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Uncovering potential biomarkers and metabolic pathways in systemic lupus erythematosus and lupus nephritis through integrated microbiome and metabolome analysis

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Abstract

Objective This study aims to explore the relationship between gut microbiota and fecal metabolomic profiles in patients with systemic lupus erythematosus (SLE), with and without lupus nephritis (LN), in order to identify potentially relevant biomarkers and better understand their association with disease progression.

Methods Fecal samples from 15 healthy controls (HC) and 36 SLE patients (18 SLE-nonLN and 18 SLE-LN) were analyzed using 16S rRNA gene sequencing and untargeted metabolomics. Differential microbial taxa and metabolites were identified using Linear Discriminant Analysis Effect Size (LEfSe) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Receiver Operating Characteristic (ROC) curve analyses were used to assess the potential clinical relevance of selected metabolites.

Results Beta diversity analysis demonstrated distinct microbial clustering between groups ($p < 0.05$). SLE-LN samples showed an increased relative abundance of Proteobacteria and decreased Firmicutes compared to SLE-nonLN. Metabolomic profiling identified multiple differentially abundant metabolites, with notable enrichment in primary bile acid biosynthesis pathways (e.g., Glycocholic acid, AUC = 0.951). In the SLE-nonLN group, increased Glycoursodeoxycholic acid levels (AUC = 0.922) were observed in pathways related to taurine and hypotaurine metabolism. Correlation analysis indicated a negative association between *Escherichia-Shigella* and bile acid levels ($p < 0.01$).

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Conclusion This integrative analysis suggests that patients with SLE and LN harbor distinct gut microbiota and metabolomic profiles. The identified microbial taxa and metabolites may have potential as non-invasive biomarkers and could contribute to a better understanding of SLE pathogenesis and progression.

Keywords SLE, LN, Gut microbiota, Metabolomics, Biomarkers, Fecal samples, 16S rRNA sequencing, Metabolic pathways

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by the immune system's aberrant attack on healthy tissues. Such dysregulation leads to systemic inflammation and multiorgan damage [1]. SLE manifests with heterogeneous clinical presentations and variable disease severity, affecting multiple organs. The global prevalence of SLE ranges from 30 to 50 cases per 100,000 individuals [2]. In China, the reported prevalence is 6.17 per 100,000 males and 67.78 per 100,000 females [3]. Lupus nephritis (LN), a severe renal complication of SLE, is characterized by immune-mediated kidney damage with diverse pathological types and significant clinical manifestations, making it one of the most serious forms of SLE [4, 5]. Despite extensive research, the underlying pathogenesis of LN remains incompletely understood. A primary goal in SLE management is preventing irreversible organ damage, which requires identifying key molecular contributors to disease progression [6]. Accurate diagnosis, timely intervention, and early relapse management are crucial for effective LN treatment. Although renal biopsy is the gold standard for LN diagnosis, its invasiveness limits its utility for continuous disease monitoring, underscoring the need for reliable non-invasive biomarkers [7, 8]. Current routine biomarkers, such as serum creatinine and complement component C3b, have limited utility in assessing LN disease activity or facilitating real-time diagnosis [9].

Emerging evidence implicates the gut microbiota as a critical modulator of autoimmune processes, including SLE [10, 11]. Gut microbiota metabolize dietary components into bioactive metabolites that modulate systemic immune responses [12]. Microbial metabolites, such as short-chain fatty acids and bile acids, are key mediators of host-microbiota interactions [13, 14]. For example, Zhang et al. reported that fecal samples from SLE patients exhibited significantly elevated metabolic activities, including enhanced amino acid biosynthesis, vitamin B1 metabolism, nitrogen cycling, tryptophan degradation, and cyanoamino acid metabolism, compared to healthy controls [15]. However, single-omics approaches (e.g., metagenomics or metabolomics alone) fail to capture the complexity of disease mechanisms. Integrative analysis of gut microbiota and host metabolome dynamics provides a holistic understanding of microbial and metabolic interactions in pathogenesis [16]. Despite progress in SLE metabolomic profiling, studies exploring microbiota-metabolome interplay, particularly in differentiating SLE with nephritis (SLE-LN) from SLE without nephritis (SLE-nonLN), remain limited [17].

In this study, we applied 16S rRNA gene sequencing and liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based untargeted metabolomics to analyze fecal samples from SLE patients (SLE-LN and SLE-nonLN) and healthy controls (HC). Our objective was to identify potential biomarkers and explore gut microbiota-metabolome interactions to provide novel insights into the mechanisms underlying SLE pathogenesis and progression.

Materials and methods

Sample collection

We recruited 36 patients diagnosed with SLE from Nanjing Drum Tower Hospital, affiliated with Nanjing University Medical School. All patients were newly admitted and were not receiving immunosuppressive therapy at the time of sample collection. Diagnosis was based on the 1997 revised classification criteria of the American College of Rheumatology (ACR) and further confirmed through clinical evaluation, including serological markers and renal biopsy where applicable. The cohort consisted of 18 SLE-LN and 18 SLE-nonLN. Clinical characteristics, including laboratory test results and medical history, were obtained from the hospital's electronic medical records system. Additionally, 15 age-, gender-, and BMI-matched HC were recruited from the hospital's health examination center. These individuals had no prior history of autoimmune diseases, infections, metabolic disorders, or malignancies. All participants provided written informed consent, and the study was approved by the Ethics Committee of Nanjing Drum Tower Hospital. Fecal samples were collected using sterile containers upon admission and immediately processed to maintain sample integrity. Each sample was divided into two aliquots and immediately stored at -80 °C for subsequent 16S rRNA sequencing and untargeted metabolomics analysis. The overall study design is illustrated in Fig. 1.

DNA extraction and 16S rRNA sequencing

Total genomic DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) with modifications, including an extended 10-minute bead-beating step to enhance the lysis of Gram-positive bacteria. DNA purity (A260/A280 ratio 1.8-2.0) and integrity were verified via

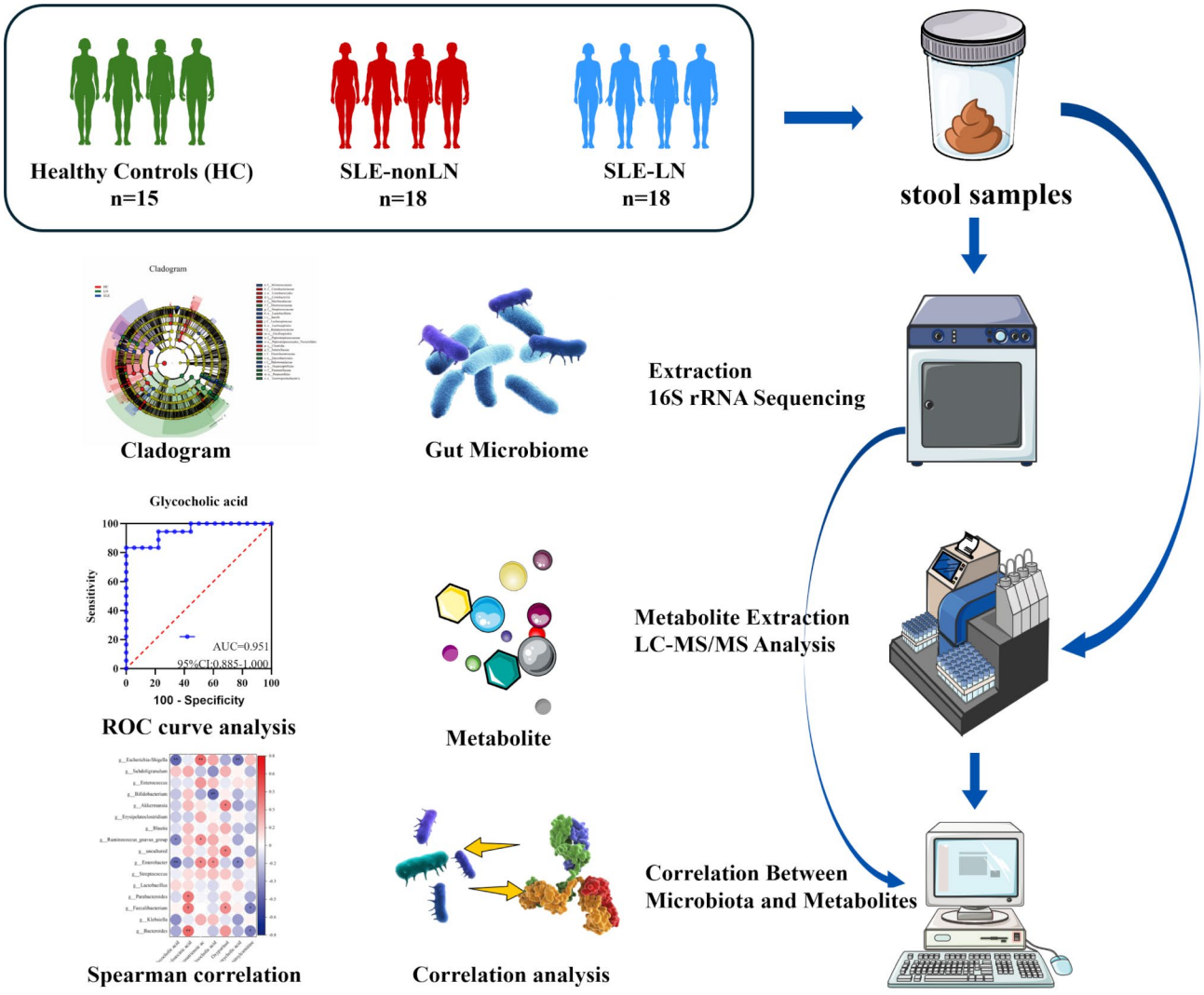

Fig. 1 Grouping Design and Analysis Flowchart

NanoDrop 2000 spectrophotometry and 1% agarose gel electrophoresis. The V3-V4 region of the 16 S rRNA gene was amplified using primers 341 F/806R under the following conditions: 98 °C for 1 min; 30 cycles of 98 °C for 10 s, 55 °C for 30 s, 72 °C for 30 s; final extension at 72 °C for 5 min. PCR products were purified (AxyPrep DNA Gel Extraction Kit) and quantified (Qubit dsDNA Assay Kit). Equimolar pooled libraries were sequenced on an Illumina MiSeq PE300 platform (2 × 300 bp) in a single run to minimize batch effects.

Sequencing data processing and microbial diversity analysis

Raw sequencing reads were preprocessed using Cutadapt (v4.0) to trim adapters and remove low-quality bases. Quality filtering was performed with FASTP (v0.23.4), retaining reads with Phred scores ≥ 20 and lengths ≥ 200 bp. Paired-end reads were merged using FLASH (v1.2.11; min overlap = 20 bp, max mismatch = 0.1). Further filtering steps were conducted to remove ambiguous bases (N), sequences with homopolymer runs (> 8 bp), and chimeric reads using USEARCH (v11.0). Operational taxonomic units (OTUs) were de novo clustered at 97% similarity using VSEARCH (v2.21.1) and taxonomically assigned via the QIIME2 Naïve Bayesian Classifier (v2023.2) against the SILVA 142 database (confidence threshold = 80%). Alpha diversity (Chao, Shannon, Simpson) was calculated after rarefaction to 10,000 reads/sample. Beta diversity was assessed using weighted/unweighted UniFrac distances and visualized via principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS). Linear discriminant analysis Effect Size (LEfSe) analysis was performed using Python (v3.9.7) to identify differentially abundant taxa, applying significance thresholds of 0.05

for both the Kruskal-Wallis and Wilcoxon tests, and an LDA score threshold of 4.

Metabolite extraction and LC-MS/MS analysis

Fecal metabolites were extracted by homogenizing 20 mg of sample with 400 µL methanol: water (7:3, v/v) on ice. After sonication (10 min), vortexing (1 min), and centrifugation (12,000 × g, 10 min, 4 °C), 200 µL of supernatant was analyzed via LC-MS/MS (Waters ACQUITY UPLC HSS T3 C18 column, 1.8 µm, 2.1 × 100 mm). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B), with a gradient elution (0.4 mL/min): 0–11 min, 5–90% B; 12–14 min, 5% B. Raw data were converted to mzML format using ProteoWizard version 3.0.23136. Peak detection and retention time alignment were performed using XCMS (v4.7). Metabolites were identified through our in-house database and the Kyoto Encyclopedia of Genes and Genomes (KEGG) online database.

Statistical analysis

Constrained Principal Coordinate Analysis (CPCoA) was performed using the prcomp function in R (v4.2.3). Hierarchical clustering analysis was conducted with the ComplexHeatmap package, and results were visualized as heatmaps with dendrograms. Normalized signal intensities of metabolites were displayed as color spectra following unit variance scaling. Differential metabolites were defined by VIP ≥ 1, |Log2FC|≥1, and $p < 0.05$. Data were log2-transformed and mean-centered prior to orthogonal partial least squares discriminant analysis (OPLS-DA). Functional and pathway analysis was conducted using the KEGG database, with pathways considered significantly enriched at $p < 0.05$.

Results

Altered microbiota composition among groups

The DNA from fecal samples of 15 HC, 18 SLE-nonLN, and 18 SLE-LN patients was examined using 16 S rRNA gene sequencing. Species accumulation curves (Fig. S1) and rarefaction curves (Fig. S2) indicated sufficient sequencing depth and coverage. To assess bacterial diversity differences across the three groups, both within-sample (alpha) and between-sample (beta) diversity metrics were calculated. No significant differences in ACE, Chao, Shannon, and Simpson indices were observed among the HC, SLE-nonLN, and SLE-LN groups (Fig. 2A-D), suggesting a lack of significant variations in microbial

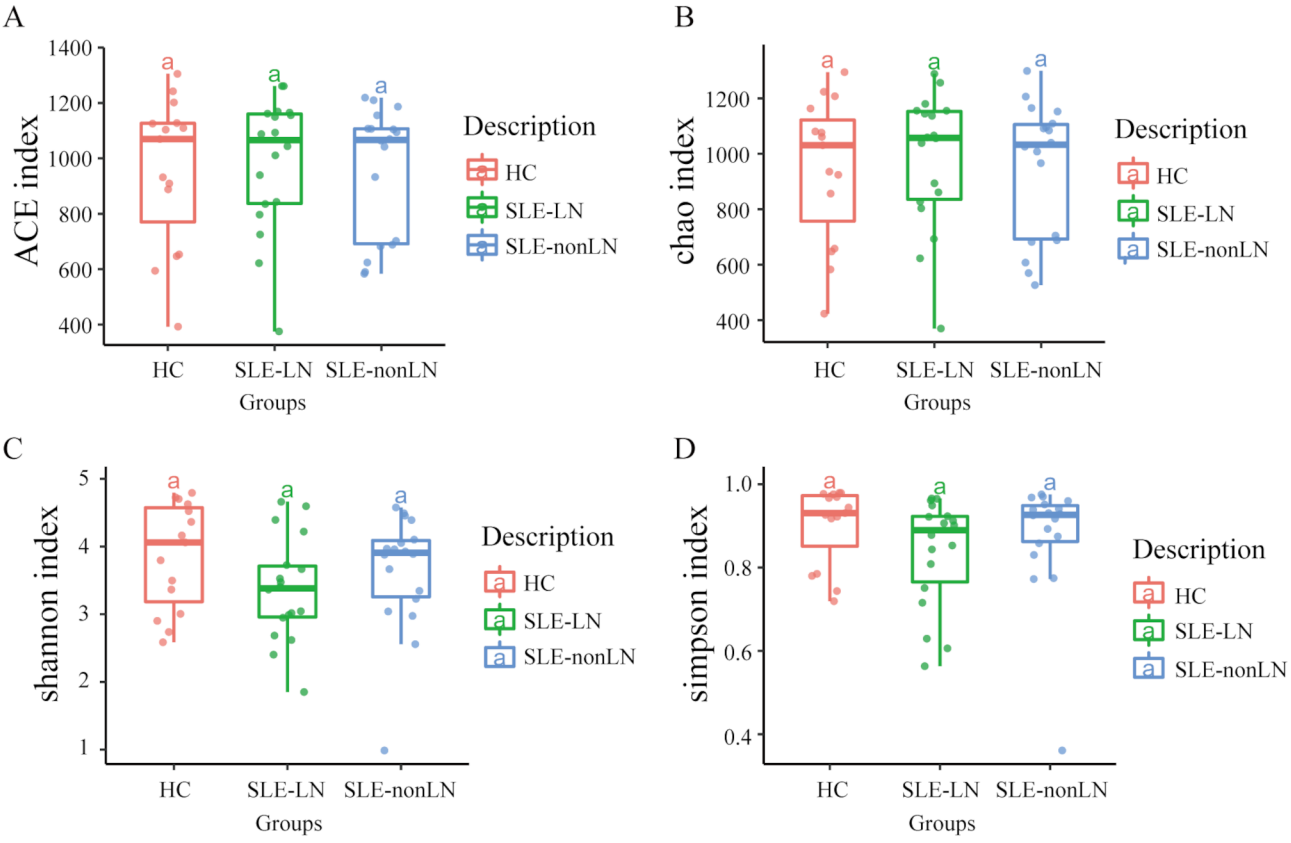

Fig. 2 Alpha diversity analysis across HC, SLE-nonLN, and SLE-LN groups. The ACE (**A**), Chao (**B**), Shannon (**C**), and Simpson (**D**) indices represent different measures of bacterial diversity among the three groups

richness or diversity. In contrast, beta diversity analysis using CPCoA and NMDS demonstrated distinct clustering patterns between SLE groups (nonLN, LN) and HC (Fig. S3A-B). These findings suggest that although alpha diversity differences were not significant, SLE influenced gut microbiota composition.

Altered microbial composition associated with SLE

At the phylum level, Firmicutes was the predominant phylum in all groups, with a significantly higher relative abundance in SLE-nonLN (59.08%) compared to HC (49.35%) and SLE-LN (39.50%, Fig. 3A). Conversely, Proteobacteria abundance was markedly elevated in SLE-LN (28.02%) versus SLE-nonLN (12.93%) and HC (23.18%). At the genus level, the relative abundance of *Faecalibacterium* was highest in the HC group (10.31%) and progressively declined in the SLE-nonLN and SLE-LN groups (4.06% and 1.90%, respectively). Conversely, *Bacteroides* abundance was lower in the HC group (9.57%) and increased in the SLE-nonLN and SLE-LN groups (11.26% and 14.73%, respectively). Compared to the HC group, the genera *Blautia* decreased, whereas *Streptococcus*, *Enterococcus*, *Akkermansia*, and *Lactobacillus* were enriched in both the SLE-nonLN and SLE-LN groups (Fig. 3B).

LEfSe analysis among groups

LEfSe identified 27 differentially abundant taxa across groups (LDA > 4, $p < 0.05$), with 6 taxa enriched in the SLE-nonLN group, 11 in the SLE-LN group, and 10 in the HC group. SLE-nonLN was characterized by Bacilli and Lactobacillales, while SLE-LN showed enrichment of Enterobacteriaceae, Enterobacterales, Proteobacteria, Gammaproteobacteria, *Escherichia_Shigella*, and *Escherichia_coli* (LDA score > 4.7). HC exhibited higher Clostridia abundance (Fig. 4A-B). Pairwise comparisons confirmed *Enterobacter* as the most distinct genus among groups (Kruskal-Wallis $p < 0.001$; Fig. S4), with *Escherichia-Shigella* significantly differentiating SLE-LN from SLE-nonLN ($p = 0.0208$, Fig. S5).

Changes in metabolome and key metabolites

Gut microbial metabolites play a crucial role in modulating host physiological functions. To characterize metabolic alterations among HC, SLE-nonLN, and SLE-LN groups, metabolite abundance in fecal samples was analyzed using LC-MS. Untargeted metabolomics revealed distinct clustering among HC, SLE-nonLN, and SLE-LN groups via OPLS-DA, with clear separation between groups (Fig. 5A). Permutation tests validated the model's robustness, with the Y-intercept of the regression line for Q^2 values below zero ($R^2Y = 0.995$, $Q^2 = 0.788$; Fig. 5B). A total of 177 metabolites were differentially abundant in HC vs. SLE-LN, 159 in HC vs. SLE-nonLN, and 94 in SLE-nonLN vs. SLE-LN (VIP ≥ 1, $p < 0.05$, |Log2FC| ≥ 1). Differential metabolites were identified based on the OPLS-DA model and visualized using volcano plots (Fig. 5C).

Metabolic pathways analysis

Pearson correlation analysis of the top 50 differential metabolites (ranked by VIP scores) revealed distinct co-regulation patterns among HC, SLE-nonLN, and SLE-LN groups (Fig. S6). KEGG pathway enrichment analysis identified taurine and hypotaurine metabolism as the most significantly altered pathway in SLE-nonLN vs. HC, followed by primary bile acid biosynthesis and histidine metabolism (Fig. 6A). In SLE-nonLN vs.

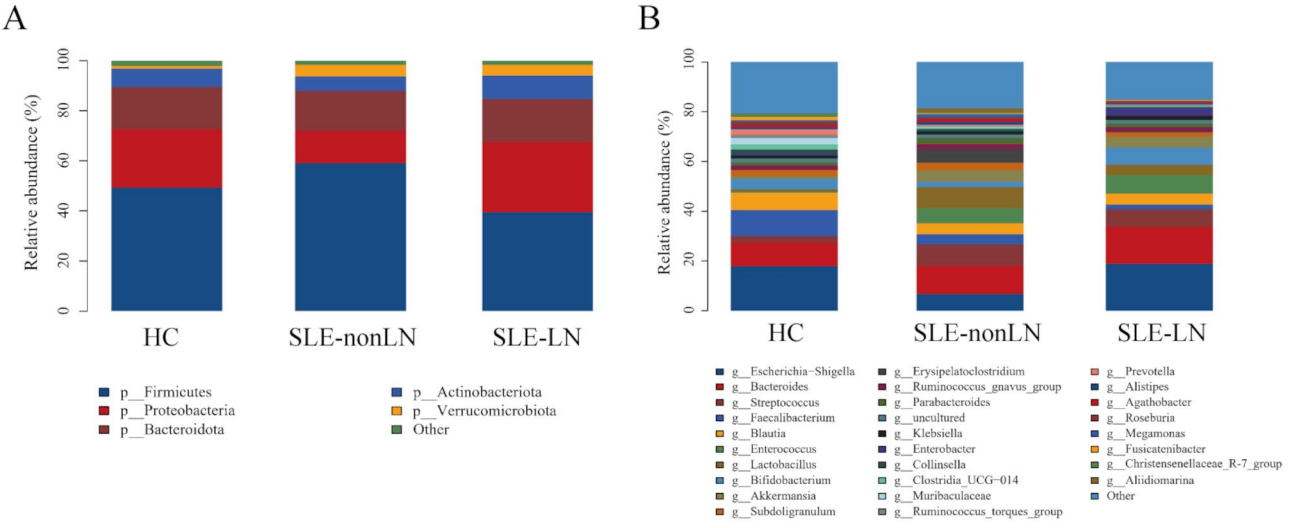

Fig. 3 Altered gut microbiota composition among HC, SLE-nonLN, and SLE-LN groups. (**A**) Relative abundances at the phylum level. (**B**) Relative abundances at the genus level

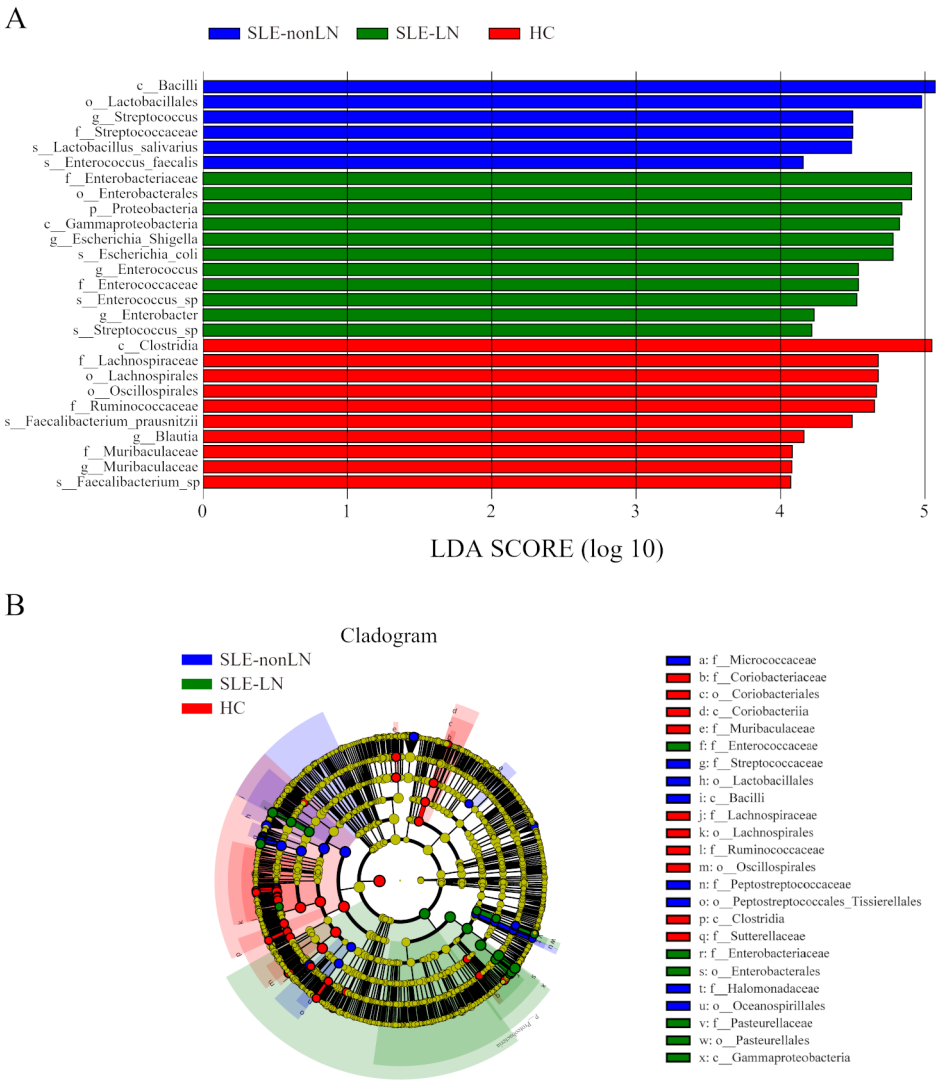

Fig. 4 Microbial community analysis using LEfSe. (**A**) Histogram of LDA scores showing differentially abundant microbial taxa (LDA score > 4). (**B**) Cladogram depicting specific differential taxa

SLE-LN comparisons, key pathways included primary bile acid biosynthesis, thiamine metabolism, and sulfur metabolism (Fig. 6B). After excluding metabolites of dietary or pharmaceutical origin (e.g., Hydroxychloroquine, Myristoleic acid, Polygodial, Furosemide), seven metabolites were consistently altered across all groups (Table 1). Heatmap analysis highlighted three metabolites—Glycocholic acid, Glycochenodeoxycholic acid, and 5,8,11-Eicosatrienoic acid—that were dysregulated in both HC vs. SLE-nonLN and SLE-nonLN vs. SLE-LN comparisons (Fig. S7).

Identification of metabolite biomarkers to distinguish SLE-LN from SLE-nonLN

Receiver operating characteristic (ROC) was conducted to evaluate the discriminatory power of key metabolites in distinguishing SLE-LN from SLE-nonLN. Seven differential metabolites involved in major metabolic pathways were assessed, revealing that Glycocholic acid (AUC = 0.951), Glycochenodeoxycholic acid (AUC = 0.827), Oxypurinol (AUC = 0.769), Methylsuccinic acid (AUC = 0.685) and 5,8,11-Eicosatrienoic acid (AUC = 0.716) exhibited varying degrees of classification accuracy between SLE-nonLN and SLE-LN (Fig. 7A-E). Among them, Glycocholic acid and Glycochenodeoxycholic acid exhibited the strongest predictive capability. For SLE-nonLN vs. HC discrimination, Glycine deoxycholic acid (AUC = 0.844) and Glycochenodeoxycholic acid (AUC = 0.922) exhibited high accuracy, whereas Soyasaponin I (AUC = 0.689) showed limited utility (Fig. S8).

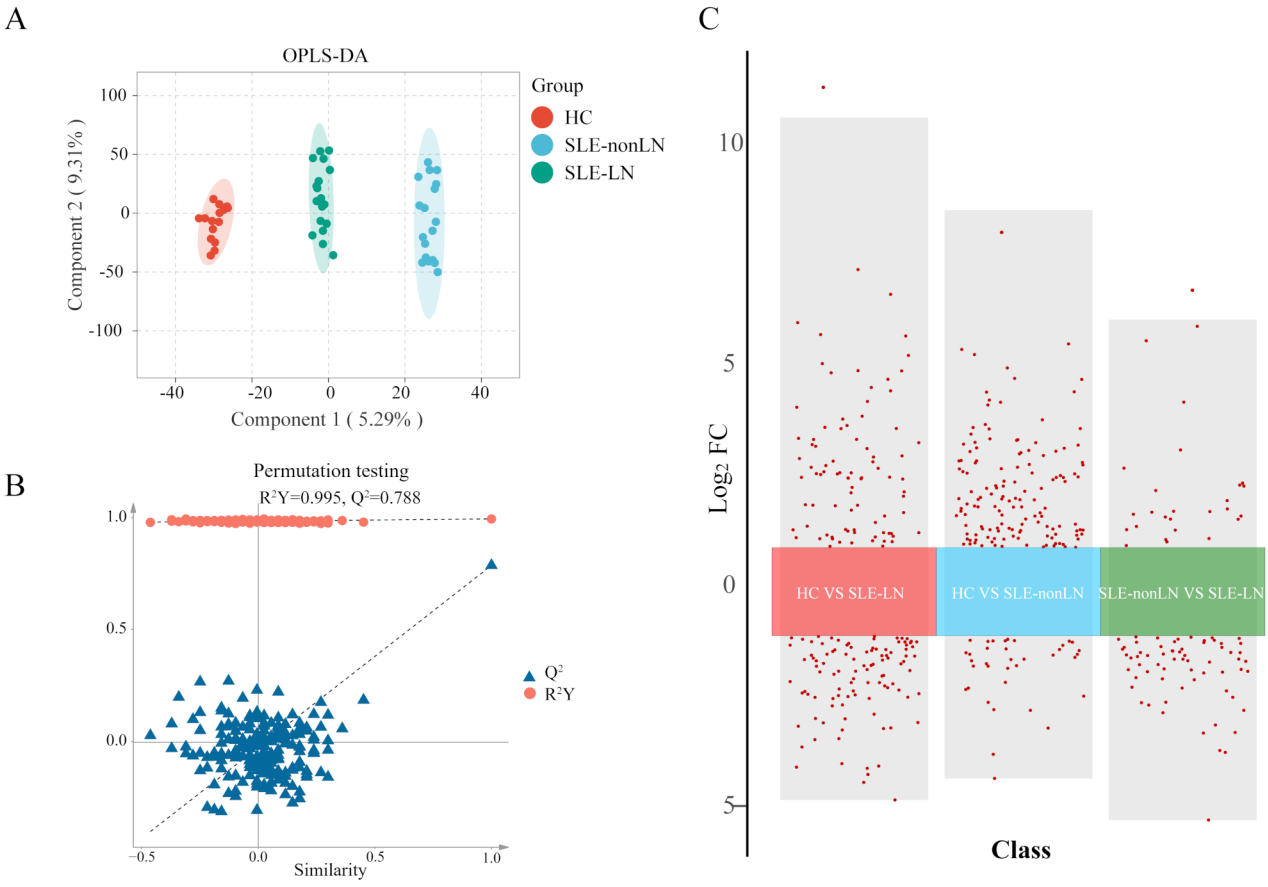

Fig. 5 Metabolic profile analysis. (**A**) OPLS-DA score plot demonstrating significant differences in metabolic profiles among groups. (**B**) Permutation test for model validation. The x-axis represents the correlation coefficient, and the y-axis represents the predictive performance of the model. (**C**) Volcano plot of differential metabolites. The x-axis denotes the groups, while the y-axis represents the $\log_2$ fold change of metabolites. Metabolites upregulated in each group are shown in red

Correlation between microbiota and metabolites

Spearman's correlation analysis between the top 16 microbial genera and key metabolites revealed significant associations. The correlation heatmap (Fig. 8A) highlighted key relationships: *Escherichia-Shigella* and *Enterobacter* abundances were negatively correlated with bile acids (Glycochenodeoxycholic acid and Glycocholic acid), but positively correlated with 5,8,11-eicosatrienoic acid. Additionally, *Bacteroides*, *Faecalibacterium*, and *Parabacteroides* were positively correlated with Methylsuccinic acid, indicating potential metabolic interactions. Notably, *Escherichia-Shigella* was inversely associated with Glycochenodeoxycholic acid, Glycocholic acid and Stachydrine in HC vs. SLE-nonLN comparisons, while *Streptococcus* correlated positively with Glycocholic acid (Fig. 8B). Furthermore, Soyasaponin I demonstrated a negative correlation with *Subdoligranulum*, *Agathobacter*, and *Faecalibacterium*, while showing a significant positive correlation with *Erysipelatoclostridium* ($p < 0.01$). These findings suggest that alterations in gut microbial composition significantly impact host metabolic profiles, particularly in the regulation of bile acid metabolism. Notably, key genera such as *Escherichia-Shigella* and *Enterobacter* appear to contribute substantially to bile acid synthesis, further underscoring their potential role in SLE pathogenesis.

Discussion

SLE is a chronic autoimmune disease that primarily affects young women and leads to systemic inflammation. LN, a severe renal complication of SLE, significantly worsens patient prognosis [18]. While our previous work identified tRNA-derived small noncoding RNAs (tsRNAs) as potential biomarkers for LN [19, 20], the gut microbiota-metabolome axis remains underexplored in SLE progression. In the present study, we integrated 16S rRNA sequencing with untargeted metabolomics to explore gut microbial and metabolic alterations across different stages of SLE. Our findings reveal stage-specific microbiota and metabolite patterns, which may provide new insights into SLE pathophysiology and support the identification of non-invasive biomarkers.

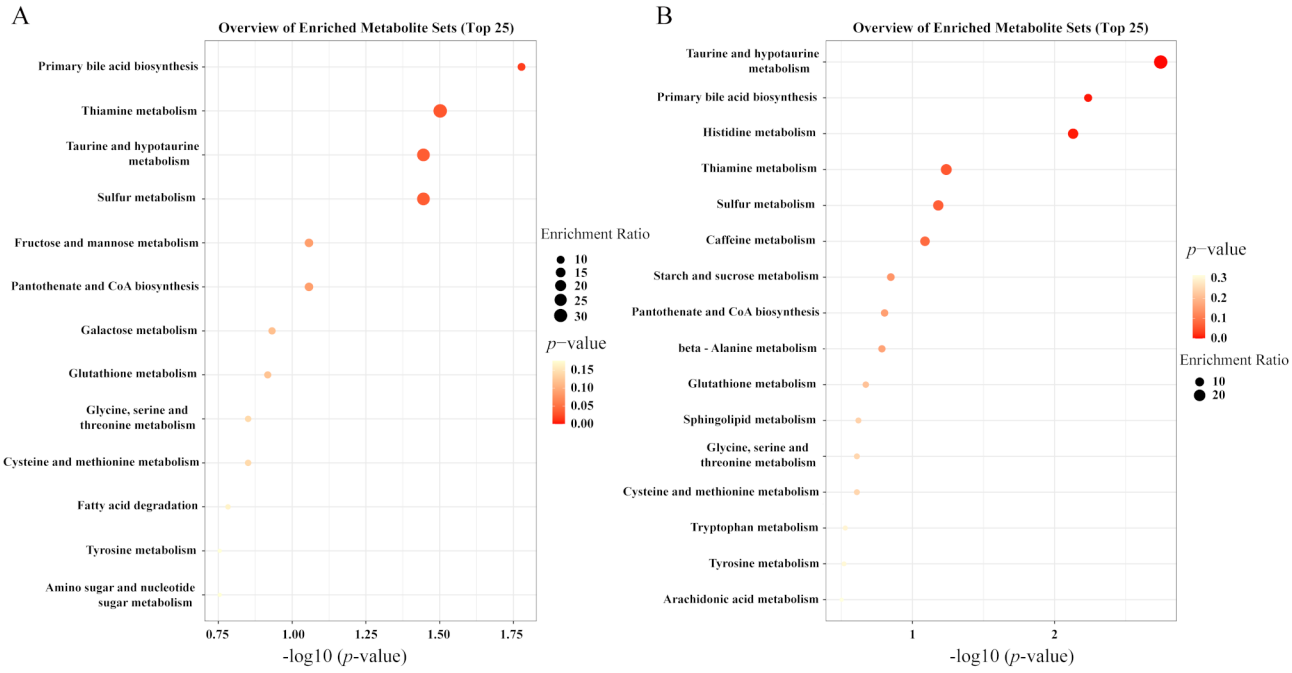

Fig. 6 KEGG pathway enrichment analysis. KEGG pathway enrichment analysis highlighting major metabolic pathways affected in (**A**) HC vs. SLE-nonLN and (**B**) SLE-nonLN vs. SLE-LN comparisons. The y-axis lists the enriched pathways, while the x-axis represents the enrichment factor (ratio of significantly altered metabolites to total metabolites in a given pathway). The bubble size corresponds to the number of enriched metabolites, whereas the color gradient denotes statistical significance, with darker shades indicating higher significance

Table 1 Metabolites with intergroup differences in fecal samples

Compounds	Class	HC vs. SLE-nonLN				SLE-nonLN vs. SLE-LN			
		VIP	FC	*p*	Trend	VIP	FC	*p*	Trend
Glycocholic acid	Bile acids	2.99	5.06	0.00	↑	3.10	0.37	0.00	↓
5,8,11-Eicosatrienoic acid	Fatty Acyls	1.66	0.31	0.01	↓	1.80	3.11	0.00	↑
Stachydrine	Amino acid and Its metabolites	1.23	4.31	0.03	↑	—	—	—	—
Glycine deoxycholic acid	Organic acid And Its derivatives	1.83	11.02	0.04	↑	—	—	—	—
Glycochenodeoxycholic acid	Bile acids	2.52	8.88	0.00	↑	2.56	0.28	0.01	↓
Methylsuccinic acid	Organic acid And Its derivatives	—	—	—	—	1.06	0.35	0.02	↓
Palmitoylcarnitine	Alkaloids	—	—	—	—	1.28	2.78	0.03	↑
Ursocholic acid	Bile acids	—	—	—	—	1.41	6.95	0.02	↑
Oxypurinol	Heterocyclic compounds	—	—	—	—	1.54	0.15	0.04	↓

Notes: ↑ Represents upregulation of metabolite; ↓ represents downregulation of metabolite

Abbreviations: VIP, variable importance in the projection; FC, fold change

The gut microbiota, functioning as a dynamic "virtual organ," plays pivotal roles in immune regulation, metabolic homeostasis, and barrier integrity [21]. In our study, alpha-diversity metrics remained stable in SLE-nonLN compared to HC, whereas beta-diversity analysis revealed significant compositional shifts between SLE groups and HC. These results suggest that alterations in microbial composition, rather than overall richness, may be associated with disease status. Further subgroup comparisons revealed differing microbial patterns between SLE-nonLN and SLE-LN, with SLE-LN characterized by an increased abundance of taxa previously linked to inflammatory phenotypes.

At the phylum level, we observed a shift from Firmicutes to Proteobacteria during disease progression. Firmicutes were predominant in SLE-nonLN (59.08%), while Proteobacteria were significantly enriched in SLE-LN (28.02%). This contrasts with a Spanish cohort reporting Bacteroidetes enrichment in SLE [22], likely reflecting geographic and dietary influences on microbial ecology. The depletion of Firmicutes (particularly butyrate-producing *Faecalibacterium*) and enrichment of Proteobacteria (e.g., *Escherichia-Shigella*) in SLE-LN may contribute to gut barrier dysfunction, permitting translocation of pro-inflammatory metabolites (e.g., lipopolysaccharides) into systemic circulation—a mechanism

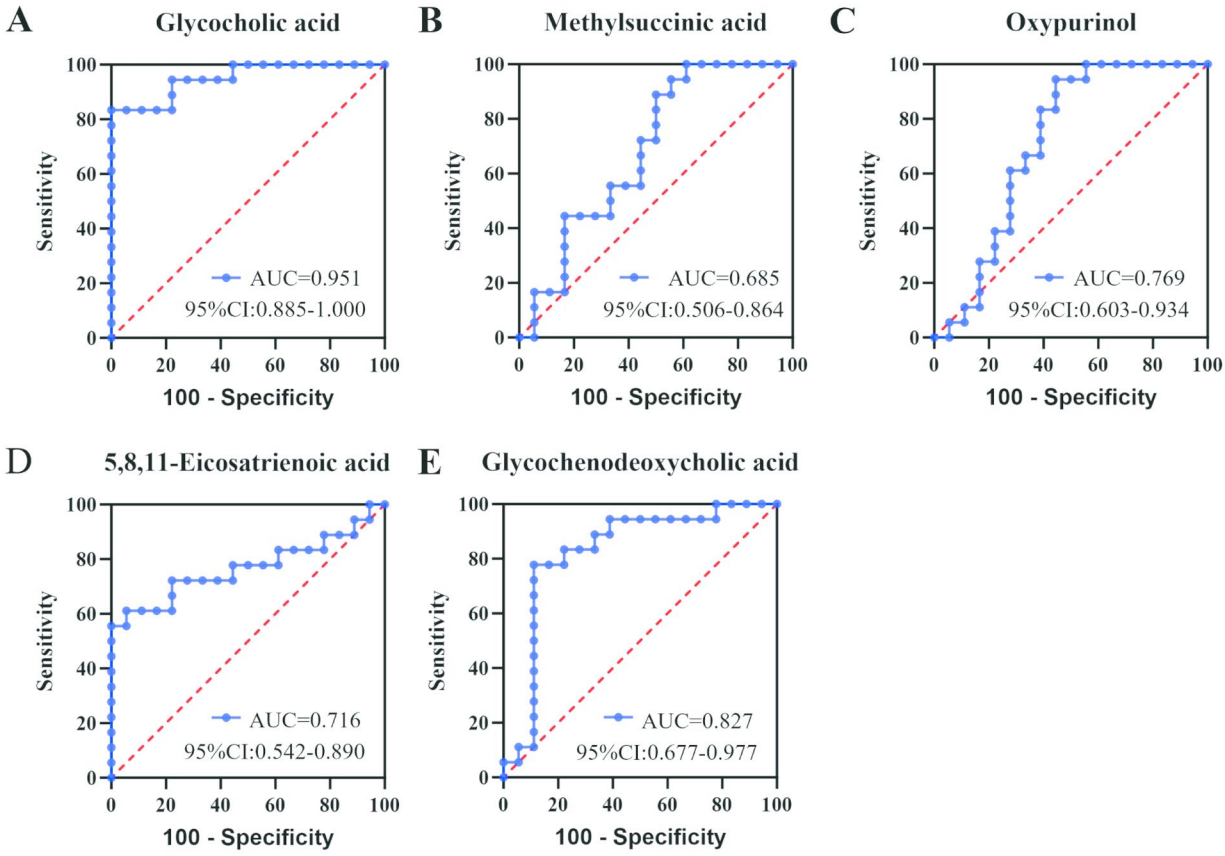

Fig. 7 ROC analysis of potential biomarkers distinguishing SLE-LN and SLE-nonLN. (**A**) Glycocholic acid, (**B**) Methylsuccinic acid, (**C**) Oxypurinol, (**D**) 5,8,11-Eicosatrienoic acid, (**E**) Glycochenodeoxycholic acid

implicated in LN-related renal inflammation [23]. Similar phylum-level dysbiosis patterns observed in systemic sclerosis and rheumatoid arthritis further support the potential involvement of these taxa in autoimmune dysregulation [24–26].

LEfSe analysis further delineated key taxonomic differences. Class Bacilli and order Lactobacillales were enriched in SLE-nonLN, whereas SLE-LN samples showed a predominance of Enterobacteriaceae and Gammaproteobacteria. These findings are consistent with previous reports indicating increased fecal Lactobacillus abundance in SLE patients [27, 28]. Notably, preclinical models have demonstrated strain-specific effects of Lactobacillus spp., with *L. reuteri* exhibiting protective immunomodulatory effects [29], while other strains may exacerbate autoimmunity [30]. In our cohort, the consistent enrichment of Enterobacteriaceae, Enterobacterales, and *Escherichia-Shigella* in SLE-LN (LDA > 4.7, $p < 0.05$) underscores the potential contribution of these taxa to disease severity. While the exact mechanisms remain to be elucidated, their expansion in LN patients suggests they may serve as markers of microbial dysbiosis associated with disease progression [31, 32].

Metabolomics, with its high resolution and sensitivity, has emerged as a powerful tool for unraveling disease-specific metabolic perturbations [33, 34]. Our untargeted fecal metabolomics analysis identified profound alterations in lipid and amino acid metabolism across SLE stages, underscoring their pivotal roles in disease progression. Notably, Mead acid (5,8,11-Eicosatrienoic acid), a biomarker of essential fatty acid deficiency [35, 36], was significantly elevated in SLE-LN compared to SLE-nonLN (FC = 3.11, $p = 0.004$). Elevated Mead acid levels, often indicative of a deficiency in dietary essential fatty acids, particularly arachidonic acid, may contribute to autoimmune disease progression [37]. These findings align with Zhang et al.'s report of Mead acid as a prognostic marker in LN [38], suggesting its potential utility for identifying SLE patients at higher risk of renal involvement.

Changes in lipid metabolism are closely associated with lipid-induced nephrotoxicity and play a significant role in SLE-LN pathophysiology. Bile acids, essential regulators of lipid metabolism, mediate this process through TGR5 modulation [39]. Previous studies have reported correlations between specific bile acids—including

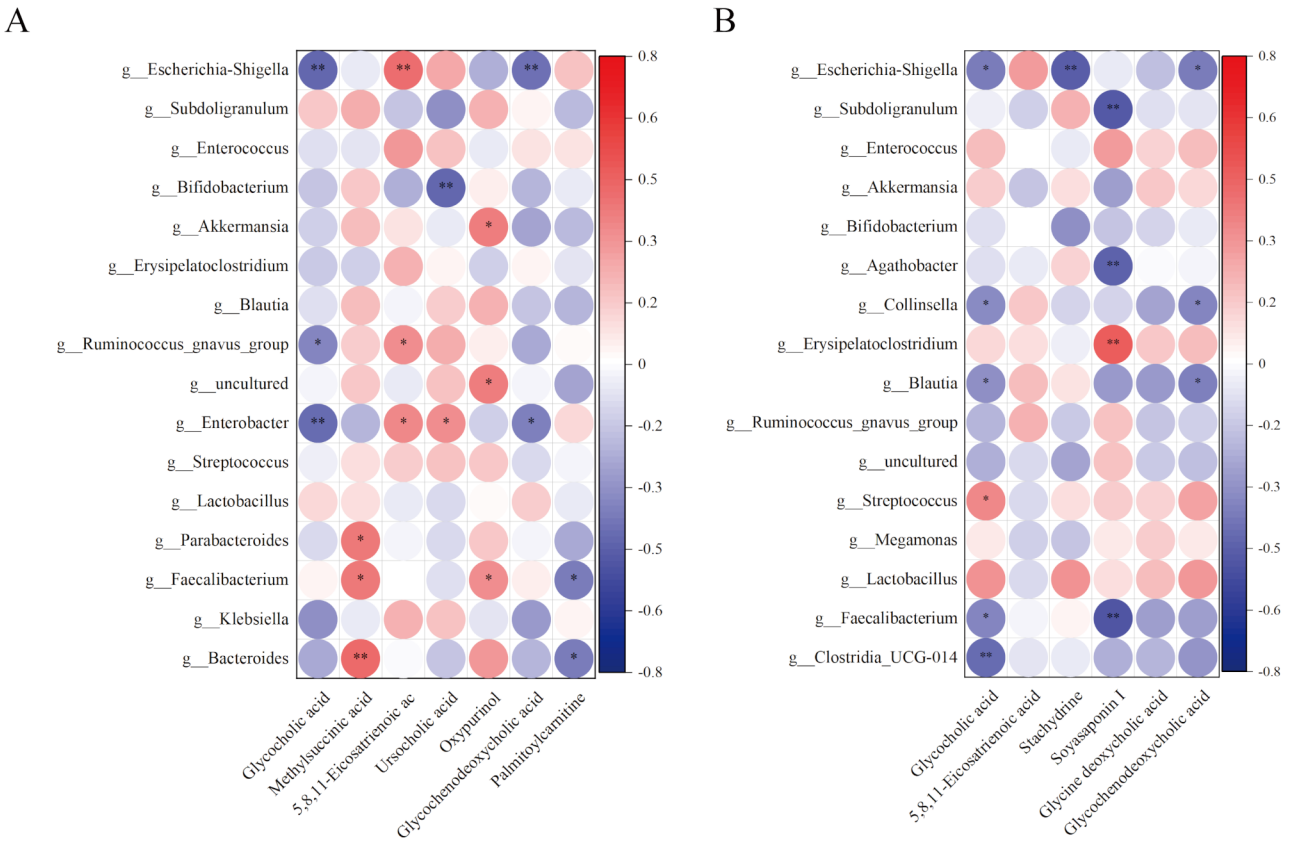

Fig. 8 Spearman correlation analysis between gut microbiota and differential metabolites. (**A**) Correlation analysis in SLE-nonLN and SLE-LN groups. (**B**) Correlation analysis in HC and SLE-nonLN groups. The x-axis represents differential metabolites, while the y-axis denotes bacterial genera identified at the 16 S rRNA gene level. Red indicates a positive correlation, whereas blue represents a negative correlation. Asterisks indicate statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$

Glycocholic acid and Glycochenodeoxycholic acid—and SLE disease activity [40]. In our cohort, both metabolites were significantly elevated in SLE-nonLN compared to HC (FC = 5.06 and 8.88, respectively), suggesting potential involvement in early-stage disease. These elevations may be linked to dysregulation of primary bile acid biosynthesis and lipid handling in SLE [41]. Interestingly, both bile acids showed markedly reduced levels in SLE-LN (FC = 0.37 and 0.28, respectively), implying a shift in bile acid metabolism during disease progression. While ROC analysis indicated high classification performance for these metabolites in distinguishing SLE subgroups (AUC = 0.951 and 0.827), their diagnostic utility warrants further validation in larger, independent cohorts. These findings support the relevance of bile acid metabolism in SLE and highlight Glycocholic acid and Glycochenodeoxycholic acid as promising candidates for non-invasive monitoring of disease progression [42].

Beyond lipid dysregulation, we observed stage-specific shifts in amino acid metabolism. SLE-nonLN patients exhibited elevated fecal levels of glucogenic amino acids (e.g., glycine, proline), indicative of a metabolic shift toward gluconeogenesis—a potential adaptation to chronic inflammation-induced energy demands [43]. In contrast, glycine and proline levels were reduced in SLE-LN patients compared to those with SLE-nonLN. This reduction was accompanied by distinct shifts in gut microbial composition, suggesting a potential association between amino acid metabolism and the progression of disease. Among the taxa showing the most notable differences across the groups were Escherichia-Shigella and Enterobacter, both of which were significantly more abundant in the SLE-LN group ($p < 0.001$). *Escherichia-Shigella* exhibited a negative correlation with bile acid metabolites, including Glycochenodeoxycholic acid and Glycocholic acid, across all three groups. Similarly, *Enterobacter* was negatively correlated with nearly all differential metabolites, such as Pterine, L-cysteine, and Glycoursodeoxycholic acid, which are strongly associated with bile acid biosynthesis and thiamine metabolism pathways. These findings suggest that the expansion of these microbial taxa may contribute to alterations in host metabolic pathways linked to disease severity. Nevertheless, further experimental studies are needed to

confirm these associations and clarify the underlying mechanisms.

However, this study has several limitations. The relatively small sample size reduces the statistical power of subgroup analyses and may limit the generalizability of our findings. Additionally, although participants were newly admitted and not receiving immunosuppressive therapy at the time of sampling, it was not feasible to completely rule out prior medication effects, which may have impacted the gut microbiota and metabolome. Future studies should incorporate larger and more diverse patient cohorts, including those with other autoimmune or inflammatory conditions, to validate and expand upon these findings. Longitudinal analyses are also warranted to explore dynamic changes in microbiome–metabolome interactions throughout the course of SLE and LN progression.

Conclusion

This study identified distinct alterations in gut microbiota composition and fecal metabolite profiles in patients with SLE, particularly those with LN. SLE was associated with increased Firmicutes and decreased Proteobacteria, while SLE-LN showed enrichment of potentially pro-inflammatory taxa such as Enterobacteriaceae (e.g., *Escherichia-Shigella*), which may be linked to renal inflammation. Metabolomic profiling revealed stage-specific metabolic shifts, including elevated levels of Mead acid and reduced concentrations of key bile acids such as Glycocholic acid and Glycochenodeoxycholic acid in SLE-LN patients. Notably, the abundance of *Escherichia-Shigella* was inversely correlated with key bile acid levels, suggesting possible microbiota–metabolite interactions relevant to LN progression. While causal relationships cannot be inferred, these findings may enhance our understanding of the microbiome–metabolome axis in SLE and support the potential use of non-invasive biomarkers for monitoring and stratifying disease severity.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03995-5.

Supplementary Material 1

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Author contributions
SYC, XJC and ZYW wrote the main manuscript text and AK prepared Figs. 2, 3 and 4. PY, HS and YT designed the study. All authors reviewed the manuscript.

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Data availability
The amplicon sequencing data are available in the NCBI Sequence Read Archive (SRA) database (BioProject: PRJNA1163353). The detailed data and materials are available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1163353.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital (approval number: 2022-461-02) and in accordance with national law and the Helsinki Declaration of 1975 (in its current, revised form). All participants provided written informed consent to participate before enrolment.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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