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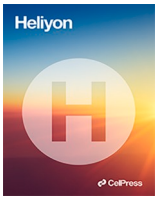

Research article

The mechanism of bile acid metabolism regulating lipid metabolism and inflammatory response in T2DM through the gut-liver axis

Yan Wang [a,1], Bohan Lv [a,1], Nannan Liu [a], Siyu Tao [a], Jinfang Dou [a], Jun Li [b], Ruxue Deng [a], Xiuyan Yang [a], Guangjian Jiang [a,c,*]

[a] *Traditional Chinese Medicine School, Beijing University of Chinese Medicine, Beijing, China*
[b] *Department of Endocrinology, Beijing He ping li Hospital, Beijing, China*
[c] *Affiliated Hospital of Traditional Chinese Medicine of Xinjiang Medical University, Xinjiang, China*

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ABSTRACT

Aims: The main objective of this study was to analyze the changes of intestinal microflora and how bile acid metabolic pathways affect lipid metabolism in T2DM through the gut-liver axis.
Methods: Firstly, 16S rRNA sequencing, metabolomics and transcriptomic sequencing were performed on plasma and feces of clinical subjects to determine the changes of intestinal flora and its metabolites. Finally, T2DM mice model was verified *in vivo*.
Results: T2DM patients have significant intestinal flora metabolism disorders. The differential fecal metabolites were mainly enriched in primary bile acid biosynthesis and cholesterol metabolism pathways in T2DM patients. After verification, the changes in gut microbiota and metabolites in T2DM patients (including up-regulated bacteria associated with BA metabolism, such as *lactobacillus* and *bifidobacterial*, and down-regulated bacteria capable of producing SCFAs such as *Faecalibacterium, Bacteroides, Romboutsia* and *Roseburia*); and the changes in the flora and metabolites that result in impairment of intestinal barrier function and changes of protein expression in the blood, intestine and liver of T2DM patients (including FGFR4↑, TRPM5↑ and CYP27A1↓, which are related to BA and lipid metabolism homeostasis, and TLR6↑, MYD88↑ and NF-κB↑, which are related to inflammatory response). These aspects together contribute to the development of further disorders of glucolipid metabolism and systemic inflammation in T2DM patients.
Conclusions: Changes in intestinal flora and its metabolites may affect lipid metabolism and systemic inflammatory response in T2DM patients through the gut-liver axis mediated by bile acids.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by impaired glucose and lipid metabolism and posing a challenge throughout life [1]. Dyslipidemia due to abnormal lipid metabolism is a prominent characteristic of diabetes [2]. Moreover,

* Corresponding author.
E-mail address: bucmjiang@163.com (G. Jiang).
[1] These authors have contributed equally to this work.

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abnormal lipid metabolism can also lead to a series of complications of T2DM and imposes an economic burden on the individuals. Previous studies have found that patients with diabetes have approximately three times the risk of dying from chronic liver disease, mainly attributable to Non-alcoholic fatty liver disease (NAFLD), which is associated with abnormal lipid metabolism and abnormal lipid accumulation in the liver [3,4].

Bile acids (BAs) are one of the major metabolites of the intestinal microbiota, and the production and excretion of BAs are essential for maintaining cholesterol homeostasis. Increased taurine conjugated BAs induce signaling through two different receptors, namely the steroid X receptor (FXR, especially in the gut) and the G-protein-coupled BA receptor TGR5(systemic), which reactivate brown adipose tissue and systemic glucose control through intestinal FXR-TGR5 cross-inker [5], resulting in weight loss and improved metabolism. Studies have shown that dysregulation of BAs metabolism due to dysbiosis of the gut microbiota can lead to hyperlipidemia and hyperglycemia [6,7]. In addition, the BAs axis itself is susceptible to inflammatory and metabolic interventions, so the BAs axis may constitute a mutual regulatory circuit in inflammation.

It is noteworthy that BAs can alleviate diabetes-induced cognitive impairment through enteric-hepato-brain axis-mediated intermittent fasting [8,9], which can directly or indirectly improve dietary habits and metabolic disorders in T2DM. FXR is a sufficient and necessary substance to reverse insulin resistance in rats and has been shown to stimulate the gut satiety pathway [10]. Abnormal secondary BA biosynthesis can mediate uncontrolled eating behavior *via* the gut-brain axis [11]. Study has pointed out that targeting the liver-brain axis can promote BA resynthesis and effectively reduce metabolic and neurocognitive disorders caused by obesity [12]. Although the understanding of the gut microbial-metabolite-host axis in T2DM is still evolving, key questions about the relationship between gut, liver, and brain tissue with gut microbiota metabolites in T2DM have not been resolved.

Based on the above evidence, we hypothesized that BAs may regulate lipid metabolism in T2DM through the gut-liver-brain axis. Multi-omics approaches have made it possible to study the mechanisms of complex metabolic diseases like obesity or diabetes in greater detail [13], including genomic, transcriptomic, metabolomic data [14]. Therefore, in this study, 16S rRNA sequencing, non-targeted metabolomics and transcriptomics were used to sequence samples of clinical T2DM subjects and T2DM mice to determine the differential intestinal flora, differential metabolites and key enrichment pathways. In animal experiments, transient receptor potential cation channel subfamily M member 5 (TRPM5) has been verified to regulate T2DM lipid metabolism, inflammatory responses and appetite through the gut-liver-brain axis *via* BAs metabolism pathway.

2. Materials and methods

2.1. Statement of Ethics and information about the subjects

Ethics committee approval was obtained from The Ethics Committee of Beijing University of Chinese Medicine (2017BZHYLL0105). Participants, enrolled from the Beijing He ping li Hospital, agreed to participate in the experiment after they were fully informed of its purpose and procedure. Inclusion and exclusion criteria are presented in Table 1. Subjects were divided into two groups: N group (n = 64) and the T2DM group (n = 58). Afterwards, fasting venous blood and fecal samples were collected from all subjects and stored at −80 °C until analysis.

2.2. Untargeted metabolomics study

Fecal samples (100 mg) were individually grounded with liquid nitrogen and resuspended with methanol and formic acid. After centrifugation, the supernatant was injected into the LC-MS/MS system analysis. LC-MS/MS analyses were performed using a Vanquish UHPLC system (Thermo Fisher) coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher). Samples were injected onto an Hyperil Gold column (100 × 2.1 mm, 1.9 μm) using a 16min linear gradient at a flow rate of 0.2 mL/min. Then, the raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) to perform peak alignment, peak picking, and quantitation for each metabolite. And then peaks were matched with the mzCloud (https://www.mzcloud.org/), mzVault and MassList database to obtained the accurate qualitative and relative quantitative results. Subsequently, these metabolites were annotated using the KEGG database (http://www.genome.jp/kegg/), HMDB database (http://www.hmdb.ca/) and Lipidmaps database (http://www.lipidmaps.org/). Finally, subsequent analysis was performed using metaX and R programming language.

Table 1
Inclusion and exclusion criteria.

	Inclusion Criteria	Exclusion Criteria
Subjects with T2DM	Diagnosed with T2DM; Diagnosed T2DM for at least three months.	Diagnosed with type 1 diabetes, secondary diabetes, gestational diabetes or unknown type of diabetes; Patients with stage III hypertension or myocardial infarction; Patients with severe primary diseases; Patients with serious complications, such as infection and diabetic ketoacidosis.
Healthy Subjects	FPG <5.6 mmol/L; Without acute or chronic disease, and medication free.	Subjects with a family history of diabetes; Subjects with hypertension or other cardiovascular and cerebrovascular diseases; Subjects currently taking medications.

2.3. qRT-PCR and transcriptome sequencing

First, we isolated the total RNA using TRIzol and tasted for purity using the NanoPhotometer spectrophotometer. After the assessment of RNA concentration and integrity, rRNA was removed and RNA sequencing libraries were prepared using the Ribo-Zero rRNA Removal Kit and NEBnext ultra ENA library prep kit, respectively. Subsequently, library quality was determined using Agilent 2100 system and quantified using quantitative real-time PCR (qRT-PCR). Finally, the libraries were sequenced on the Illumina Hiseq 2000 platform. The detailed experimental methods referred to our previous article [15].

2.4. Fecal 16S rRNA sequencing

16S rRNA sequencing was done using previously published methods in the laboratory of Novegene (Beijing, China) [16]. All of operating procedures strictly followed aseptic principles. Sequencing of the 16S rRNA gene was performed on an Illumina MiSeq, and data obtained with Illumina MiSeq amplicon sequencing was analyzed with QIIME 1. 8. 0 for the microbiota profiles. Sequences with ≥97 % similarity were assigned to the same Operational Taxonomic Unit (OTU) by the UCLUST algorithm in QIIME. The representative sequences of each OTU were used for taxonomic identification and phylogenetic analysis. DIAMOND software (V0.7.9) was used to compare the sequences of bacteria, fungi, archaea and viruses extracted from NCBI NR database to determine the number and abundance of species genes in each sample at different taxonomic levels. T-test, Metastats and LDA Effect Size (LEfSe) analysis were used to find the species of intergroup differences. Functional annotation of Unigenes was carried out, and the functional databases included KEGG (https://www. kegg.jp/), eggNOG (http://eggnogdb. embl.de/) and CAZy (http://www.cazy.org/) collect the relative abundance of different functional levels under the annotated functions of different functional databases. According to the results of functional annotation and gene abundance table, the number of genes in each sample in each classification level was obtained. Annotated gene count, total relative abundance display, abundance clustering heat map display, metabolic pathway analysis, etc.

2.5. Animals experiment

The experiment was ethically approved by the Animal Ethics Committee of the Beijing University of Chinese Medicine (BUCM-4-2018112901-4051). Twenty male C57BL/6J mice (8-weeek-old at the beginning of the experiment were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.) were housed with temperature and humidity controlled and 12h light/12h dark cycle. After one week of adaptation, ten mice were randomly allocated into normal control group and fed a standard diet. The other ten mice as T2DM group were fed with high-fat diet (HFD, 24 % Proteins, 24 % fats, and 41 % carbohydrates) for eight weeks. After 12h fasting, STZ solution (100 mg/kg) was injected intraperitoneally into the T2DM group mice to induce T2DM model. And the mice of control group were injected with the same volume of citric sodium citrate buffer. After seven days, mice with fasting blood glucose (FBG) ≥11.1 mmol/L were considered to T2DM. Finally, all mice were sacrificed, and the plasma, liver, brain and the colon were collected for subsequent analysis.

2.6. RNA extraction and qRT-PCR

Total mRNA was extracted from peripheral blood, livers and colons of mice using TRIzol total RNA isolation reagent. After that, cDNA was prepared by cDNA synthesis kit (ThermoScientific, Waltham, MA, USA). cDNA input was normalized to β-actin. Annealing temperature was 60 °C for all primers. Relative gene expression was calculated using the ΔΔCT method. The primers used for the PCR were list in Supplementary Table 1.

2.7. Hematoxylin eosin (H&E) staining and immunohistochemistry

Formalin-fixed liver, brain and colon tissues were embedded in paraffin, sectioned and stained with hematoxylin eosin as well as immunohistochemistry (IHC) staining. IHC for FGFR4, CYP46A1, NF-κB, Claudin-1 and Occludin were detected using the rabbit FGFR4, CYP46A1, NF-κB, Claudin-1 and Occludin monoclonal antibodies (dilution1:500). Microscopy (Olympus, U-LH100-3 Tokyo, Japan) was used to examine the tissues. And the positive immunohistochemical reaction was measured by Image Pro Plus.

2.8. Western blot analysis

Total proteins were isolated from liver, brain and colon tissues by RIPA protein lysate (SOLA Biosciences), then the protein was quantified using the BCA protein quantitative kit (Pierce Biotechnology). Protein samples of 40 μg each were resolved by the SAS-PAGE gel, transferred onto PVDF membranes, and subsequently incubated with corresponding primary antibodies (1:1000) and secondary antibodies (1:5000). Chemiluminescence was detected using enhanced chemiluminescence detection reagents. β-actin was used as the internal control.

2.9. Statistical analysis

All statistical analysis in this study were carried out in R 3.5.3 and SPSS 23.0 (IBM). The experimental data are presented as the mean ± standard deviation. Correlation analysis was performed using Kendall correlation analysis or Spearman correlation analysis.

Student's t-test was conducted to compare between the two groups. Statistically significant differences were indicated by $P < 0.05$. Each experiment was repeated for three technical replicates.

3. Results

1. Clinical individuals with T2DM exhibited notable lipid metabolism abnormalities.

In total, 58 T2DM patients and 64 healthy subjects were enrolled in this study. All T2DM patients met current diagnostic criteria for T2DM. Table 2 lists the characteristics of all subjects. There were no statistical differences in age. There was a significant increase in fasting plasma glucose (FPG) and triglyceride (TG) levels, and a decrease in high density lipoprotein cholesterol (HDL-C) level in the T2DM group compared with the normal (N) group.

2. The most significantly enriched KEGG pathways and the differential metabolites are focused on the BA metabolism pathways.

First, we performed fecal metabolomics analysis in T2DM patients compared with normal subjects. The results showed that 363 (including 74 up-regulated and 289 down-regulated metabolites) and 253 (including 137 up-regulated and 116 down-regulated metabolites) significantly different metabolites were screened in positive and negative-ion mode in T2DM patients compared with normal subjects, respectively.

We further performed KEGG enrichment analysis on the differential metabolites. The results are shown in Fig. 1. The most significantly enriched KEGG pathways in the positive-ion mode were Primary BA biosynthesis, Endocrine resistance, and Cholesterol metabolism. The most significantly enriched pathways in the negative-ion mode were Porphyrin and chlorophyll metabolism and Steroid biosynthesis. Among them, abnormalities in BAs metabolism and cholesterol metabolism are strongly associated with the development of T2DM and its complications.

Therefore, we focused on the primary BA synthesis and cholesterol metabolism pathways and performed further analysis. The metabolomic results showed that the differential metabolites enriched to both primary BA synthesis and cholesterol metabolism pathways were Taurocholic acid (TCA), Glycocholic acid (GCA) and Glycochenodeoxycholic Acid (GCDCA). And all the above three metabolites were significantly downregulated in T2DM group compared with N group ($P < 0.05$) (Supplementary Table 2).

3. Bifidobacterium bifidum and Lactobacillus lactis are the most significantly up-regulated microbiota in the T2DM group, which regulate BA metabolism.

Based on the 16s sequencing results, a total of 2386 OTUs were obtained for T2DM compared to normal subjects. we subsequently performed a *t*-test analysis between groups to identify species with significant differences ($P < 0.05$). The results indicated that at the genus level: T2DM vs N, the significantly up-regulated bacteria were *Lactobacillus, Bifidobacterium, Blautia, Megasphaera, Anaerostipes* and *Erysipelatoclostridium*; the significantly down-regulated bacteria were *Faecalibacterium, Bacteroides, Romboutsia,* unidentified *Ruminococcaceae, Alistipes, Roseburi, Alloprevotella, unidentified Prevotellaceae, Barnesiella,* and *Tyzzerella* (Fig. 1E). Among them, the most significantly up-regulated microbiota in the T2DM group were *Bifidobacterium bifidum* and *Lactobacillus lactis*, both BSH-rich microbiota, that catalyze the catabolism of conjugated BAs and regulate BAs metabolism.

4. Ruseburia and Romboutsia may regulate intestinal BAs metabolism in T2DM patients through short-chain fatty acids.

To gain a deeper understanding of the relationship between intestinal microbiota and metabolites, we performed a Pearson correlation analysis of genus-level significantly different bacteria in the intestinal flora with metabolites involved in primary BA biosynthesis and cholesterol metabolism from metabolome sequencing results. The results showed that *Roseburia* was significantly and positively associated with TCA, GCDCA and GCA, *Romboutsia* and *Erysipelatoclostridum* were significantly and positively associated with GCA and GCDCA, and *Tyzzerella* was significantly and positively associated with GCDCA (Fig. 1F). We found that *Ruseburia* and *Romboutsia* varied to a greater extent between the T2DM and N groups, so we speculate that the changes in these two bacteria may have

Table 2
Characteristics of study subjects.

	N	DM	*P*-value
Number	64	58	–
Male/female	32/32	32/26	–
Age	54.72 ± 11.48	55.62 ± 0.04	0.645
FPG	5.59 ± 1.13	10.52 ± 4.11	< 0.001
TC	5.36 ± 1.19	4.97 ± 1.20	0.081
TG	1.60 ± 0.91	2.54 ± 2.15	0.002
LDL-C	3.16 ± 0.95	2.87 ± 0.83	0.082
HDL-C	1.36 ± 0.37	1.20 ± 0.44	0.034
ALT	22.90 ± 14.09	25.80 ± 14.94	0.334
AST	23.90 ± 10.23	22.57 ± 9.91	0.554

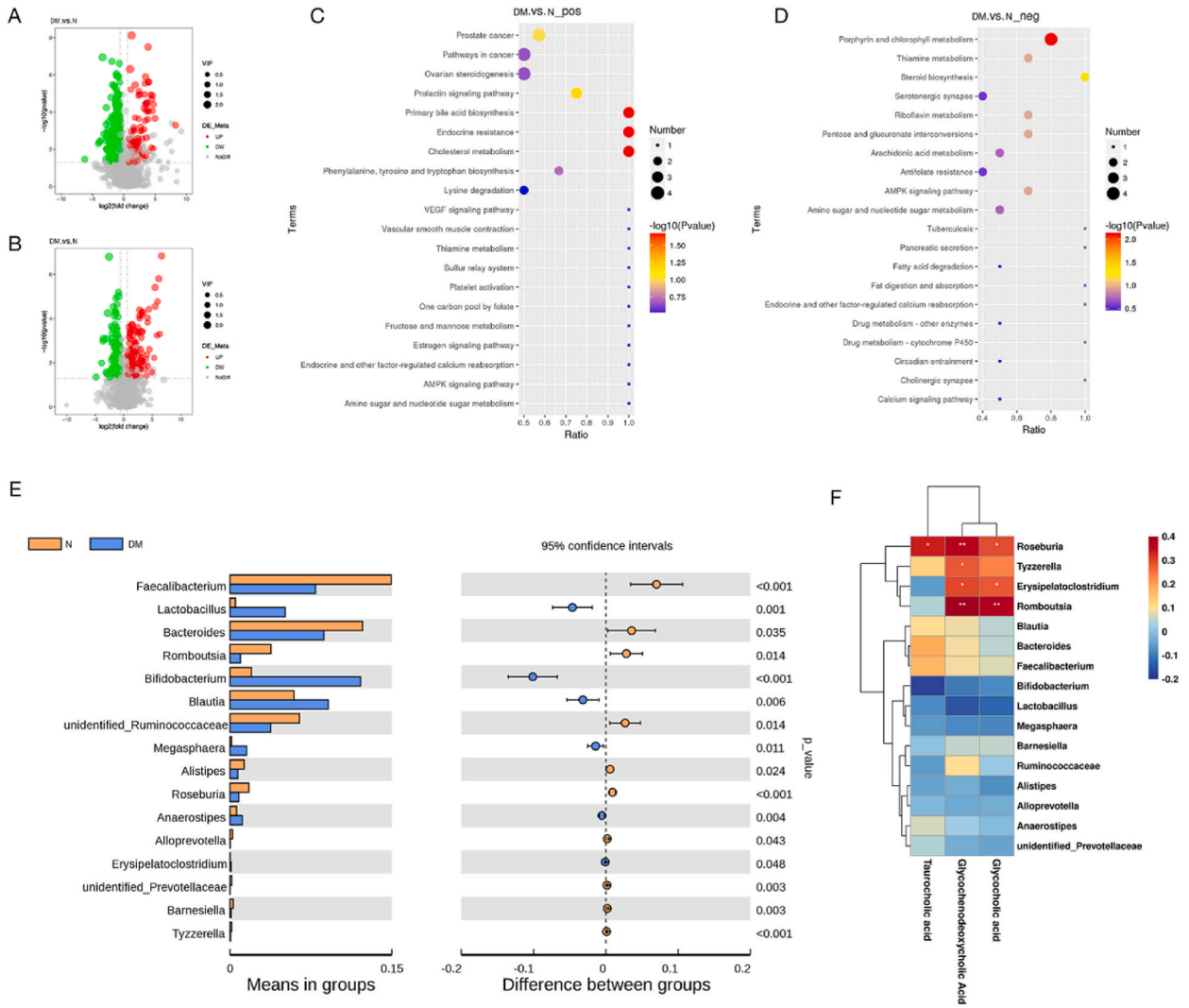

Fig. 1. The results of fecal metabolomics analysis, 16s sequencing analysis and correlation analysis. (A) The volcano plot of the DM vs. N group in positive ion mode; (B) The volcano plot of the DM vs. N group in negative ion mode; (C) KEGG analysis results of metabolites in positive ion mode; (D) KEGG analysis results of metabolites in negative ion mode; (E) Discrepancy of the microbiota composition between DM and N group (genus level); (F) Heatmap graph of correlation analysis result between metabolomics and intestinal microbiota: Red: positive correlation, blue: negative correlation. *Correlation is significant at $0.01 \leq P < 0.05$; **Correlation is significant at $P < 0.001$.

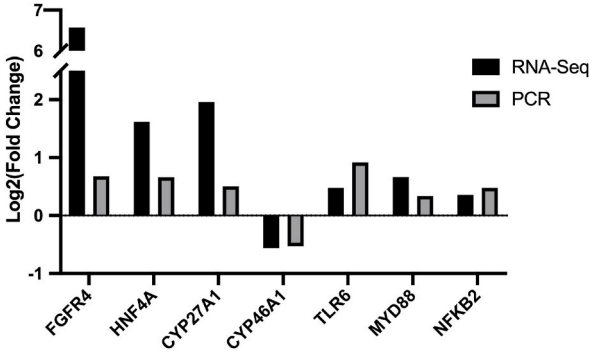

Fig. 2. Sequencing and quantitative PCR. Sequencing and quantitative PCR for FGFR4, HNF4A, CYP27A1, CYP46A1, TLR6, MYD88 and NFκB2. The quantitative PCR results were consistent with the sequencing data.

some relationship with the altered BA metabolism in the intestine of T2DM patients. In addition, both *Ruseburia* and *Romboutsia* are capable of producing short-chain fatty acids (SCFAs), especially butyric acid, which can affect intestinal permeability, cause intestinal inflammation, and play an important role in the development of complications of T2DM.

5. Abnormal BA metabolic pathway is closely related to inflammation in T2DM patients.

Since the metabolites of the gut flora such as BAs and SCFAs can be taken up by the intestine and enter the blood circulation, we further investigated the results of serum transcriptomic sequencing in T2DM patients and normal subjects. We found that genes related to BA metabolism as well as inflammation were differentially expressed in the peripheral blood of T2DM patients, including FGFR4, HNF4A, CYP27A1, CYP46A1, TLR6, MYD88, and NF-κB (Supplementary Table 3). We subsequently validated the sequencing results using qRT-PCR. The PCR results were consistent with the transcriptome sequencing results, confirming the reliability of the transcriptional sequencing results (Fig. 2).

6. Correlation Analysis of Gut Microbiota, Metabolomics and Transcriptomic: Differential flora may up-regulate inflammatory response in T2DM through BA metabolism pathway

To further understand the association between transcriptomic, intestinal microbiota and metabolomics results, we performed a combined multi-omics analysis. The results in Fig. 3 showed that *Lactobacillus* was significantly associated with MYD88, NF-κB, CYP27A1 and FGFR4, *Bifidobacterium* was significantly associated with MYD88 and NF-κB, and *Romboutsia* was significantly associated with CYP27A1. In the network plot, it can be seen that the *Roseburia* and *Romboutsia,* which are associated with butyric acid production and intestinal inflammation, were significantly correlated with changes in BAs metabolites in the intestine, while *Lactobacillus* and *Bifidobacterium*, which are closely related to the process of BA metabolism, were significantly correlated with changes in the expression of inflammation-related genes MYD88 and NF-κB. These results illustrated the relevance of BAs metabolism to the inflammatory process and the complexity of metabolic regulation by the gut microbiota during the development of diabetes.

7. T2DM mice had hepatic steatosis and up-regulated inflammatory markers FGFR4, NF-κB, and TRPM5 associated to aberrant BA metabolism.

We constructed a T2DM mice model, hoping to further understand the changes of the above pathways. The liver HE staining results showed that the liver of T2DM mice developed hepatocyte ballooning and switched to an adipocyte-like state (Fig. 4). While in the N group, the structure of hepatic lobules was clear and intact, and the liver cells were round and normal size with clear nucleoli. These

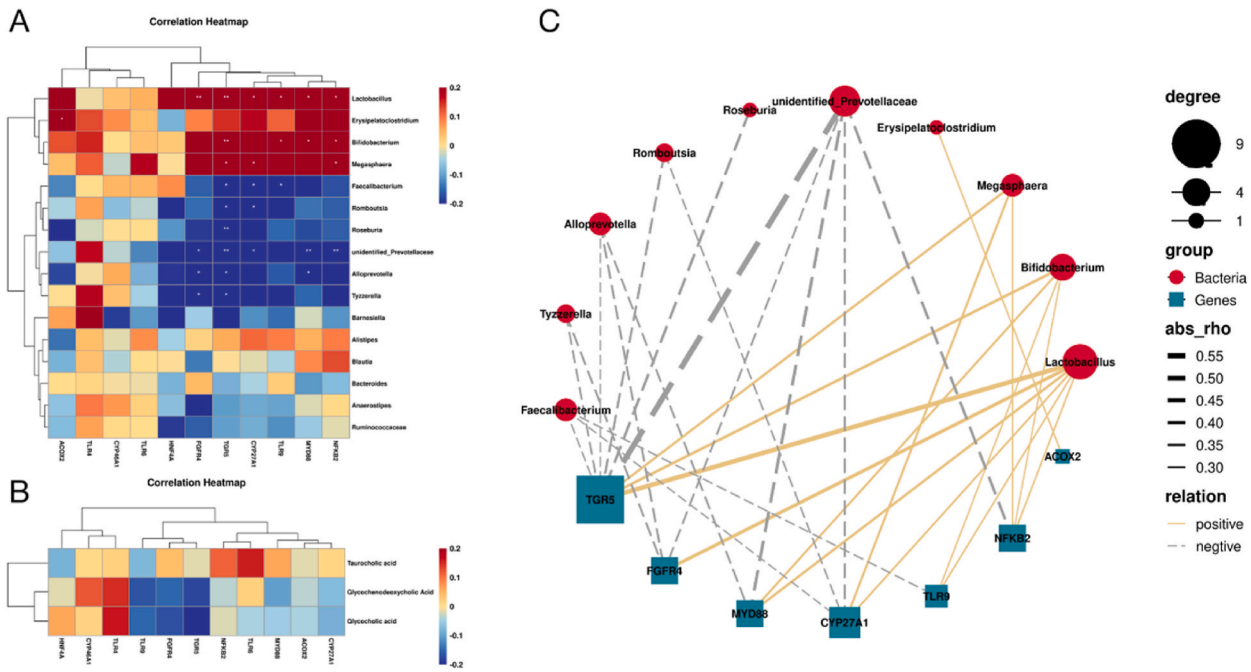

Fig. 3. Heatmap and network graphs of correlation analysis result between transcriptomic, metabolomics and intestinal microbiota. (A, B) heatmap of correlation analysis results between transcriptomic and intestinal microbiota (A), and between transcriptomic and metabolomics (B); Red: positive correlation, blue: negative correlation. *Correlation is significant at $0.01 \leq P < 0.05$; **Correlation is significant at $P < 0.001$. (C) network graph pf correlation analysis results.

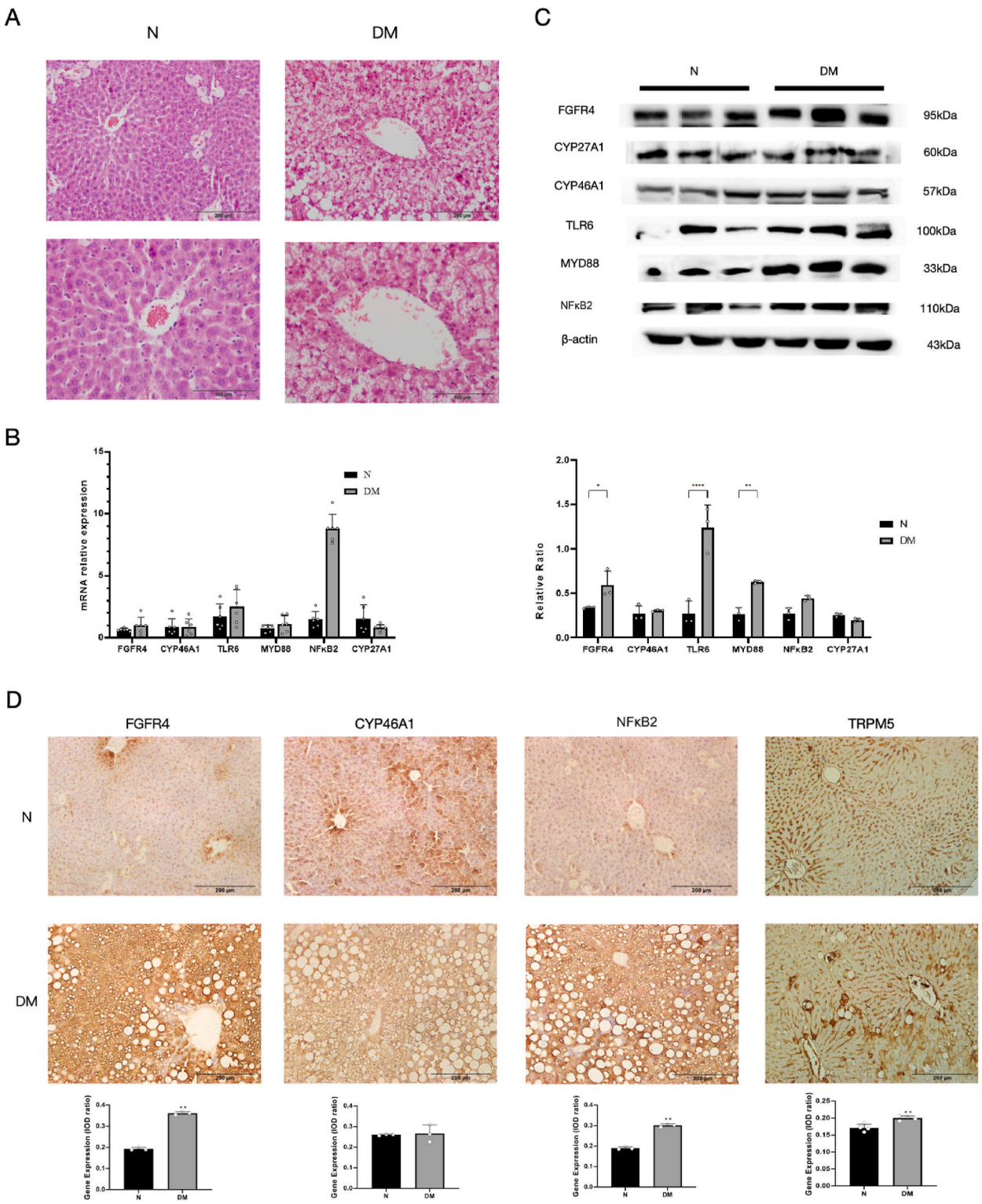

Fig. 4. The results of H&E staining, qRT-PCR, Western blot and immunohistochemistry in the liver. (A) H&E stains; (B) PCR analysis for FGFR4, CYP46A1, CYP27A1, TLR6, MYD88 and NF-κB2 between DM and N group; (C) results of Western blot analysis. Upper: Western blot; Lower: relative density from the Western blot; (D) Immunohistochemistry of FGFR4, CYP46A1, NF-κB2 and TRPM5.

results indicate that hepatic steatosis may occur during the development of T2DM.

According to the results of multi-omics sequencing in T2DM patients, changes in their intestinal microbiota may cause abnormal BAs metabolism, which leads to abnormal lipid metabolism in the liver. Thus, the expression of key genes, which were differentially

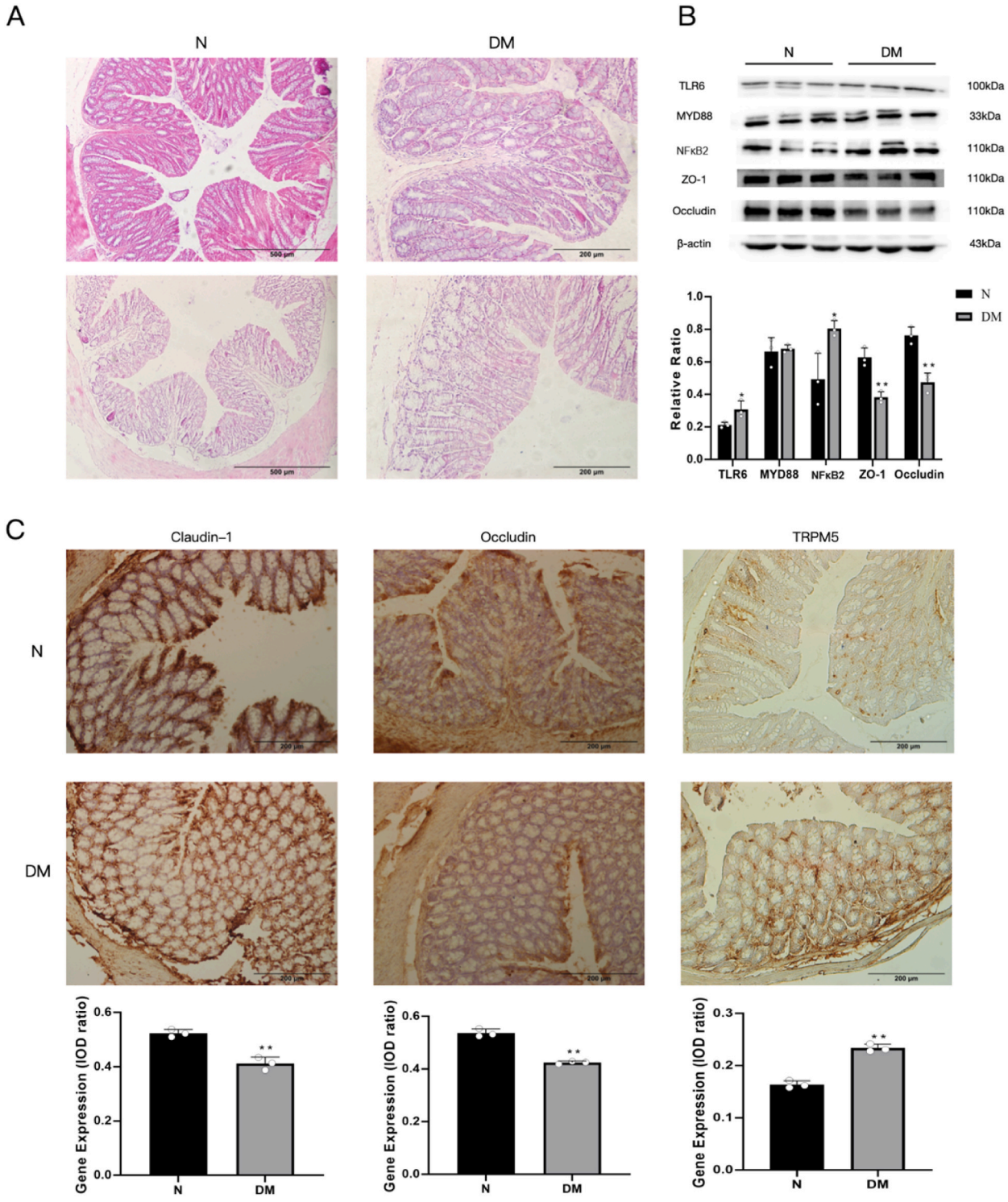

Fig. 5. The results of H&E staining, Western blot, and immunohistochemistry in the colon. (A) H&E stains; (B) results of Western blot analysis. Upper: Western blot; Lower: relative density from the Western blot; (C) Immunohistochemistry of Claudin-1, Occlusin and TRPM5.

expressed in T2DM patients, were tested in the liver of T2DM mice. PCR results showed that the expression of FGFR4, TLR6, MYD88 and NF-κB were up-regulated, while CYP27A1 and CYP46A1 were down-regulated in the liver of T2DM mice. The Western blot results showed that the expression levels of FGFR4, TLR6, MYD88 and NF-κB were significantly increased ($P < 0.01$ or $P < 0.05$) and the expression levels of CYP27A1 and CYP46A1 were decreased in the liver of the T2DM mice compared with the control group. In addition, immunohistochemistry was performed for FGFR4, CYP46A1, NF-κB and TRPM5 proteins. And the results showed that the expression levels of FGFR4, NF-κB and TRPM5 were significantly elevated in the liver, while the changes of CYP46A1 were not significant in the T2DM group compared with the N group ($P > 0.05$).

8. Significantly inflammatory factors up-regulation and morphological injury was observed in colon tissues of T2DM mice.

In addition, we also examined the changes in morphology and gene expression in colonic tissue of T2DM mice. HE staining results suggested that the T2DM group presented crypt distortion, loss of goblet cells, epithelial damage and inflammatory cell infiltration (Fig. 5). Western blot and immunohistochemistry results showed that TLR6, NF-κB and TRPM5 proteins were significantly up-regulated, while ZO-1, Occludin and Claudin-1 proteins were significantly down-regulated in the T2DM group compared with the N group in colon.

4. Discussion

Using untargeted metabolomics, we uncovered the different metabolic profiles between the T2DM and N groups. Pathway analysis of differential metabolites revealed that Primary BA biosynthesis and Cholesterol metabolism pathway were the most significantly enriched pathways. Liver is the most important organ for cholesterol and BA synthesis. The main metabolic pathway of cholesterol, BA biosynthesis, plays an important role in maintaining a healthy level of cholesterol in the liver and the blood. BAs are signaling molecules that are important in regulating metabolism and inflammation. The abnormal BAs are inked to insulin secretion changes, leading to T2DM [17,18]. In addition, the BA biosynthesis is also associated with intestinal inflammation and increased mucosal permeability. Therefore, in our study, the presence of changes in pathways related to cholesterol and BA metabolisms in T2DM patients may indicate the mechanisms of lipid metabolism abnormalities and intestinal inflammation that accompany T2DM patients.

In addition, we conducted 16S rRNA sequencing of fecal total RNA to investigate the changes in microbiological composition of T2DM patients and healthy controls. Our results showed that, compared to healthy controls, patients with T2DM showed a significant increase in the relative abundance of *Bifidobacterium, Lactobacillus, Blautia* and *Megesphaera*, and a remarkable decrease of *Faecalibacterium, Bacteroides, Romboutsia* and *Roseburia.* Gut microbiome is a key player in intestinal and physiological homeostasis, immunity, energy metabolism and glucose and lipid metabolism [19]. BAs are one of the major metabolites of intestinal microbes. The interaction between gut microbiota and BAs plays an important role in regulating energy metabolism and cholesterol and BA homeostasis [20–22]. In the presence of bile salt hydrolase (BSH) containing intestinal flora, conjugated BAs are converted to free BAs and influence the size and composition of the BA pool through the enterohepatic circulation [20,23]. BSH activity is mainly attributed to *lactobacillus* and *bifidobacterial* [23,24]. In our results, significant upregulation of *Bifidobacterium* and *lactobacillus* occurred in the intestine of T2DM patients. Meanwhile, the metabolome results showed that the levels of TCA, GCA and GCDCA, which are conjugated BAs, were significantly decreased in T2DM group compared with N group. It suggests that the changes in the intestinal flora of T2DM patients may have caused a decrease in the levels of conjugated BAs in their intestines, thus affecting the energy and lipid metabolism of the organism.

We further performed a combined multi-omics analysis and found that bacteria associated with BAs metabolism were significantly correlated with inflammation-related genes. Therefore, to further elucidate the interactions between various changes during the development of T2DM, we constructed an animal model of T2DM. Huang et al. showed that decreased BSH enzyme activity and increased conjugated BAs in the intestine can inhibit the intestinal FXR-FGF15 signaling pathway, leading to a decrease in FGF15/FGF19, resulting in the production of BAs in the liver [25]. FGF15/FGF19 is able to activate its receptor FGFR4 in the liver, which acts to reduce hepatic BA synthesis by inhibiting BA synthetic enzymes CYP7B1 and CYP27A1 [26]. In our study, elevated FGFR4 protein expression and reduced CYP27A1 protein expression of the T2DM mouse model resulted in decreased synthesis of BAs and lipid accumulation in the liver. This may be an intrinsic mechanism for the abnormal lipid metabolism caused by changed in BA metabolism in T2DM.

On the other hand, we found a downregulated expression of *Faecalibacterium, Bacteroides, Romboutsia* and *Roseburia* in the T2DM group, which are bacteria capable of producing SCFAs [27,28]. Carbohydrate enzymation by intestinal bacteria can produce SCFAs, including acetate, propionate and butyrate, etc. SCFAs are a major energy source for enterocyte and are important for maintaining normal intestinal function and the morphology and function of colonic epithelial cells [29]. A decline in SCFAs production, causing intestinal barrier dysfunction [30,31]. Butyrate can serve as an important energy source for colon cells, and is also involved in inhibiting inflammation and enhancing barrier function [29,32]. It can inhibit the mRNA expression of pro-inflammatory cytokines in the mucosa by suppressing the activation of NF-κB [33]. In addition, chronic inflammation and increased intestinal permeability are not only strongly associated with the development of T2DM, but can also contribute to the transfer of inflammatory/antigenic/toxic substances from gut lumen to liver, thereby affecting the hepatic lipid metabolism homeostasis [34–36]. In our study, significant down-regulation of SCFAs-producing flora may have contributed to the development of intestinal inflammation and weakened intestinal barrier function in T2DM patients, leading to further systemic inflammation and disruption of glucose and lipid metabolism.

The results of the combined multi-omics analysis showed that the bacteria associated with the production of SCFAs were significantly correlated with changes in BA metabolites. This suggests a complex interaction between multiple pathways in the development

of T2DM. It also illustrates that the comp lex metabolic changes in the T2DM patients contribute to the high susceptibility of T2DM to multiple complications. Down-regulation of bacteria such ad *Bacteroides, Romboutsia* and *Roseburia* can lead to a decrease in the production of SCFAs. Many studies have shown that decreased concentrations of SCFAs are associated with intestinal inflammation [37–39]. Among them, butyrate can activate G protein-coupled receptors (GPRs), thus inhibiting the downstream NF-κB signaling pathway, reducing the secretion of pro-inflammatory factors, suppressing the inflammatory response, and protecting the intestinal mucosal barrier [40]. In addition, Toll-like receptors (TLR) are pattern recognition receptors present on many cell types, and MYD88/NF-κB-mediated cell signaling is one of the common pathways involved in TLR activation. It has been shown that *Faecalibacterium, Bacteroides* or their metabolites are able to be recognized by TLRs, resulting in subsequent effects [41]. Moreover, tight junction proteins (TJ proteins), including ZO-1, ZO-2, Occludin and Claudin-1, located between the adjacent intestinal epithelial cells, are an essential mechanical barrier, which plays a crucial role in the regulation of intestinal epithelial barrier integrity and intestinal permeability [42,43]. Previous studies have described that inflammatory cytokines and bacterial antigens can affect TJ proteins expression, thus altering intestinal permeability [44]. In our study, up-regulated expression of TLR6, MYD88 and NF-κB genes were observed in the peripheral blood of T2DM patients, while TLR6 expression was up-regulated in both the intestine and liver of T2DM model mouse, and led to elevated expression of its downstream NF-κB. Furthermore, the expression of ZO-1, Occludin and Claudin-1 proteins was decreased in the T2DM mice, suggesting the barrier function of the intestinal mucosa exhibited damage. Collectively, these results suggest that changes in the intestine of T2DM patients may affect intestinal barrier function and increase the extent of the inflammatory response, leading to further metabolic disorders.

TRPM5 is a newly cloned gene from gustatory cells and is a non-selective monovalent cation channel that is activated by an increase in intracellular Ca^{2+}. TRPM5 is a common downstream signaling pathway for sweet, bitter and fresh tastes and is widely expressed in liver, brain, small intestine, thymus, spleen and kidney. Another study showed that TRPM5 is expressed on pancreatic β-cells, suggesting that TRPM5 may be involved in the release and action of insulin [45]. The role of TRPM5 has been investigated by knockout mouse models. $Trpm5^{-/-}$ mice lack type II taste sensation and have reduced glucose-induced insulin secretion. TRPM5 expression levels are reduced in obese, leptin-signaling-deficient mice, and TRPM5 mutations are associated with type II diabetes and metabolic syndrome [46]. In addition, TRPM5 is expressed in BA-sensitive cluster cells in the intestine and is able to be activated by BAs and short-chain fatty acids to exert a regulatory role in inflammation and BA metabolism [47,48]. We therefore hypothesized that TRPM5 may play an important role in the abnormal lipid metabolism in T2DM. Our results showed that TRPM5 expression was upregulated in the colon and liver tissues of mice. Therefore, TRPM5 may play a regulatory role in BAs metabolism and lipid metabolism in T2DM through the gut-liver axis.

Therefore, based on the multi-omics results, we hypothesize that the abnormalities in lipid metabolism in patients with T2DM may be due to the following reasons: 1. Changes in gut microbiota and metabolites in T2DM patients (including up-regulated bacteria associated with BA metabolism, such as *lactobacillus* and *bifidobacterial*, and down-regulated bacteria capable of producing SCFAs such as *Faecalibacterium, Bacteroides, Romboutsia* and *Roseburia*); 2. Changes in the flora and metabolites that result in impairment of intestinal barrier function and changes of protein expression in the blood, intestine and liver of T2DM patients (including FGFR4↑, TRPM5↑ and CYP27A1↓, which are related to BA and lipid metabolism homeostasis, and TLR6↑, MYD88↑ and NF-κB↑, which are related to inflammatory response). These aspects together contribute to the development of further disorders of glucolipid metabolism and systemic inflammation in T2DM patients.

Overall, in this study, we used a multi-omics approach to T2DM patients to reveal possible links between intestinal microbiota, metabolites and transcriptomic. However, the present study has several limitations. First, because liver and colon tissues from T2DM subjects were not available, we selected peripheral blood for transcriptome sequencing. This resulted in differences in the expression of some relevant genes in the peripheral blood of subjects and animal tissues (like CYP27A1). Since the expression of genes varies in tissues throughout the body, more studies are needed to investigate the exact mechanism. In addition, the regulatory mechanisms of gut microbiota and their associated metabolites are quite complex. To further clarity the effects of intestinal microbiota on the changes of gene expression and phenotype in T2DM patients, approaches such as gut microbiota depletion and fecal microbiota transplantation could be used in future studies. Despite the need for further specific investigation, our study has elucidated the possible mechanisms of the role of changes in gut microbiota and metabolites in the development of T2DM and its lipid metabolism abnormalities, and highlighted the utility of combination 16S rRNA sequencing, metabolomics, and transcriptome sequencing to investigate the mechanisms of T2DM.

Ethics approval and consent to participate

Ethics committee approval was obtained from The Ethics Committee of Beijing University of Chinese Medicine (2017BZHYLL0105). Participants, enrolled from the Beijing He ping li Hospital, agreed to participate in the experiment after they were fully informed of its purpose and procedure. And the experiment was ethically approved by the Animal Ethics Committee of the Beijing University of Chinese Medicine (BUCM-4-2018112901-4051).

Consent for publication

Not applicable.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yan Wang: Writing – original draft. **Bohan Lv:** Writing – original draft. **Nannan Liu:** Visualization, Data curation. **Siyu Tao:** Resources, Methodology, Formal analysis. **Jinfang Dou:** Resources, Methodology, Formal analysis, Data curation. **Jun Li:** Software, Resources, Data curation. **Ruxue Deng:** Resources, Methodology, Investigation. **Xiuyan Yang:** Visualization, Validation, Supervision. **Guangjian Jiang:** Funding acquisition.

Declaration of competing interest

Dear Editors, We would like to submit the enclosed manuscript entitled “The mechanism of bile acid metabolism regulating lipid metabolism and inflammatory response in T2DM through the gut-liver axis”, which we wish to be considered for publication in “Heliyon”.

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my-coauthors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35421.

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