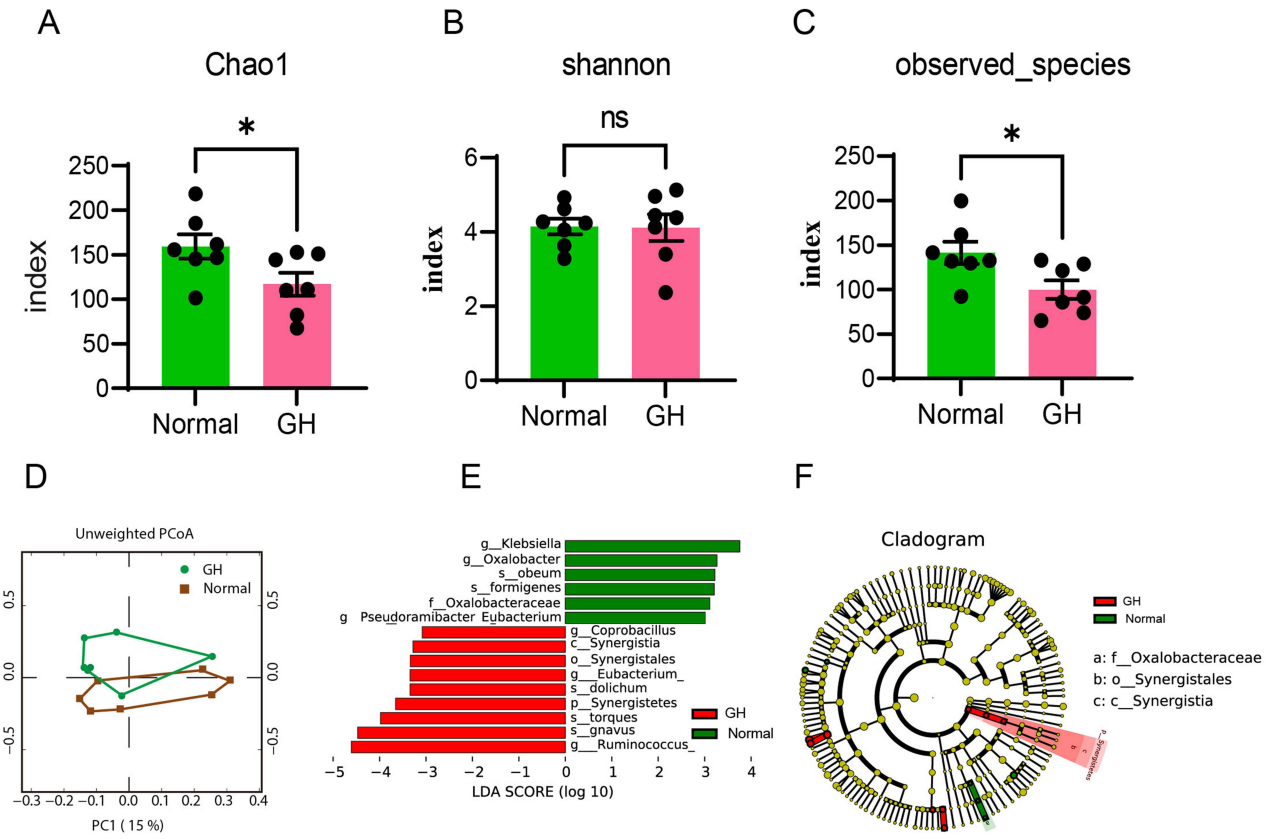

Figure 4 Identification of taxonomic biomarkers in two sample groups. (**A**–**C**) Alpha index of biome diversity in different samples, including Chao1 (**A**) shannon (**B**) and observed_species (**C**). (n = 7, two-tailed Student's *T*-test), Data represent mean ± SD *$P < 0.05$ and ns means no significance difference. (**D**) Heterogeneity of gut microbiota between the two groups analyzed by PCoA. (**E**) Identification of the top 15 differential bacterial communities between the two groups by LEfSe algorithm. (**F**) The LefSe evolutionary branch diagram shows the evolutionary relationship between different bacterial communities.

suggested that there were differences in intestinal flora between the GH group and the normal group, and a series of markers were obtained.

Differences in Intestinal Lipid Metabolites of GH Patients

Given the diverse array of metabolites produced by gut microbiota, we employed untargeted metabolomics to investigate the differences in fecal metabolites between patients with GH and healthy controls. The Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) demonstrated significant differences in fecal metabolite profiles between GH patients and normal subjects, and the reliability of the OPLS-DA model was confirmed without signs of overfitting, as indicated by the Variable Importance in Projection (VIP) score of ≥1 (Figure 5A). By comparing the differential lipid profiles of the two groups, we generated a volcano plot of lipid metabolites (Figure 5B), applying differential screening criteria of $\log_2$FC ≥1, P-value <0.05, and VIP value ≥1. Notably, compared to normal samples, TG (49:2/FA16:1), ChE (22:6), and PS (18:0/20:0) were found to be upregulated, whereas LPE (20:2), LPE (20:1), LPI (20:4), and LPC (20:2) were downregulated. Furthermore, correlation analysis of the differential metabolites revealed a strong positive correlation between LPE (20:1) and LPE (20:2), while LPC (20:2) and ChE (22:6) exhibited a negative correlation. In the figure, blue represents a positive correlation between two metabolites, red indicates a negative correlation, and the completeness of each pie chart reflects the correlation coefficient (Figure 5C). Lastly, we utilized a heatmap to illustrate the expression levels of these differential metabolites (Figure 5D). Collectively, these observations suggested significant differences in the intestinal microbiota metabolites between the GH group and the normal group.

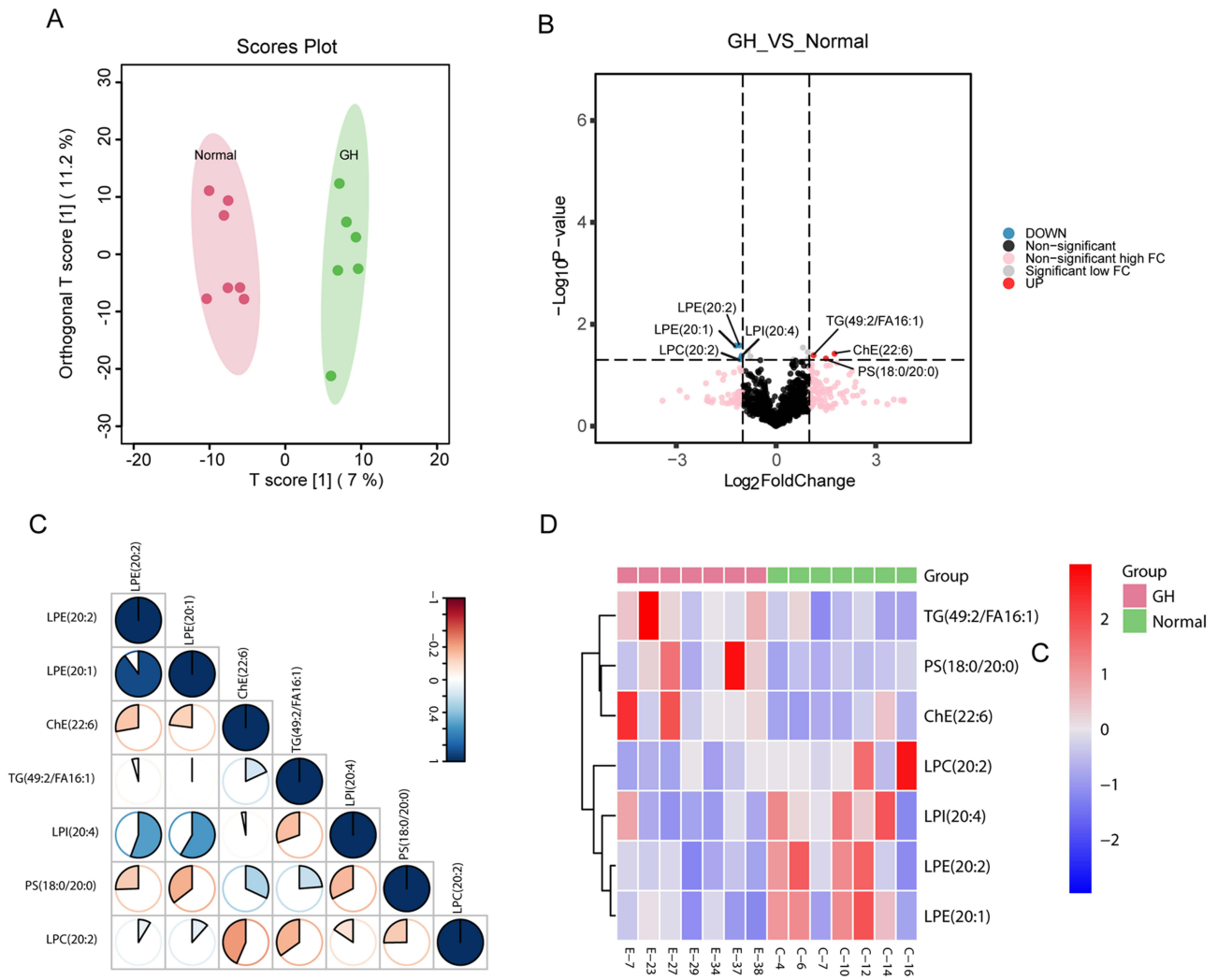

Figure 5 Non-targeted lipid metabolome characterization in feces. (**A**) The scatter plots show the differences in fecal metabolites between the two groups. (**B**) The volcano plot shows the distribution characteristics of seven significantly differential metabolites between the GH group and the normal group. (**C**) Pie chart shows the correlation distribution of the seven significantly differential metabolites between the two groups. (**D**) The heat map shows the expression characteristics of the differential metabolites.

Differences in Blood Lipid Metabolites of GH Patients

Metabolites produced by the gut enter the bloodstream for utilization by the body. We subsequently investigated the expression profiles of blood metabolites. OPLS-DA results suggested significant differences in blood metabolites between GH patients and normal controls, with the OPLS-DA model being reliable and free from overfitting; VIP≥1 (Figure 6A). Through comparative analysis of the two groups of differential lipids, a volcano plot of differential lipid metabolites was obtained. The criteria for differential screening were set as $\log_2$FC ≥ 1; P-value < 0.05, and VIP value ≥ 1, compared with normal samples. The top 10 with the largest differences were displayed, including nine up-regulated differential lipids: TG (56:6/FA18:1), TG (56:7/FA18:1), PC (16:0/16:0), TG (56:7/FA16:0), TG (58:8/FA18:1), TG (56:6/FA20:4), TG (57:2/FA18:1), TG (56:6/FA22:6), TG (58:7/FA22:6); and one down-regulated differential lipid: ChE (18:2) (Figure 6B). The correlation analysis revealed that, unlike fecal metabolites, the expression of blood metabolites predominantly exhibited strong correlations (r>0.5) (Figure 6C). The heatmap displayed the expression patterns of differential metabolites in the blood (Figure 6D). These observations suggested that there were differences in blood metabolites between the GH group and the normal group.

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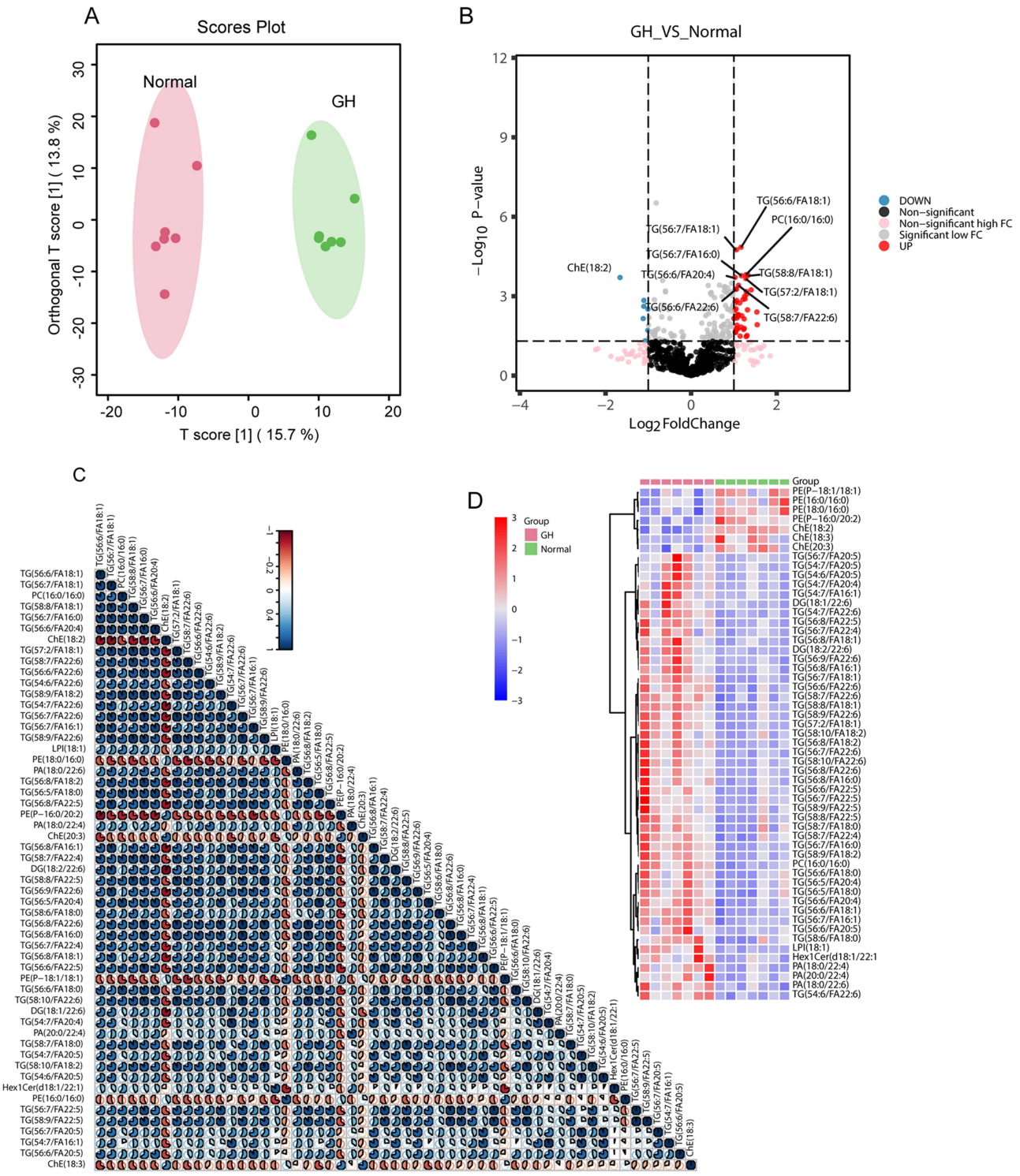

Figure 6 Non-targeted lipid metabolome characterization in blood. (**A**) The scatter plots show the differential results of blood metabolites between the two groups. (**B**) The volcano plot shows the distribution characteristics of 10 significantly differential metabolites between the GH group and the normal group. (**C**) Pie chart shows the correlation distribution of 10 significantly differential metabolites between the two group. (**D**) The heat map shows the expression characteristics of differential metabolites.

Correlations Between Microbial Composition, Lipid Metabolism and Inflammatory Response

To study the correlations between microbial composition, lipid metabolism and inflammatory response. We first compared the relationship between blood metabolites and fecal metabolites and found that there were two metabolites

that were differentially expressed in either blood or feces (Figure 7A). We then visualized the expression characteristics of these two metabolites, and we found that PE (O-16:0/20:2) showed inconsistent trends between the two groups (Figure 7B), while TG (54:5/FA22:5) showed an up-regulation trend in different groups (Figure 7C). Next, correlation analysis was performed using TG (54:5/FA22:5) with differential flora and inflammatory factors detected by ELISA. The correlation analysis demonstrated that, after adjusting for *p*-values, *f_Oxalobacteraceae* was significantly negatively correlated with IL17 and TG (54:5/FA22:5) in blood, but significantly positively correlated with *g_Oxalobacter* and *s_formigenes*. (Figure 7D). This result suggested that intestinal flora may aggravate the inflammatory response in GH patients by affecting metabolism.

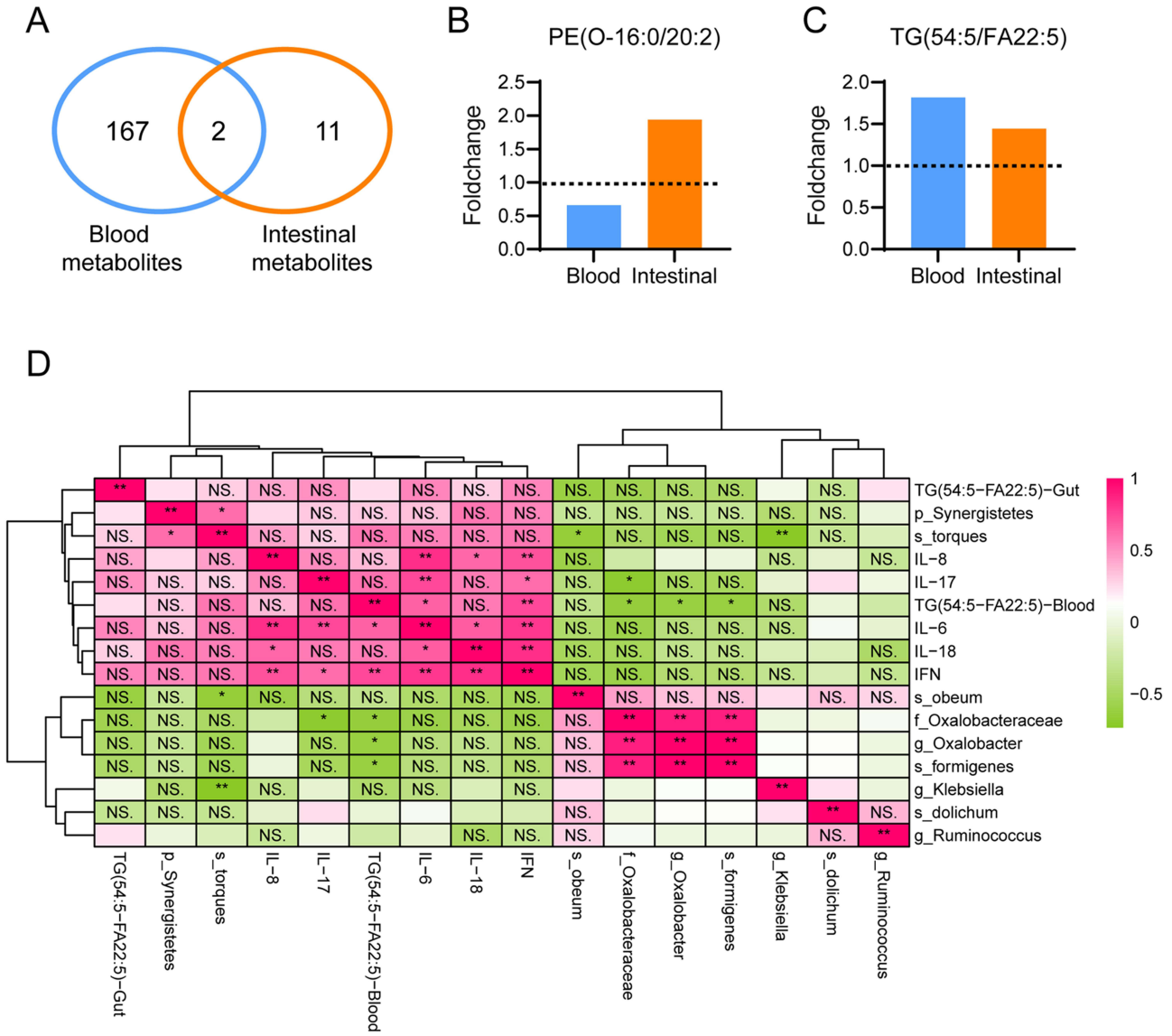

Figure 7 Relationships among microbes, metabolites, and inflammatory factors. (**A**) Venn diagram showing a comparative analysis between blood metabolome and fecal metabolome. (**B**) Differential expression of the metabolite PE (O-16:0/20:0) in blood versus feces, with the vertical axis representing the fold change (GH/normal). (**C**) Differential expression of the metabolite TG (54:5/FA22:5) in blood pressure versus feces, with the vertical axis representing the fold change (GH/normal). (**D**) The heatmap shows the correlation between differential bacteria, differential metabolites and the expressions of pro-inflammatory cytokines, with correlation analysis performed using the Benjamini & Hochberg method. **P* < 0.05, ***P* < 0.01 and NS. means no significance.

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Discussion

Pregnant women with GH are at a heightened risk for developing preeclampsia, which is a leading cause of increased morbidity and mortality for both the fetus and the mother.[31] Therefore, elucidating the underlying cause of GH may contribute to mitigating the risk of adverse maternal and fetal outcomes. In this study, we investigated the intricate interaction network between the intestinal microbiota, fecal and plasma metabolites, and plasma cytokines in both normal and GH pregnant women.

The inflammatory response is a pathological characteristics of GH. Immune cells and cytokine signaling pathways play a crucial role in mediating communication between the mother and fetus, thereby promoting a healthy pregnancy.[32,33] Conversely, abnormal inflammatory responses induced by maternal diseases can adversely effect the developing fetus., and a normal, healthy pregnancy requires the homeostasis of proteins involved in the inflammatory response.[25,32,34] Babbette LaMarca et al reported in a rat model that additional IL-6 infusion can promote the development of GH, which is consistent with our observation of elevated IL-6 levels in GH patients.[35] Moreover, IL-8 and TNF-α have been identified as effective markers for GH.[36,37] However, in our study, we found that while TNF-α levels were elevated in GH, the increase was not statistically significant. Previous studies have also noted the prevalence of elevated proinflammatory cytokines in GH.[27] These findings illustrate the heterogeneity of the pathological features of GH and underscore the role of the inflammatory response the condition. Additionally, we observed significant increases in IL-17, IL-18, and IFN-γ, which are also proinflammatory cytokines, in GH patients.[38] Although validated in mice, unfortunately, there are differences in physiological metabolism and disease pathology mechanisms between mice and humans. This may lead to different manifestations in human patients due to species differences. Future research should incorporate cross-species multi-omics analysis to further reduce the research bias caused by species disparities.

The imbalance of microbial composition and inflammatory response may be explained by feedback regulation[9,39] Therefore, we speculated that the microbial diversity of the intestinal flora may be changed in patients with GH. Our results indicate that the α-diversity index of the intestinal flora is significantly reduced in GH patients, which aligns with previous reports.[18,40] There is a consensus that the intestinal microbiota plays a crucial role in host immune regulation, directly or indirectly influencing intestinal epithelial immunity and ultimately impacting systemic immune regulation.[41,42] To investigate whether changes in immune status in GH are influence by intestinal microbiota, we analyzed the characteristics of intestinal microbiota and plasma immune indicators in pregnant women with GH compared to normal pregnant women, and performed correlation analyses. Compared to normal pregnant women, *s_torques* was significantly increased in GH, further correlation analysis showed *s_torques* was positively correlated with the expression of inflammatory factors IL-6, IL-18, IFN-γ. Our results are consistent with previous reports indicating that disturbances in intestinal flora can lead to alterations in systemic inflammatory responses.[43] A prior study demonstrated that *Ruminococcus torques* could disrupt intestinal structure in GH patients, and further functional enrichment analysis suggested a potential link to fatty acid metabolism.[20] These results suggest that gut microbiota may alters inflammatory responses, possibly mediated by fatty acid metabolism.

Current views suggest that metabolites play a crucial role in mediating interactions with the immune system.[44,45] We subsequently analyzed lipid metabolomics data derived from the feces and blood of GH patients. Through comparative analysis, we found that the expression trend of TG (54:5/FA22:5) in both feces and blood were consistent, shoeing significantly increased in the GH group. Alterations in metabolite levels may be influenced by the composition of the gut microbiota.[46–48] The changes in the TG (54:5/FA22:5) spectrum may exacerbate the pathological process through mechanisms such as lipid metabolism disorders, release of inflammatory mediators, and oxidative stress. Correlation analysis indicated that *f_Oxalobacteraceae* was significantly negatively correlated with IL-17 and TG (54:5/FA22:5) in blood, while it exhibited a significant positive correlation with *g_Oxalobacter* and *s_formingenes*. This findings suggested that metabolites may play a pivotal role in the complex interactions between the gut microbiota and host responses.[49] Meanwhile, f-Oxalobacteraceae may exert anti-inflammatory effects through multiple mechanisms, including the production of short-chain fatty acids, enhancement of intestinal barrier function, modulation of immune responses, and generation of anti-inflammatory metabolites.

Although our results are consistent with previous findings, unfortunately, this study is observational in nature. Although we conducted associations between the microbiota, metabolism, and immunity, these findings still need experimental validation.

Simultaneously, this study provides new biomarkers for the early prediction and diagnosis of gestational hypertension. In the future, by modulating the gut microbiota, exploring its association with metabolic functions, and conducting multi-omics research, it may bring breakthrough progress in the prevention and treatment of gestational hypertension.

Conclusion

Our ELISA results demonstrated that the inflammation level of GH patients was aggravated, and the characteristics of intestinal flora were significantly different from those of the normal group. In addition, the correlation analysis between the metabolome and the microbiome suggested that the intestinal flora in the GH group was significantly positively correlated with the pro-inflammatory response factors in the blood. In summary, our results suggest that the intestinal flora may cause the aggravation of the inflammatory response in GH patients by affecting metabolism.

Data Sharing Statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The study conformed to the Declaration of Helsinki and has been approved by the Ethics Committee of Liaocheng People's Hospital (authorization number: 2022263). All patients provided written informed consent prior to the study. All animal experiments were performed under the guidelines evaluated and approved by the ethics committee of Institutional Animal Care and Use Committee of Liaocheng People's Hospital. (authorization number: 2022263).US National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed in this study.

Consent for Publication

All authors read the manuscript and approved it for publication.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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