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Research article

A study on the relationship between gut microbiota and intrahepatic cholestasis of pregnancy

Li-wen Liu [a], Yan Chen [b], Liu-jing Zhu [b], Qun-xiang Xu [c], Shaolin Xu [d], Yanling Ding [d], Biao Yin [e,*]

[a] Department of Anesthesia, The Reproductive Hospital of Guangxi Zhuang Autonomous Region, Nanning City, 530000, China
[b] Department of Obstetrical, Guangzhou Women and Children's Hospital Liuzhou Hospital, Liuzhou City, 545000, China
[c] Department of Breast, Guangzhou Women and Children's Hospital Liuzhou Hospital, Liuzhou City, 545000, China
[d] Department of Laboratory, Guangzhou Women and Children's Hospital Liuzhou Hospital, Liuzhou City, 545000, China
[e] Department of Eugenic Genetics, The Reproductive Hospital of Guangxi Zhuang Autonomous Region, Nanning City, 530000, China

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ABSTRACT

Objective: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disease associated with a high incidence of complications in the mid and late stages of gestation. This study investigates differences in the composition of intestinal flora among pregnant women diagnosed with ICP, employing Illumina MiSeq high-throughput sequencing technology.

Methods: This case-control study obtained patient data from the hospital information system (HIS) and the laboratory information system (LIS). Fecal samples were collected from 25 pregnant women who did not undergo intestinal preparation before delivery between December 2020 and March 2021. Whole-genome analysis was performed. PCR was used to amplify the 16S rRNA V3–V4 variable region, which was then sequenced. Alpha and beta diversity were computed, and the maternal intestinal flora's abundance and composition characteristics were analyzed. Differences in intestinal flora between the two sample groups were examined.

Results: Bacteroides and Proteobacteria exhibited positive correlations with TBIL and IBIL. Betaproteobacteria, Gammaproteobacteria, and Erysipeiotrichi showed positive correlations with TBIL, IBIL, and DBIL, while *Lactobacillus*, *Delftia*, and *Odoribacter* demonstrated positive correlations with ALT.

Conclusion: The ICP group displayed significantly higher levels of total bile acid and ALT compared to the control group. The intestinal flora composition comprised four primary phyla: Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria. ICP patients exhibited a lower relative abundance of intestinal flora across different levels of community composition when compared to the control group. Specific correlations between certain intestinal flora and clinical liver parameters were identified.

Intrahepatic cholestasis of pregnancy (ICP) is a liver ailment exclusive to pregnancy, predominantly manifesting complications during the mid and late gestational phases. The primary clinical symptom is itching, accompanied by elevated levels of total bile acids in serum. The global prevalence of ICP ranges from 0.2% to 2%, contingent on geographic and racial variances within the study

* Corresponding author. Department of Eugenic Genetics, The Reproductive Hospital of Guangxi Zhuang Autonomous Region, No.3 Longyuan Road, Qingxiu District, Nanning City, 530000, China.
E-mail address: yinbiaooo33@163.com (B. Yin).

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population [1]. Some women with ICP encounter increased postpartum bleeding due to decreased intestinal bile acid levels, impairing vitamin K absorption and diminishing clotting factor synthesis. ICP can exert more severe repercussions on the perinatal infant than maternal complications [2]. Bile acids influence the volume of the placental chorionic cavity, leading to various changes, including trophoblastic edema. These changes disrupt oxygen and nutrient exchange between the mother and the perinatal fetus, resulting in intrauterine hypoxia, restricted growth, and intrauterine fetal demise. Perinatal mortality among ICP patients is 6–10 times higher than infants born to unaffected mothers. Additionally, there is a risk of meconium contamination of amniotic fluid, intracranial hemorrhage in newborns, neonatal depression, and respiratory distress syndrome [3,4]. In Chinese mothers with bile acid imbalances, the incidence of preterm birth is approximately 20% [5,6]. A significant 2014 UK national cohort study revealed that severe ICP is associated with a significantly increased risk of preterm birth, stillbirth, and neonatal unit admission for treatment compared to a control group [7].

ICP's etiology remains elusive, with studies suggesting the potential involvement of factors such as hormonal secretion levels, patient genetics, immune function, and environmental conditions in its pathogenesis [8]. Some research has employed animal models to investigate estrogen's cholestatic effects and its hepatotoxicity implications [9,10]. Related research has indicated higher progesterone metabolite levels in pregnant women with ICP, hinting at an adverse association between these metabolites and ICP [11]. Patients with a familial history of ICP (92%) face a greater risk of recurrent ICP than sporadic cases (40%) [12]. Genetic variations affecting bile acid synthesis and transport pathways have also been associated with ICP progression. In one study, mutations in the hepatocyte transporter protein ABCB4 were identified in over 15% of ICP cases [13]. Environmental factors also exert a notable influence on ICP dynamics. A cross-sectional cohort study in Chile demonstrated a correlation between ICP prevalence and seasonal variations, with the lowest incidence recorded in the summer months. This seasonal trend aligned with higher plasma selenium levels during summer, implicating diet in ICP's pathogenesis [14].

Numerous researchers have explored the connection between gut microbiota and health. The gut microbiota, constituting approximately 76% of the human microbiota, offers diversity and abundance. It contributes to digestion, facilitates the elimination of harmful substances in the intestines, enhances intestinal barrier function, metabolizes bile acids, and detoxifies toxins [15–18]. Additionally, gut microorganisms synthesize essential vitamins and amino acids for human requirements. They are also involved in sugar and protein metabolism while regulating the human immune system, a pivotal aspect of maintaining overall well-being [19,20]. Dysbiosis of the intestinal flora has been linked to various cholestasis-related diseases, including cirrhosis and cholangitis [21,22]. Multiple studies have suggested a correlation between the gut microbiota and bile acid metabolism. The gut microbiota participates in various biological processes, promoting bile acid metabolism. Sayin et al. demonstrated that the gut microbiota contributes to the conversion of unconjugated primary bile acids into secondary bile acids through a series of enzymatic reactions [23]. The enterohepatic cycle of bile acids involves the formation of primary bile acids, including bile acids and chenodeoxycholic acid (CDCA), conjugated with glycine and taurine, followed by secretion into the biliary system. Upon entering the intestine, these bile acids undergo enzymatic reactions with intestinal enzymes, resulting in secondary bile acids such as deoxycholic acid, lithocholic acid, and a small quantity of ursodeoxycholic acid (UDCA) [24]. Bile acids play a pivotal role in maintaining the integrity of the intestinal mucosal barrier function and inhibiting bacterial translocation. Disruption of the gut microbiota can lead to increased intestinal mucosal permeability, allowing endotoxins to enter the liver via the enterohepatic circulation, thereby triggering a hepatic inflammatory response [25]. Furthermore, the gut microbiota can influence bile acid synthesis in the liver by alleviating farnesoid X receptor (FXR) inhibition in the ileum [23]. An animal study even demonstrated that probiotics can ameliorate disorders related to bile acid metabolism in pregnant rats [26]. The composition of bile acids in the intestine is closely intertwined with the intestinal flora and maintains a dynamic equilibrium. Alterations in the gut microbiota can result in changes in bile acid diversity and vice versa. An imbalance between the intestinal flora and bile acids can lead to hepatitis, which, in turn, can exacerbate the imbalance between the two. Consequently, the gut flora and bile acids interact through the enterohepatic cycle. This interaction's dysregulation also occurs in pathological conditions, including diet-induced obesity [27], cholestatic liver disease [28], gastrointestinal inflammation, and cancer [29]. Some studies employing 16S rRNA sequencing have unveiled distinctions in the gut microbiota characteristics of ICP patients compared to healthy individuals [30,31]. Nevertheless, evidence is currently insufficient to establish causal relationships or ascertain whether gut microbiota changes result from diseases or contribute to ICP development. The specific types of gut microbiota involved and the mechanisms by which gut microbiota impacts ICP pathogenesis remain enigmatic. In this study, we explore the potential association between ICP and specific gut microbiome characteristics, aiming to elucidate the gut microbiome's role in ICP pathogenesis.

Our team gathered fecal samples from individuals, conducted 16S rRNA sequencing on qualified samples, and amplified the V3 and V4 variable regions of the amplification products. Subsequently, we determined the phylum, order, family, and genus of the gut flora in both sample groups, assessing their composition characteristics. Through the examination of changes in intestinal flora biodiversity and their correlation with ICP progression, we aimed to introduce novel ideas and methods for managing ICP, with a focus on reducing maternal hemorrhage and ensuring the well-being of both mothers and infants.

1. Information and methods

1.1. General information

We conducted a case-control study and gathered participant information from the Hospital Information System (HIS) and Laboratory Information System (LIS). Fecal samples were selected from 25 pregnant women who had not undergone intestinal preparation before delivery between December 2020 and March 2021, obtained from a biological bank. These samples were divided into two

groups: Group I: Comprising 17 diagnosed ICP mothers. Group N: Consisting of eight normal pregnant women (the control group). This study received approval from the hospital ethics committee.

1.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) Age between 20 and 40 years with singleton pregnancies. (2) American Society of Anesthesiologists (ASA) grade I-II. (3) Patients who provided signed informed consent to participate in the study.

The exclusion criteria were as follows: (1) Patients who received antibiotics within the last three months, as antibiotics can impact gut microbiota. (2) Patients who consumed probiotic preparations, including probiotic-containing foods, within the last three months, as probiotics can affect gut microbiota. (3) Patients with other liver diseases such as gallstones, hepatotoxic drug usage, hepatitis, and inflammatory bowel disease. (4) Patients with hepatitis B or pregnancy-related conditions like gestational hypertensive syndrome, gestational diabetes mellitus, pre-eclampsia, or other pregnancy-related syndromes. (5) Patients who declined to participate in the study.

The diagnostic criteria for ICP were as follows: (1) Biochemical indicators: Serum total bile acid levels exceeding 10–40 μmol/L (2) Clinical symptoms: Presence or absence of pruritus and jaundice.

1.3. Sample collection

All patients provided informed consent and tested negative for COVID-19. Stool samples were collected before delivery without bowel preparation and processed for the 16S rRNA assay of intestinal flora (see Supplementary Material 1). Fresh mid-posterior internal stool samples were collected immediately upon defecation and stored at −80 °C within 30 min of collection. To prevent damage to the bacterial flora, we avoided repeated freezing and thawing during the freezing period. Blood samples were collected from patients on an empty stomach, and serum levels of total bilirubin (TBILI), direct bilirubin (DBILI), indirect bilirubin (IBILI), total bile acids, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were compared.

1.4. Observation indicators

The 16S rRNA analysis of intestinal flora, obtained from patient fecal samples, determined the taxonomic rank at the phylum, order, family, and genus levels, along with their composition characteristics. Patient fasting blood samples were collected to compare levels of serum total bilirubin (Tbil), direct bilirubin (Dbil), indirect bilirubin (IBil), total bile acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other biochemical indicators.

1.5. Analysis of 16S rRNA

Detailed 16S rRNA analysis is presented in Supplementary Material 1.

1.6. Statistical analysis

All statistical analyses were conducted using SPSS (20.0, SPSS Inc., Chicago, Illinois, USA). Prior to analysis, the Shapiro-Wilk test assessed data normality. With the exception of birth weeks, all other features exhibited a normal distribution. Group differences were determined through Student's t-tests, with statistical significance set at $p < 0.05$. Genera's relative abundance differences were assessed using t-tests. The relationship between genera exhibiting a prevalence rate of ≥10% and clinical parameters was ascertained through the execution of a GLM analysis. For this purpose, the R package "lmmADMB" and a negative binomial distribution predicated on the target variable were employed to standardize the OTU data prior to the GLM analysis. Consequently, this analytical module standardized the OTU sequence numbers across samples in the OTU table, ensuring that the total of OTU sequence numbers for each sample was uniform. This standardization involved merely scaling each sample up or down proportionally based on its relative abundance, thereby preserving the original shape of the data distribution. The R software package "vegan" or the redundancy analysis method (RDA) was employed to examine the association between intestinal flora and relevant clinical parameters.

Table 1
Maternal age and gestational week in both groups($x \pm s$).

Group	Number of cases (cases)	Age (years)	Gestational week (weeks)
Group I	17	30.8 ± 4.7	38.9 ± 1.0
Group N	8	28.1 ± 6.4	39.8 ± 1.0
P	/	0.469	0.647

2. Results

2.1. Comparison of general maternal conditions between the two groups

The age and gestational weeks of the two groups of pregnant women showed no statistically significant differences ($P > 0.05$) (Table 1).

We observed statistically significant differences in total bilirubin and ALT levels between the two groups of pregnant women ($P < 0.05$). However, there were no significant differences in AST, TBILI, DBILI, and IBILI ($P > 0.05$) (Table 2).

2.2. Sequencing information

A total of 1,229,256 16S rRNA sequences were obtained through deep sequencing using the Illumina MiSeq platform, with 250 bp double-end sequencing. Following the removal of low-quality sequences, an average of 49,280 sequences per sample were retained.

2.3. Comparison of the gut flora species between ICP patients and healthy individuals

We employed the 16S rRNA amplification technique to analyze the fecal samples of 17 ICP mothers and eight healthy individuals (controls). Depending on the presence of OTUs within the samples, unique or identical OTUs were identified, and a Venn diagram was constructed to analyze the OTUs that were unique to or shared among the different sample groups. This analysis facilitated a comparison of the composition of the two sample groups at the OTU level, including their overlap (Fig. 1). The group with ICP and the normal control group shared 342 OTU species, while each group also possessed unique OTU species (Fig. 1), indicating a divergence in species composition between the two groups of flora.

2.4. Characteristics of the composition of the intestinal flora among ICP patients and healthy individuals at each level

2.4.1. Differences in the composition of the maternal intestinal flora between the two groups at the phylum level

In total, we detected 27 phyla (refer to Supplementary Material 2). Predominant phyla included Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, Tenericutes, Spirochaetes, Synergistota, Fusobacteria, Lentisphaerota, Cyanobacteria, Acidobacteria, Chloroflexota, Fibrobacterota, Thermotogota, Nitrospirota, and more.

The abundance of intestinal flora at the phylum level was notably lower in ICP patients than in healthy controls. In ICP patient samples, we identified 19 phyla, with *Firmicutes* (76%), Actinobacteria (14.6%), *Bacteroidetes* (7%), and *Proteobacteria* (1.9%) being the most prevalent (Supplementary Material 3).

Healthy control samples contained 25 phyla, with *Firmicutes* (63.7%), Actinobacteria (17.6%), *Bacteroidetes* (7.8%), and *Proteobacteria* (5.6%) being the dominant phyla. Other groups displayed varying proportions at the phylum level.

The samples underwent cluster analysis, and a cluster tree of the two sample groups was constructed based on similarities or differences between them. Species of interest at the taxonomic level (the top 20 species in absolute abundance by default) were selected to perform sample clustering (see Supplementary Material 4). Horizontal clustering was conducted to analyze taxonomic information, ascertain differences between samples, and identify clustering patterns among species or samples. A heat map of the clustering tree of samples at the phylum level was generated, demonstrating that the gut microbiota of pregnant women primarily comprised four phyla: Thick-walled Firmicutes, Actinobacteria, Bacteroidetes, and Proteobacteria, with all four phyla present in over 96% of samples.

2.4.2. Differences in the composition of the intestinal flora between the two groups of patients at the class level

We identified 61 classes (See Supplementary Material 5), mainly comprising Clostridia, Actinobacteria, Bacteroidia, Bacilli, Erysipelotrichi, Coriobacteria, Gammaproteobacteria, Verrucomicrobacteria, Verrucomicrobiae, Betaproteobacteria, Cloacamonae, Spirochaetes, Spirochaetes, Cloacaemonae, Mollicutes, Deltaproteobacteria, Alphaproteobacteria, and Fusobacteria.

The abundance of intestinal flora at the class level was lower in ICP patients compared to normal controls. We detected 34 classes in the samples of ICP patients. Six classes exhibited higher abundance in the intestinal flora, with the class *Clostridia* accounting for the largest proportion (65%) of the intestinal flora, followed by *Actinobacteria* (10.8%), *Bacteroidia* (7.17%), *Bacilli* (6.14%), *Erysipelotrichi* (4.97%), and *Coriobacteriia* (3.8%) (see Supplementary Material 6).

On the other hand, we identified 53 classes in the samples of the normal control group. Eight classes represented a higher proportion of the intestinal flora, with *Clostridia* (57.45%) being the most prominent, followed by *Actinobacteria* (12.46%), *Bacteroidia*

Table 2
Comparison of maternal laboratory findings between the two groups($x \pm s$).

Group	total bile acids(umol/L)	ALT(U/L)	AST(U/L)	TBILI(umol/L)	DBILI(umol/L)	IBILI(umol/L)
Group I	19.6(12.7,20.0)	14.3(8.0,14.0)	20.3(14.0,23.0)	6.3 ± 2.1	2.2 ± 0.6	4.1 ± 1.7
Group N	2.9 ± 1.2	7.8 ± 1.8	15.2 ± 2.4	6.7(4.5,7.7)	2.1(1.7,2.5)	4.6(2.9,5.1)
t	4.797	2.469	1.835	0.261	0.679	0.522
p	< 0.001	0.014	0.067	0.794	0.497	0.602

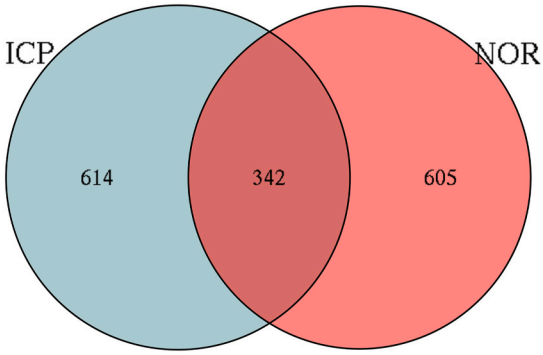

Fig. 1. Wayne diagram of common or endemic species. Note: Wayne plots show the number of OTUs that are common or unique across samples, and circles represent subgroups.

(7.79%), *Bacilli* (2.94%), *Erysipelotrichi* (3.17%), *Coriobacteriia* (5.13%), *Gamma Aspergillus* (5%), *Gammaproteobacteria* (5%), and *Verrucomicrobiae* (3.08%).

The heat map of the clustering tree of samples at the class level revealed that the gut microbiota of pregnant women consisted of seven major classes, including *Clostridia, Actinobacteria, Bacteroidia, Bacilli, Erysipelotrichi, Coriobacteriia, and Gamma Aspergillus* (see Supplementary Material 7).

2.4.3. Differences in the composition of the intestinal flora between the two groups of patients at the order level

Members belonging to Clostridiales, Bifidobacteriales, Bacteroidales, Lactobacillales, Erysipelotrichales, Coriobacteriales, Enterobacteriales, Verrucomicrobiales, Turicibacterales, Burkholderiales, Cloacamonales, Sphaerochaetales, Acholeplasmatales, Actinomycetales, Desulfovibrionales, Pseudomonadales, Bacillales, etc., were identified by us (Supplementary Material 8).

We identified 67 orders in the samples collected from ICP patients. Among them, seven orders constituted a high proportion of the intestinal flora, with Clostridiales accounting for the largest share (65%), followed by *Bifidobacteriales* (10.69%), *Bacteroidales* (7.17%), *Lactobacillales* (5.46%), *Erysipelotrichales* (4.97%), *Coriobacteriales* (3.8%), and *Enterobacteriales* (1.36%) (see Supplementary Material 9).

On the other hand, we detected 87 orders in the samples collected from the normal control group. Eight orders represented a higher proportion of the intestinal flora, with Clostridiales (56.5%) being the most prevalent, followed by Bifidobacteriales (12.31%), *Bacteroidales* (7.79%), Coriobacteriales (5.13%), Enterobacteriales (4.79%), *Erysipelotrichales* (3.17%), *Verrucomicrobiales* (3.1%), *Lactobacillales* (2.62%), and other orders at the order level. The abundance of intestinal flora was higher in normal control samples.

2.4.4. Differences in the composition of the intestinal flora between the two groups at the family level

In this study, 188 bacterial families were identified (refer to Supplementary Material 10), with predominant members, including *Lachnospiraceae, Ruminococcaceae, Bifidobacteriaceae, Erysipelotrichaceae, Coriobacteriaceae, Streptococcaceae, Bacteroidaceae, Enterobacteriaceae, Clostridiaceae, Prevotellaceae, Verrucomicrobiaceae, Veillonellaceae, Porphyromonadaceae, Turicibacteraceae, Enterococcaceae, Paraprevotellaceae, Mogibacteriaceae*, among others.

In the samples from ICP patients, 12 families of intestinal flora were found to constitute a significant proportion of the sample. Notably, Lachnospiraceae represented the largest fraction (41.35%), followed by subsequent proportions of Lachnospiraceae (15.62%), Lachnospiraceae (10.69%), Lachnospiraceae (4.97%), Lachnospiraceae (4.47%), and Lachnospiraceae (3.8%) (Supplementary Material 11).

Conversely, in the normal control group, *Lachnospiraceae* (34.1%), *Ruminococcaceae* (14.53%), *Ruminococcaceae* (12.31%), *Erysipelotrichaceae* (4.87%), and other flora were observed to have a higher abundance at the family level.

2.4.5. Differences in the composition of the intestinal flora between the two groups of patients at the genus level

A total of 333 genera were identified (Supplementary Material 12), predominantly comprising Blautia, Bifidobacterium, Coprococcus, Faecalibacterium, Gemmiger, Streptococcus, Bacteroides, Roseburia, Ruminococcus, Collinsella, Prevotella, Clostridium, Akkermansia, among others.

The composition of intestinal flora in ICP patients was found to be similar to that of normal controls at the genus level, albeit with varying proportions across different genera. Fifteen genera were significantly represented in the intestinal flora of ICP patients, with *Blautia* being the most abundant (20.4%), followed by *Bifidobacterium* (10.69%), *Coprococcus* (6.88%), and *Faecalibacterium* (6.3%). In the normal control group, 19 genera were predominantly represented, with Blautia accounting for the highest proportion (13.56%), and other bacterial genera demonstrating similar abundance levels (Supplementary Material 13).

The heat map and clustering tree of the samples at the genus level revealed that the gut microbiota composition of pregnant women was primarily comprised of 16 genera: *Blautia, Bifidobacterium, Coprococcus, Faecalibacterium,* etc. (Supplementary Material 14).

2.5. Comparison of gut flora abundance between ICP patients and controls

The comparison of the absolute abundance of OTUs in two subgroups was conducted, and representative sequences of OTUs of interest were selected for phylogenetic analysis utilizing the ggtree package of the R software. One OTU exhibiting the highest abundance was deemed representative, followed by the selection of the top 50 OTUs by abundance. The abundance of intestinal flora was found to be significantly lower in ICP patients than in healthy pregnant women in the control group. It was observed that the abundance of all six major phyla was elevated in healthy pregnant women (Supplementary Material 15).

2.6. Correlation between intestinal flora and liver function parameters

Adjustments were made to the conditions to elucidate the correlation between OTUs and the clinical parameters of ICP patients and controls. The association between specific microbiota and liver function parameters was established. The findings indicated that:

(1) *Bacteroidetes* and *Proteobacteria* exhibited a positive correlation with TBIL and IBIL (Fig. 2);
(2) *Betaproteobacteria*, *Gammaproteobacteria* and *Erysipelotrichi* were positively correlated with TBIL, IBIL, and DBIL. (Fig. 3);
(3) *Lactobacillus*, *Delftia*, and *Odoribacter* showed a positive correlation with ALT. (Fig. 4).

3. Discussion

Intrahepatic cholestasis of pregnancy (ICP) is recognized as the most prevalent liver disease specific to pregnancy and resolves soon after delivery [32]. However, the etiology of ICP remains poorly understood. It is thought to be multifactorial, involving genetic, environmental, and hormonal factors influencing the development and severity of the disease. The mechanisms through which ICP elevates the risk of preterm delivery, fecal contamination of the amniotic fluid, and fetal demise remain unclear [33]. ICP is a transient condition with no long-term morbidity associated. The recurrence rate of ICP can be very high (90%), with recurrences tending to be more severe and occurring earlier in the gestational weeks of subsequent pregnancies.

Cholestasis is characterized as a stagnation of bile flow, resulting either from impaired secretion or obstruction downstream, leading to the retention of bile components. In cholestasis, bile acids accumulate in hepatocytes, leading to abnormal levels of bile acids in the peripheral blood. The condition is influenced by hormones, and it is more common in patients taking progesterone [34]. The accumulation of progesterone metabolites can overload certain hepatic transport systems, leading to a decrease in hepatic bile acid output. Thus, the hyperestrogenic and hyperprogesterone state during pregnancy is considered to play a role in the development of intrahepatic cholestasis (ICP), which predominantly occurs in late pregnancy when serum estrogen and progesterone concentrations are at their highest. Familial aggregation, ethnicity, and high recurrence rates suggest that genetic factors have a significant influence on the development of ICP.

The relationship between bile acids and bacteria is intricate. The composition of intestinal bile acids is shaped by bacterial metabolism, which, in turn, can affect the size and composition of the intestinal flora, playing a crucial role in maintaining intestinal homeostasis. Gram-positive bacterial strains, such as *Lactobacillus, Enterococcus, Bifidobacterium,* and *Clostridium*, present in the intestine, are capable of producing bacterial enzymes that catalyze the hydrolysis of amide bonds between glycine or taurine conjugated to the steroid nucleus, a process known as deconjugation. This process makes bile salt substrates available for further modification by the intestinal bacterial flora, an essential component of bile biotransformation [35,36]. The 7α/β-dehydroxylation process transforms primary bile salts into secondary bile salts. These biotransformations take place in the human colon, where deoxycholate and cholestate are the primary bile salts found in human feces, and only a select few *Clostridium difficile*-like bacteria possess 7α/β-dehydroxylation activity. Numerous enteric bacteria, including *Clostridium, Streptococcus, Bacillus, Eubacterium,* and *Escherichia coli*, can oxidize and isomerize hydroxyl groups at the C3, C7, and C12 positions of bile salts to produce isothiolates. These altered bile acids (differential isomers and isomers) are typically returned to the liver for repair before being reabsorbed [36].

In the absence of microbial conversion, the diversity of the bile salt pool diminishes. The intestinal flora plays a crucial role in actively regulating bile salt synthesis: bacterial metabolism decreases the levels of taurine-coupled rhamnolipids, an antagonist of the

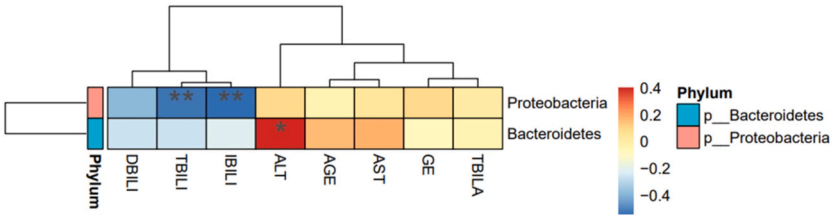

Fig. 2. Heat Map of the Relationship between Gut Flora and Clinical Parameters at the Phylum Level. Note: Clinical parameters on the x-axis and species on the y-axis. R-values (rank correlation) and P-values were obtained by calculation. Different colors indicate different R values. If the P-value is less than 0.05, it is marked with *. The legend on the right shows the color range of different R values, while the color bar on the left indicates the phylum classification of the species. * 0.01 $P < 0.05$, ** 0.001 $P < 0.01$, ***$P < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

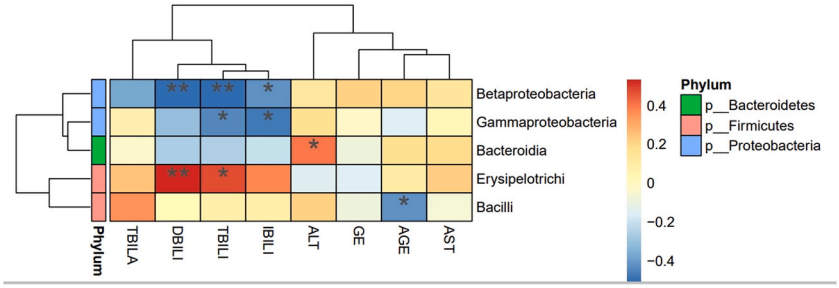

Fig. 3. Heat map of the relationship between gut flora and clinical parameters at the class level.

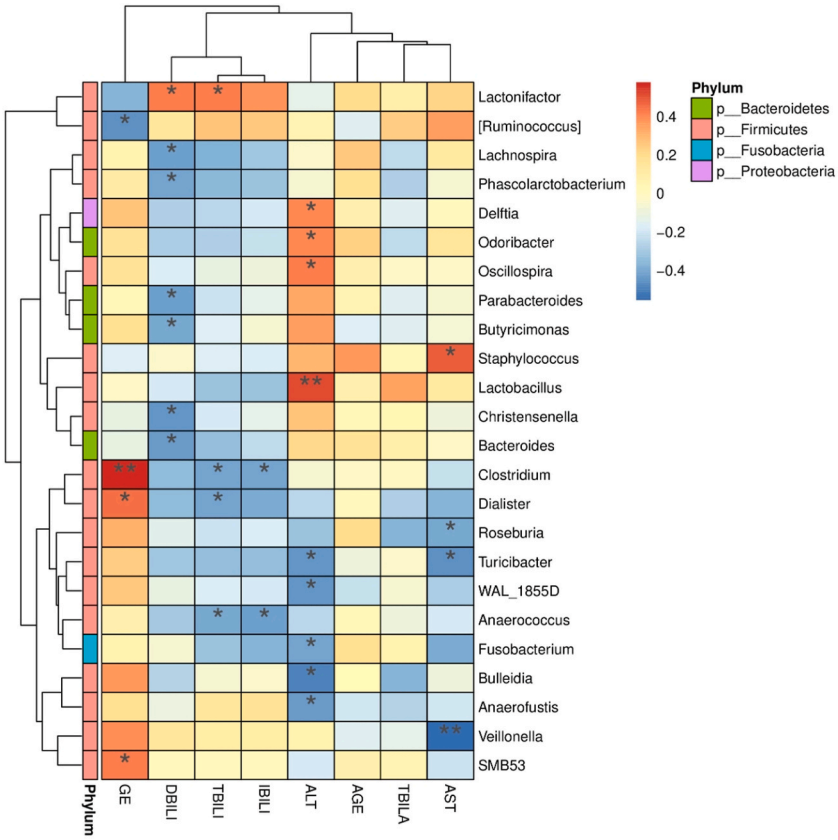

Fig. 4. Heat map of the relationship between gut flora and clinical parameters at the genus level.

farnesoid X receptor (FXR), which in turn inhibits FXR signaling in the intestine. This signaling pathway reduces the expression of cholesterol 7α-hydrolase, the rate-limiting enzyme necessary for bile acid synthesis, thereby diminishing primary bile acid synthesis. Consequently, the intestinal flora regulates secondary bile acid metabolism and may influence primary bile acid production by mitigating FXR inhibition [37]. The volume of bile released into the gut can impact intestinal colonization: low levels of bile salts promote the proliferation of Gram-negative bacteria, including pathogens, while high levels favor the proliferation of Gram-positive bacteria [38].

The gut microbiota of ICP patients undergoes changes due to a reduction in intestinal bile caused by bile stasis. In ICP patients, there is an increase in the abundance of *Lactobacillus* and *Flavonifractor*. Toscano et al. observed a decrease in the abundance of *Flavontonifractor* in healthy volunteers following the oral administration of the probiotic *Lactobacillus kefiri* LKF01 (DSM32079) for one month [39]. Tedesco et al. suggested a mechanism through which lactobacilli may ameliorate cholestatic liver disease [40]; hence, enhancing the abundance of lactobacilli could represent a self-regulation strategy. However, this may also aggravate liver diseases such as alcohol-dependent and non-alcohol-dependent liver diseases, cirrhosis, and primary sclerosing cholangitis [41]. Experimental results indicated that the abundance of gut microbiota at different classification levels was lower in ICP patients compared to the control group. The percentages of *Firmicutes*, *Clostridia*, *Clostridiales*, *Lachnospiraceae*, and *Blautia* in the microbiota were higher in ICP

patients than in healthy mothers, while the percentages of *Actinobacteria*, *Actinobacteriaceae*, *Bifidobacteriales, Bifidobacteriaceae*, and *Bifidobacterium* increased, influencing bile acid metabolism and altering the composition of the intestinal bile pool. The microbiota in the gut of the participants predominantly consisted of four phyla: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, found in 96% (24/25) of the samples. The relative abundance of gut bacteria at different taxonomic levels was lower in ICP patients than in the control group. By analyzing the OTUs, a correlation was found between specific gut microbiota compartments and certain liver function parameters. *Bacteroidetes* and *Proteobacteria* were positively correlated with TBIL and IBIL. *Betaproteobacteria*, *Gammaproteobacteria*, and *Erysipelotrichi* were positively correlated with TBIL, IBIL, and DBIL. *Lactobacillus*, *Delftia*, and *Odoribacter* showed positive correlations with ALT. Thus, supplementing probiotics during pregnancy may be effective in treating ICP, but it remains unclear whether the increase in lactobacilli in the intestine is a "good" self-regulation or a "bad" disease -driving force. The application of lactobacilli as probiotics during pregnancy requires careful consideration.

Whether changes in gut flora lead to alterations in the composition of intestinal bile, causing bile acids to return to the liver via the enterohepatic circulation and resulting in hepatic cholestasis, or whether the reduction in bile release into the gut due to maternal cholestasis leads to changes in gut flora, thereby resulting in maternal symptoms and perinatal risks, requires further investigation. A more comprehensive understanding of the interactions between bacteria and bile acids may unveil novel strategies for treating gastrointestinal and hepatobiliary disorders associated with altered flora, as well as new approaches to combat bacterial infections.

4. Conclusion

In summary, this study comprehensively evaluated the causal relationship between gut microbiota and ICP. The results demonstrated positive correlations between Bacteroides and Proteobacteria with TBIL and IBIL; Betaproteobacteria, Gammaproteobacteria, and Erysipelotrichi with TBIL, IBIL, and DBIL; and *Lactobacillus, Delftia*, and *Odoribacter* with ALT. A correlation was identified between specific gut microbiota compartments and certain liver function parameters. Total bile acids and ALT were significantly elevated in the ICP group. The maternal gut flora was comprised of four main phyla: *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. The relative abundance of bacteria within these phyla was decreased in the gut flora of ICP patients, and a correlation was observed between specific phyla of gut flora and certain liver function parameters. This study provides robust evidence of a causal relationship between the relative abundance of various groups of gut microbes and ICP. The findings may enhance the understanding of ICP's occurrence and suggest new avenues for evaluating microbial subtypes and treatment methods for ICP. To elucidate the impact of probiotics on ICP and the precise mechanisms of their effects, further research based on the outcomes of meticulously designed randomized controlled trials is necessary.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of The Reproductive Hospital of Guangxi Zhuang Autonomous Region(No.2020
058), written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

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CRediT authorship contribution statement

Li-wen Liu: Writing – original draft, Methodology, Investigation. **Yan Chen:** Methodology, Investigation, Data curation. **Liu-jing Zhu:** Methodology, Investigation, Formal analysis. **Qun-xiang Xu:** Validation, Supervision, Software, Resources. **Shaolin Xu:** Validation, Supervision, Software, Resources. **Yanling Ding:** Supervision, Software, Resources, Project administration. **Biao Yin:** Writing – review & editing, Software, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25861.

References

[1] A.M. Wood, E.G. Livingston, B.L. Hughes, J.A. Kuller, Intrahepatic cholestasis of pregnancy: a review of diagnosis and management, Obstet Gynecol Surv 73 (2018) 103, https://doi.org/10.1097/OGX.0000000000000524.

[2] A.P. Kenyon, C.N. Piercy, J. Girling, C. Williamson, R.M. Tribe, A.H. Shennan, Obstetric cholestasis, outcome with active management: a series of 70 cases, BJOG 109 (2002) 282, https://doi.org/10.1111/j.1471-0528.2002.01368.x.

[3] E. Zecca, D. De Luca, M. Marras, A. Caruso, T. Bernardini, C. Romagnoli, Intrahepatic cholestasis of pregnancy and neonatal respiratory distress syndrome, Pediatrics 117 (2006) 1669, https://doi.org/10.1542/peds.2005-1801.

[4] L. Brouwers, M.P. Koster, G.C. Page-Christiaens, H. Kemperman, J. Boon, I.M. Evers, A. Bogte, M.A. Oudijk, Intrahepatic cholestasis of pregnancy: maternal and fetal outcomes associated with elevated bile acid levels, Am J Obstet Gynecol 212 (2015) 100, https://doi.org/10.1016/j.ajog.2014.07.026.

[5] M. Reyihanguli, H.Y. Liu, W.H. Han, Y.F. Luo, A.Q. Hu, Y. Wang, H.L. Wang, H.Y. Xiong, Y.J. Zheng, [Influence of intrahepatic cholestasis during pregnancy on the incidence of preterm birth], Zhonghua Liu Xing Bing Xue Za Zhi 38 (2017) 1415, https://doi.org/10.3760/cma.j.issn.0254-6450.2017.10.024.

[6] J.A. Marathe, W.H. Lim, M.P. Metz, W. Scheil, G.A. Dekker, W.M. Hague, A retrospective cohort review of intrahepatic cholestasis of pregnancy in a South Australian population, Eur J Obstet Gynecol Reprod Biol 218 (2017) 33, https://doi.org/10.1016/j.ejogrb.2017.09.012.

[7] V. Geenes, L.C. Chappell, P.T. Seed, P.J. Steer, M. Knight, C. Williamson, Association of severe intrahepatic cholestasis of pregnancy with adverse pregnancy outcomes: a prospective population-based case-control study, Hepatology 59 (2014) 1482, https://doi.org/10.1002/hep.26617.

[8] F.A. Crocenzi, A.D. Mottino, J. Cao, L.M. Veggi, E.J. Pozzi, M. Vore, R. Coleman, M.G. Roma, Estradiol-17beta-D-glucuronide induces endocytic internalization of Bsep in rats, Am J Physiol Gastrointest Liver Physiol 285 (2) (2003 Aug) G449–G459.

[9] F.R. Simon, J. Fortune, M. Iwahashi, I. Qadri, E. Sutherland, Multihormonal regulation of hepatic sinusoidal Ntcp gene expression, Am J Physiol Gastrointest Liver Physiol 287 (2004) G782, https://doi.org/10.1152/ajpgi.00379.2003.

[10] Y. Yamamoto, R. Moore, H.A. Hess, G.L. Guo, F.J. Gonzalez, K.S. Korach, R.R. Maronpot, M. Negishi, Estrogen receptor alpha mediates 17alpha-ethynylestradiol causing hepatotoxicity, J Biol Chem 281 (2006) 16625, https://doi.org/10.1074/jbc.M602723200.

[11] F. Lammert, H.U. Marschall, A. Glantz, S. Matern, Intrahepatic cholestasis of pregnancy: molecular pathogenesis, diagnosis and management, J Hepatol 33 (2000) 1012, https://doi.org/10.1016/s0168-8278(00)80139-7.

[12] M. Savander, A. Ropponen, K. Avela, N. Weerasekera, B. Cormand, M.L. Hirvioja, S. Riikonen, O. Ylikorkala, A.E. Lehesjoki, C. Williamson, K. Aittomäki, Genetic evidence of heterogeneity in intrahepatic cholestasis of pregnancy, Gut 52 (2003) 1025, https://doi.org/10.1136/gut.52.7.1025.

[13] E. Jacquemin, D. Cresteil, S. Manouvrier, O. Boute, M. Hadchouel, Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy, Lancet 353 (1999) 210, https://doi.org/10.1016/S0140-6736(05)77221-4.

[14] H. Reyes, M.E. Báez, M.C. González, I. Hernández, J. Palma, J. Ribalta, L. Sandoval, R. Zapata Selenium, Zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile, J Hepatol 32 (2000) 542, https://doi.org/10.1016/s0168-8278(00)80214-7.

[15] G. Clarke, R.M. Stilling, P.J. Kennedy, C. Stanton, J.F. Cryan, T.G. Dinan, Minireview: gut microbiota: the neglected endocrine organ, Mol Endocrinol 28 (2014) 1221, https://doi.org/10.1210/me.2014-1108.

[16] V. Iebba, V. Totino, A. Gagliardi, F. Santangelo, F. Cacciotti, M. Trancassini, C. Mancini, C. Cicerone, E. Corazziari, F. Pantanella, S. Schippa, Eubiosis and dysbiosis: the two sides of the microbiota, New Microbiol 39 (2016) 1.

[17] F. Sommer, J.M. Anderson, R. Bharti, J. Raes, P. Rosenstiel, The resilience of the intestinal microbiota influences health and disease, Nat Rev Microbiol 15 (2017) 630, https://doi.org/10.1038/nrmicro.2017.58.

[18] V. Iebba, M. Nicoletti, S. Schippa, Gut microbiota and the immune system: an intimate partnership in health and disease, Int J Immunopathol Pharmacol 25 (2012) 823, https://doi.org/10.1177/039463201202500401.

[19] H. Chung, D.L. Kasper, Microbiota-stimulated immune mechanisms to maintain gut homeostasis, Curr Opin Immunol 22 (2010) 455, https://doi.org/10.1016/j.coi.2010.06.008.

[20] F. Guarner, J.R. Malagelada, Gut flora in health and disease, Lancet 361 (2003) 512, https://doi.org/10.1016/S0140-6736(03)12489-0.

[21] J.M. Ridlon, J.M. Alves, P.B. Hylemon, J.S. Bajaj, Cirrhosis, bile acids and gut microbiota: unraveling a complex relationship, Gut Microbes 4 (2013) 382, https://doi.org/10.4161/gmic.25723.

[22] L. Liao, K.M. Schneider, E.J.C. Galvez, M. Frissen, H.U. Marschall, H. Su, M. Hatting, A. Wahlström, J. Haybaeck, P. Puchas, Amohs, J. Peng, I. Bergheim, A. Nier, J. Hennings, J. Reißing, H.W. Zimmermann, T. Longerich, T. Strowig, C. Liedtke, F.J. Cubero, C. Trautwein, Intestinal dysbiosis augments liver disease progression via NLRP3 in a murine model of primary sclerosing cholangitis, Gut 68 (2019) 1477, https://doi.org/10.1136/gutjnl-2018-316670.

[23] S.I. Sayin, A. Wahlström, J. Felin, S. Jäntti, H.U. Marschall, K. Bamberg, B. Angelin, T. Hyötyläinen, M. Orešič, F. Bäckhed, Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist, Cell Metab 17 (2013) 225, https://doi.org/10.1016/j.cmet.2013.01.003.

[24] J.M. Ridlon, D.J. Kang, P.B. Hylemon, J.S. Bajaj, Bile acids and the gut microbiome, Curr Opin Gastroenterol 30 (2014) 332, https://doi.org/10.1097/MOG.0000000000000057.

[25] T. Inagaki, A. Moschetta, Y.K. Lee, L. Peng, G. Zhao, M. Downes, R.T. Yu, J.M. Shelton, J.A. Richardson, J.J. Repa, D.J. Mangelsdorf, S.A. Kliewer, Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor, Proc Natl Acad Sci U S A 103 (2006) 3920, https://doi.org/10.1073/pnas.0509592103.

[26] V. Geenes, A. Lövgren-Sandblom, L. Benthin, D. Lawrance, J. Chambers, V. Gurung, J. Thornton, L. Chappell, E. Khan, P. Dixon, H.U. Marschall, C. Williamson, The reversed feto-maternal bile acid gradient in intrahepatic cholestasis of pregnancy is corrected by ursodeoxycholic acid, PLoS One 9 (2014) e83828, https://doi.org/10.1371/journal.pone.0083828.

[27] X. Zheng, F. Huang, A. Zhao, S. Lei, Y. Zhang, G. Xie, T. Chen, C. Qu, C. Rajani, B. Dong, D. Li, W. Jia, Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice, BMC Biol 15 (2017) 120, https://doi.org/10.1186/s12915-017-0462-7.

[28] Y. Li, R. Tang, P.S.C. Leung, M.E. Gershwin, X. Ma, Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases, Autoimmun Rev 16 (2017) 885, https://doi.org/10.1016/j.autrev.2017.07.002.

[29] V. Iebba, F. Guerrieri, V. Di Gregorio, M. Levrero, A. Gagliardi, F. Santangelo, A.P. Sobolev, S. Circi, V. Giannelli, L. Mannina, S. Schippa, M. Merli, Combining amplicon sequencing and metabolomics in cirrhotic patients highlights distinctive microbiota features involved in bacterial translocation, systemic inflammation and hepatic encephalopathy, Sci Rep 8 (2018) 8210, https://doi.org/10.1038/s41598-018-26509-y.

[30] Q. Zhan, X. Qi, R. Weng, F. Xi, Y. Chen, Y. Wang, W. Hu, B. Zhao, Q. Luo, Alterations of the human gut microbiota in intrahepatic cholestasis of pregnancy, Front Cell Infect Microbiol 11 (2021) 635680, https://doi.org/10.3389/fcimb.2021.635680.

[31] R. Li, X. Chen, Z. Liu, Y. Chen, C. Liu, L. Ye, L. Xiao, Z. Yang, J. He, W.J. Wang, H. Qi, Characterization of gut microbiota associated with clinical parameters in intrahepatic cholestasis of pregnancy, BMC Gastroenterol 20 (2020) 395, https://doi.org/10.1186/s12876-020-01510-w.

[32] C. Williamson, V. Geenes, Intrahepatic cholestasis of pregnancy, Obstet Gynecol 124 (2014) 120, https://doi.org/10.1097/AOG.0000000000000346.

[33] M.J. Bicocca, J.D. Sperling, S.P. Chauhan, Intrahepatic cholestasis of pregnancy: review of six national and regional guidelines, Eur J Obstet Gynecol Reprod Biol 2319 (2018) 180, https://doi.org/10.1016/j.ejogrb.2018.10.041.
[34] Y. Chen, A. Vasilenko, X. Song, L. Valanejad, R. Verma, S. You, B. Yan, S. Shiffka, L. Hargreaves, C. Nadolny, R. Deng, Estrogen and estrogen receptor-α-mediated transrepression of bile salt export pump, Mol Endocrinol 29 (2015) 613, https://doi.org/10.1210/me.2015-1014.
[35] S.L. Long, C.G.M. Gahan, S.A. Joyce, Interactions between gut bacteria and bile in health and disease, Mol Aspects Med 56 (2017) 54, https://doi.org/10.1016/j.mam.2017.06.002.
[36] J.M. Ridlon, S.C. Harris, S. Bhowmik, D.J. Kang, P.B. Hylemon, Consequences of bile salt biotransformations by intestinal bacteria, Gut Microbes 7 (2016) 22, https://doi.org/10.1080/19490976.2015.1127483.
[37] S.I. Sayin, A. Wahlström, J. Felin, S. Jäntti, H.U. Marschall, K. Bamberg, B. Angelin, T. Hyötyläinen, M. Orešič, F. Bäckhed, Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist, Cell Metab 17 (2013) 225, https://doi.org/10.1016/j.cmet.2013.01.003.
[38] J.S. Bajaj, P.B. Hylemon, J.M. Ridlon, D.M. Heuman, K. Daita, M.B. White, P. Monteith, N.A. Noble, M. Sikaroodi, P.M. Gillevet, Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy and is linked to cognition and inflammation, Am J Physiol Gastrointest Liver Physiol 303 (2012) G675, https://doi.org/10.1152/ajpgi.00152.2012.
[39] M. Toscano, R. De Grandi, V.L. Miniello, R. Mattina, L. Drago, Ability of Lactobacillus kefiri LKF01 (DSM32079) to colonize the intestinal environment and modify the gut microbiota composition of healthy individuals, Dig Liver Dis 49 (2017) 261, https://doi.org/10.1016/j.dld.2016.11.011.
[40] D. Tedesco, M. Thapa, C.Y. Chin, Y. Ge, M. Gong, J. Li, S. Gumber, P. Speck, E.J. Elrod, E.M. Burd, W.H. Kitchens, J.F. Magliocca, A.B. Adams, D.S. Weiss, M. Mohamadzadeh, A. Grakoui, Alterations in intestinal microbiota lead to production of interleukin 17 by intrahepatic γδ T-cell receptor-positive cells and pathogenesis of cholestatic liver disease, Gastroenterology 154 (2018) 2178, https://doi.org/10.1053/j.gastro.2018.02.019.
[41] V.B. Dubinkina, A.V. Tyakht, V.Y. Odintsova, K.S. Yarygin, B.A. Kovarsky, A.V. Pavlenko, D.S. Ischenko, A.S. Popenko, D.G. Alexeev, A.Y. Taraskina, R. F. Nasyrova, E.M. Krupitsky, N.V. Shalikiani, I.G. Bakulin, P.L. Shcherbakov, L.O. Skorodumova, A.K. Larin, E.S. Kostryukova, R.A. Abdulkhakov, S. R. Abdulkhakov, S.Y. Malanin, R.K. Ismagilova, T.V. Grigoryeva, E.N. Ilina, V.M. Govorun, Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease, Microbiome 5 (2017) 141, https://doi.org/10.1186/s40168-017-0359-2.