Characterization of Gut Microbiota Associated with Clinical Parameters in Intrahepatic Cholestasis of Pregnant Patients

Rong Li
The First Affiliated Hospital of Chongqing Medical University
xuehai chen
The First Affiliated Hospital of Chongqing Medical University https://orcid.org/0000-0001-6319-1930
Zhongzhen Liu
BGI-Shenzhen
Yan Chen
BGI-Shenzhen
Chuan Liu
BGI-Shenzhen
Lingfei Ye
BGI-Shenzhen
Liang Xiao
BGI-Shenzhen
Zhenjun Yang
BGI-Shenzhen
Jian He
BGI-Shenzhen
Wen-Jing Wang (wangwenjing@genomics.cn)
BGI-Shenzhen
Hongbo Qi (qihongbocy@gmail.com)
The First Affiliated Hospital of Chongqing Medical University

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Abstract

Background

Intrahepatic cholestasis of pregnancy (ICP) is a liver disease that specifically occurs during pregnancy. Pregnant women with ICP biochemically reflect elevated liver functions and increases in serum bilirubin levels, while clinically individuals can display symptoms of itching and have elevated risks of preterm delivery and stillbirths. We hypothesized that there linkages between gut microbiota and ICP progression exist and could be scientifically characterized.

Methods

In total, 27 patients with ICP and 31 unafflicted control patients were recruited in this study. We performed 16S rRNA gene amplicon sequencing on gut microbiota from individual fecal samples. Sequencing data was analyzed and the correlations between components of microbiota and patient ICP status were tested. Relative abundances, related metabolic pathways, and significantly different OTUs between ICP and control patients were identified.

Results

Biochemical indices including measures for bile, ALT, AST, Dbil, and Tbil, and these were found to at higher levels in ICP versus control patients. Gut microbiota in pregnant women was dominated by four major phyla and 27 core genera. PCoA analysis results indicated that there was marginal significant clustering in unweighted Unifrac distance matrices. A moderate correlation coefficient was observed between specific OTUs and measured clinical parameters of patients. When comparing relatively rare microbiota taxa, the abundance of *Butyricimonas* was lower, while *Citrobacter*, *Pseudomonas*, *Streptococcus,* and *Weissella* were higher in ICP patients than in control patients. No significant differences in the pathways between ICP and control patients were identified.

Conclusions

Our research indicated that patients with ICP have altered phylogenetic gut microbiota profiles compared to control patients without ICP, and that the composition was associated with measurements of patient clinical parameters. These alterations may be correlated with variations of the levels of patient enteric bile acids, and may play a role in the progression of ICP.

Background

Intrahepatic cholestasis of pregnancy (ICP) is a common liver disease that occurs during pregnancy. Global incidence of ICP has been reported to vary at levels fro, from 0.2 % to 2 % depending upon the sample region and ethnicity (1). Typical symptoms of ICP include itching without a rash that is typically localized to the soles of the feet and palms of the hands. Symptoms also include elevated levels of both liver enzymes and serum bilirubin. Fetal complications are more significant compared to strictly maternally associated complications. In 2014, a large prospective national cohort in the United Kingdom was examined and results indicated that women with severe ICP had significantly elevated risks of preterm delivery, stillbirth, and admissions for

treatment into neonatal units compared to unafflicted controls (2). Other symptoms that can affect the fetus as a result of ICP affliction in pregnant women include meconium-stained amniotic fluids, neonatal depression, and respiratory distress syndrome (3-5).

Although underlying mechanisms of ICP are not fully understood, several factors have been identified as being important. Reproductive hormones that encompass estrogens and progesterones have been implicated in the dynamics of pathogenesis of ICP. Several studies that have examined data for animal based models have unveiled the dynamics behind cholestatic effects of estrogen and its impact on hepatotoxity (6-8). Researchers also found that the levels of progesterone metabolites were higher in ICP afflicted patients versus unafflicted patients, implying an adverse association (9). A genetic link has also been suggested for patients afflicted with ICP, as it was found that patients with a family history of ICP had a higher risk of recurrence of ICP and symptoms than did than sporadic patients without such family histories (92 % versus 40 %) (10). Moreover, several genetic variants that are normally mainly involved in the dynamics of bile acid synthesis and in transport pathways have been implicated in as playing important roles in relation to ICP. Chief among these variants are mutations in the hepatocellular transport protein ABCB4 (MDR3), which was been found for more than 15 % of ICP cases in one study (11). There is also evidence that helps to support and which may prove that environmental factors also play important roles in the dynamics of ICP. A cross-sectional cohort study which occurred for patients in Chile had results that indicated that the prevalence of ICP was associated with seasonal variation, with the lowest recorded incidences of ICP in summer months. This seasonal variation was found to coincide with higher levels of plasma Selenium concentrations in summer compared to other months, supporting the implication that nutrition is an important factor in the dynamics of pathogenesis of ICP (12).

The dynamics of the relationship between gut microbiota and health has been increasingly extensively studied in recent years. As one of the most important factors related to individual health, gut microbiota have been implicated to play important roled in the dynamics of metabolism and in the level of immunity of hosts (13). Some of the species of gut microbiota have been demostrated to be able to provide easily absorbed short-chain fatty acids from fermenting dietary fiber; others can synthesize vitamins as well as metabolize bile acids and sterols as to benefit the hosts (14). Disturbances of the balance of the flora making up gut microbiota are have been found to be related to various diseases including inflammatory disorders, obesity, and colon cancer (15).

Crosstalk between gut microbiota and metabolism of bile acids has recently become increasingly intensively studied. Gut microbiota are involved in several processes that are improtant in the dynamics of metabolism of bile acids by deconjugation of glycine or taurine from bile acid to prevent its reuptake through the small intestine. Deconjugated primary bile acids instead enter the colon, where they can be metabolized into secondary bile acids through the coutrse of a series of enzymatic reactions. These reactions involve CYP7A1, CYP7B1, and CYP27A1 (16), which are regulated by bacteria mainly from the genera of *Clostridium* and *Eubacterium* belonging to within the Firmicutes phylum (17). Gut microbiota can also regulate bile acid synthesis indirectly via their influences upon receptors including FXR and FGF19 (16). At the same time, bile acids or FXR could help to regulate the gut microbiota community. As a detergent, bile acid is likley to be able to destroy the bacterial membrane, while also inducing transcription of anti-microbial factors through FXR, iNOS and IL-18 to induce an immune response (18). The dysregulation of microbiota-bile acid interactions also occurs in pathological states, including diet induced obesity (19), cholestatic liver disease (20), gastrointestinal inflammation, and carcinogenesis (21).

To understand whether or not gut microbiota was associated with ICP, we collected fecal samples from pregnant women diagnosed with ICP and from healthy women as the control samples. We extracted DNA from individuals stool samples and conducted 16S rRNA sequencing. We hypothesized that several potentially pathogenic bacteria, including *Enterobacteriaceae* and *Streptococcus* would have increased levels of prevalence and that contrastingly the butyric acid producing bacteria *Butyricimonas* would be inhibited in patients with ICP. The examinations of potential changes in microbial diversity might help to contribute to a better understanding of the progression of ICP and thus help to lead to better preventive measures and treatment options for patients afflicted with ICP.

Methods

Study Participants

This study was performed at the First Affiliated Hospital of Chongqing Medical University, China, between May, 2015 and February, 2016 with approval of all study aspects granted from the hospital Ethics Committee. Written informed consent were collected from all participating patients.

In total we collected samples from 27 individual patients that were confirmed to be afflicted with ICP. ICP was diagnosed and classified according to the following criteria: severe pruritus without rash; notably elevated concentrations of maternal serum bile acids (> 10 μM); absence of definitive itching-causing diseases; absence of other liver-damaging diseases, such as gallstones, hepatotoxic drug consumption, hepatitis, and inflammatory bowel diseases among others.; and no smoking and or drinking histories. Thirty-one age and BMI matched pregnant women unafflicted by ICP were recruited as controls for the study.

Sampling

A stool sample from each individual was collected after ICP diagnoses were confirmed during pregnancy or were collected during consultations and before delivery of the child (for the control samples). After collection, samples were stored at -80 °C until further processed. Blood samples were also collected after patients fasted and at the same time such as to allow comparative examinations of biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin (Tbil), and direct bilirubin (Dbil).

Stool DNA Extraction and Sequencing

DNA was extracted from stool samples following standard protocols and procedures (22). We targeted and quantified the levels of expression of amplicons of 16S rDNA resultant from the V3 and V4 regions for all qualified DNA samples. Amplicons were sequenced using the Miseq platform and 300-PE-cycles based upon standard protocols described in the literature (22).

Bioinformatics and Statistical Analysis

We performed quality control on sequenced reads with the use of an in-house developed software, which filtered for low-quality data, ambiguous bases, low complexity of reads, and adapter reads as has been previously described (22). PE-reads with acceptable levels of quality were then assembled into tags. Operational taxonomic units (OTUs) were clustered using a ≥97 % similarity threshold for tags with Uparse (version 7.0.1090) using all default settings in the Uparse OTU analysis pipeline program (23). OTUs were taxonomically

annotated using a bootstrap cutoff of 80 %, which is level that has been previously used in similarly oriented studies (24, 25). Alpha diversity was calculated using Mothur (version 1.31.2) (26). Corresponding rarefaction curves and box graphs and or histograms were plotted through use of R statistics software (27). Beta diversity was measured by Bray-Curtis, weighted , and unweighted UniFrac calculations with the function “beta_diversity.py” in the QIIME pipeline (28). A heatmap for beta diversity was produced using the R 'aheatmap' function within the 'NMF' package. Principal coordinate analysis (PCoA) analysis was performed using QIIME and an iterative algorithm, with 75 % of the total sequences randomly extracted after iterations which producedweighted and or unweighted species classifications and information about abundance. The results from PCoA were plotted using GraphPad Prism 5 software (29). Linear Discriminant Analysis Effect Size (LEfSe) (30) and Picrust (31) were performed to determine if there weredifferences in OTUs between comparisons of ICP and control patients and to predict the functional contents of the metagenome.

The relative abundance at 95 % confidence intervals for differences between ICP and control patients at a series of taxonomic levels was calculated by using a non-parametric Mann-Whitney test or by using the Benjamini-Hochberg approach for determination of the false discovery rate (FDR) and the corresponding applied correction. Spearman's rank correlation coefficients were compared between OTUs and six biochemical parameters (ALT, AST, bile, Tbil, Ibil, and Dbil) were quantified and compared using the cor.test function in the R Statistics suite with all default pararmeters. All biochemical parameters were expressed as the median and interquartile range (IQR). Non-parametric Mann-Whitney tests with resultant p-values < 0.05 were considered as statistically significant between comparisons of ICP and control patients. We used the Geom_boxplot and geom_jitter functions in the ggplot2 package in R statistics in order to draw results for the six biochemical parameters. We used the VennDiagram package and method to map OTUs in the form of a Venn diagram. Lastly, we used a Euclidean based distance algorithm and used “complete” clustering methods in R statistics in order to draw a phylogenetic tree at the level of taxonomic organization of Genus.

Results

Study Subject Characteristics

In total 27 ICP patients and 31 control patients were enrolled in our study. Basic clinical information for subjects is summarized in Table 1 and Figure 1, in which the ages were equivalent between ICP and control patients. ICP patients were sampled at ~ 35.4 weeks while the control patients were sampled at ~39.4 weeks. Median values of bile, ALT, AST, Dbil, and Tbil were all significantly higher in ICP versus control patients (Figure 1, Table 1), Ibil median values for ICP patients were ~ two times higher than were the values in control patients (3.5 versus 1.7).

Table 1. Study subject characteristics.

	ICP(n=27)		Control(n=31)		
	Median	IQR	Median	IQR	p-value
Age	28	7	29	6	0.5573
Sampling pregnancy weeks	35.4	3.5	39.4	2	1.97E-09
Bile (μmol/L)	26.6	57.1	8.1	3.3	2.56E-07
Total serum bilirubin (μmol/L)	15.3	12	6.5	2.9	7.18E-08
Indirect bilirubin (μmol/L)	3.5	4.3	1.7	5.2	0.08912
Direct bilirubin (μmol/L)	10.6	11	4.6	3.8	7.84E-08
Aspartate aminotransferase (U/L)	104	164	19	3	1.69E-06
Alanine aminotransferase (U/L)	168	285	17	9	1.28E-06

Characterizing Gut Microbiota in Women with ICP and controls

16S rRNA amplicon-based microbiome analysis was performed on stool samples from 27 ICP and 31 healthy control patients. After sequencing using the Miseq platform and preliminary data processing, clean reads were assembled into tags. Similar numbers of tags (on average 89265 ± 17127 tags for each ICP patient and 90964 ± 13947 tags for each control patient) were clustered into OTUs. There were no significant differences for the identified OTUs in comparisons between the two groups (median = 234 for ICP and 238 for control patients, Figure S1A). Alpha diversity analyses revealed that the number of OTUs in ICP patients was 9.8 % lower than were the resultant number of OTUs for control patients (p = 0.0165). Alpha rarefaction curves of numbers of observed OTUs for both patient groups showed a gradual leveling off by 60,000 sequences, while our sequences did not total greater than 400,000, suggesting there was adequate saturation at the selected rarefaction level (Figure S1B).

Collectively, the composition of gut microbiota of pregnant women was dominated by four major phyla including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, all of which occurred in more than 95 % of these samples. At the level of genus, 27 core genera, including *Faecalibacterium, Streptococcus, Escherichia,* existed in more than 95 % of the samples from pregnant women (Table S1).

The PCoA results based on overall bacterial community composition showed a marginally significant clustering in unweighted UniFrac distance matrices (Figure 2A, ANOSIM, p=0.0434), which only took the microbiota composition into account. In contrast, no significant clustering was observed in weighted UniFrac analysis (Figure 2B, ANOSIM, p=0.1578) in which more abundant OTUs were observed with an overall stronger effect.

Five measures (Chao, ace, sobs, Shannon, Simpson) were used to analyze the abundance and diversity of microbiota within samples. None of the five measures showed any significant differences for comparisons between ICP and control group patients (Figure S2, Table S2).

We next examined the correlation coefficient between OTUs and clinical parameters for both ICP and control patients. When we set up the estimates threshold above 0.4 or below -0.4, and p-value < = 0.001 (which means a moderate relationship), we observed that: 1) *Lachnospiraceae*, *Weissella*, *Citrobacter*, and *Bacillus* were positively associated with ALT; 2) *Blautia_producta*, and *Clostridiales* were positively associated with bile; 3) *Dialister*, *Ruminococcus* were negatively and *Streptococcus_luteciae*, *Clostridiales* were positively associated

with Dbil; 4) *Weissella*, *Bacillus*, *Streptococcus_luteciae* were positively associated with AST; 5) *Weissella*, *Streptococcus_luteciae*, *Clostridiales* were positively and *Oscillospira* was negatively associated with Tbil (Table 2).

Table 2. Correlation coefficient between OTUs from all subjects and clinical parameters.

OTUs	Estimates	p-value	Occ	SampCount	Catalogue	Taxonomy
Otu1090	0.408477	0.001456	56	58	ALT	Lachnospiraceae
Otu319	0.43044	0.000745	18	58	ALT	Weissella
Otu411	0.434778	0.000649	36	58	ALT	Citrobacter
Otu711	0.406692	0.001535	16	58	ALT	Bacillus
Otu319	0.414393	0.001221	18	58	AST	Weissella
Otu711	0.441002	0.00053	16	58	AST	Bacillus
Otu737	0.44422	0.000477	25	58	AST	Streptococcus_luteciae
Otu147	0.419866	0.001034	58	58	bile	Blautia_producta
Otu973	0.426097	0.000853	29	58	bile	Clostridiales
Otu20	-0.41329	0.001262	27	58	Dbil	Dialister
Otu737	0.450102	0.000393	25	58	Dbil	Streptococcus_luteciae
Otu802	-0.41767	0.001106	6	58	Dbil	Ruminococcus
Otu973	0.464376	0.000241	29	58	Dbil	Clostridiales
Otu319	0.419613	0.001042	18	58	Tbil	Weissella
Otu411	0.405553	0.001587	36	58	Tbil	Citrobacter
Otu7	0.479112	0.000142	23	58	Tbil	Prevotella_stercorea
Otu718	-0.40842	0.001459	17	58	Tbil	Oscillospira
Otu737	0.412664	0.001286	25	58	Tbil	Streptococcus_luteciae
Otu973	0.430958	0.000732	29	58	Tbil	Clostridiales

At the genus level, the abundance of *Butyricimonas* was significantly lower in the group of ICP patients compared to patients making up the control group. However, *Blautia*, *Citrobacter*, *Pseudomonas*, *Streptococcus* and *Weissella* were significantly higher in ICP versus control patients. At the species level, *Streptococcus lutecia* was higher in ICP patients. At the class level, *Bacilli* and *Gammaproteobacteria* were higher in ICP patients. At the order level, *Enterobacteriales*, *Lactobacillales,* and *Pseudomonadales* were higher in ICP patients. At the family level, *Enterobacteriaceae*, *Leuconostocaceae*, *Pseudomonadaceae,* and *Streptococcaceae* were higher in ICP patients (Figure 3, Table 3). All these bacteria were considered to be rare (mean relative abundance < 5 %) in both the ICP control patient groups.

Table 3. Differential relative abundances of bacterial in six levels with FDR P-value <0.05.

Items	Mean(ICP)	SD(ICP)	Mean(Nor)	SD(Nor)	p.value	FDR	
Bacilli	0.43725	0.590779	0.141213	0.182898	0.000676	0.015548	Class
Gammaproteobacteria	2.542838	5.428261	0.443897	0.691728	0.001971	0.022667	
Enterobacteriales	2.186482	5.326455	0.386201	0.704164	0.002905	0.032923	Order
Lactobacillales	0.378908	0.577339	0.114305	0.178683	0.000462	0.008636	
Pseudomonadales	0.003022	0.004634	0.000907	0.002216	0.000508	0.008636	
Enterobacteriaceae	2.186482	5.326455	0.386201	0.704164	0.002905	0.04067	Family
Leuconostocaceae	0.009571	0.020579	0.00033	0.001309	0.000117	0.006011	
Pseudomonadaceae	0.002195	0.003701	0.000363	0.000717	0.000322	0.006011	
Streptococcaceae	0.340405	0.566579	0.077515	0.095561	0.000273	0.006011	
Blautia	1.700667	1.161503	0.899852	0.689057	0.001317	0.026867	Genus
Butyricimonas	0.077094	0.112087	0.22485	0.30419	0.010086	0.098031	
Citrobacter	0.041611	0.074845	0.002425	0.005323	0.00014	0.007293	
Pseudomonas	0.002195	0.003701	0.000363	0.000717	0.000322	0.010948	
Streptococcus	0.327829	0.566782	0.072758	0.094323	0.001317	0.026867	
Weissella	0.009296	0.020585	0.000299	0.001305	0.000143	0.007293	
Streptococcus luteciae	0.078866	0.155461	0.00461	0.017362	0.000431	0.029739	Species

In total, 51 metabolic pathways were identified by PICRUSt (Figure 4). There were no significant differences in examinations of the pathways for comparisons between ICP and control patients.

LEfSe identified eight significant ($\log_{10}$(LDA SCORE) > = 2 or $\log_{10}$(LDA SCORE) < = -2) OTUs (i.e., *Streptococcus* (genus), *Lactobacillales* (order), *Streptococcaceae* (family), *Bacilli* (class), *Blautia* (genus), *Enterobacteriaceae* (family), *Enterobacteriales* (order), and *Gammaproteobacteria* (class)) in ICP patients group and identified 12 significant OTUs (i.e., *S24-7* (class), *Betaproteobacteria* (class), *Burkholderiales* (order), *Sutterella* (genus), *Alcaligenaceae* (family), *Bacteroides ovatus* (species), *Odoribacteraceae* (family), *Odoribacter* (genus), *Butyricicoccus* (genus), *Bacteroides eggerthii* (species), *Bacteroides caccae* (species), and *Oscillospira* (genus)) in controls (Figure 5).

Discussion

In this study, we analyzed gut microbiota in the third-trimester of pregnant women, and examined the relationship between gut microbiota and ICP for the first time. We determined correlation coefficients for comparisons between OTUs and clinical parameters, as well as determined differences between ICP control patient groups on different taxonomic levels. Additionally, we performed analyses for the prediction of metagenomic functions to distinguish differential abundances of OTUs. Our findings indicated that the observed increase of pathogenic bacteria (Enterobacteriaceae, Streptococcus) and decrease of butyrate-producing populations of bacteria (Butyricimonas) might have contributed to the progression of ICP and symptoms in patients. What's more, we identified eight significant OTUs in ICP patients and 12 significant OTUs in control patients. We combined the patients from the two groups and then examined the common bacteria collectively. We found that Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the dominant phyla, while 27 core genera including Faecalibacterium, Streptococcus, Escherichia were the dominant genera in both groups. The compositions of these taxa were found to be in accordance with a previous study focusing on microbiota

changes during pregnancy, which had results that similarly indicated that relative abundances of *Proteobacteria* and *Actinobacteria* increased as pregnancy progressed. We also found that OTUs including members of the *Enterobacteriaceae* family and *Streptococcus* genus were dominant in the third trimester (32). Another survey of fecal microbiota of a cohort of 314 young Chinese individuals was able to identify a list of 16 abundant genera, 11 of which were also included in our core genera list, further validating our data (33).

While few studies have been performed with a high focus upon the flora making up the gut microbiota in patients with ICP, some teams have studied the metagenomes in bile acid related abnormity or liver diseases. A study which examined gut microbiota for patients with cirrhosis had levels of observed bile acid entering the intestine that were low, and *Enterobacteriaceae* (the only one family belonging to Enterobacteriales) was found to have increased (34). This result was in accordance with our observations. Another study which examined primary sclerosing cholangitis (PSC) found that when the bile was inhibited from releasing to small intestine the proportions of *Blautia* increased (35). We obtained similar results in our experiments. Intestinal bile acid is one of the major regulators of gut microbiota and inhibition of the entrance of bile acid to intestines causes bacterial dysbiosis, as gram-positive members such as *Rumminococcaceae* and members of *Clostridium cluster* XVIa, which are involved insecondary fecal bile acid production and anti-inflammatory response, were inhibited (17) Contrastingly, pro-inflammatory and potentially pathogenic taxa, including *Enterobacteriaceae*, increased (34). In our study, *Butyricimonas* was inhibited in the patients in the ICP group compared to patients in the control group. As *Butyricimonas* is able to produce butyrate, one of the members of the short-chain fatty acids (SCFA), inhibition of *Butyricimonas* decreases butyrate production, further disturbs the anti-inflammatory effects, and impacts the level of intestinal barrier integrity.

Bile acids affect gut microbiota composition directly through antimicrobial effects or indirectly through impacts upon FXR-dependent antimicrobial peptides. As one of the components of the pool of bile acids, deoxycholic acid (DCA), has a strong effect upon inhibiting the growth of the microbiome and acts as a detergent upon bacterial membranes (36). In ICP patients, Ruminococcus and Blautia both had a strong correlation with serum Tbil. Enterobacteriales and Streptococcaceae composition was found to have increased, which was also commonly seen similarly based examinations of the impacts of cirrhosis upon gut microbiota. It is likely that overgrowth of potentially pathogenic bacteria including Enterobacteriaceae and Streptococcus is normally suppressed due to mutual inhibitions with healthy human bacteria, such as *Lachnospiraceae* and *Ruminococcaceae*. When the latteris is inhibited, the overgrowth of Enterobacteriaceae and Streptococcus occurs. This observation is in accordance with previous results from bacteriological cultures of fecal samples, which indicated that Escherichia coli and staphylococcus spp. had overgrowth in patients that were afflicted with cirrhosis (37).

Studies in which rats were examined suggested that after IT surgery the levels of circulating bile acid increased significantly. As a result, the homeostasis of glucose was found to have been improved. Proportional increases of *Gammaproteobacteria* in IT-operated animals were a potential contributor of the observed increase in the levels of circulating bile acids (38). Another study which examined a colitis mouse model found that *Citrobacterrodentium* infection downregulated the genes involved in bile acid biosynthesis and transportation, and increased the risk of cholestasis (39). In our study, we also found a significantly increased levesl of *Citrobacter* in the ICP patient group. Three case-reports have also indicated that severe Pseudomonas infections could lead to the development of irreversible hepatocellular cholestasis without liver cell necrosis or bile duct injury, although the mechanisms and dynamics of pathogenesis were undescribed (40, 41). Notably, the serum

level of Tbil was found to have increased dramatically in ICP patients, inferring a that there is a relationship between Tbil and Pseudomonas.

In our experimental results, women with ICP were sampled at a median time of pregnancy of 35.4 weeks (i.e. before full term) and control women were sampled at a median of 39.4 weeks (i.e. at term). A previous study which examined temporal variation in the composition ofhuman microbiota during pregnancy evaluated the communities sampled in consecutive weeks through delivery and found that there were no significant trends over gestational time ($P > 0.05$, t test) (42). Thus, in our study we felt it was appropriate and reasonable to have collected samples at different time points in the third trimester.

In conclusion, our study presented the first view of research which examined the gut microbiota of ICP afflicted patients as well as for a concomitant unafflicted control group of patients. Although the mechanisms and dynamics with regards to how phylogenetic diversity changes gut microbiota in patients afflicted with ICP remains obscure, our findings might provide new diagnostic and treatment strategies during pregnancy for this disease and the associated symptoms. Further studies are needed however that seek to identify factors impacting gut bacterial composition in ICP patients to prevent the occurrence and progression of these complications in the third trimester of pregnancy.

Conclusions

In this manuscript, we analyzed the fecal microbiota from 27 ICP patients and 31 comparable controls by 16S rRNA gene amplicon sequencing and tested the correlation between components of microbiota and ICP. Our results indicated for the first time that patients with ICP have an altered phylogenetic gut microbiota profile compared with control group. Our findings might provide the new diagnosis and cure strategies to this pregnant related disease.

Abbreviations

ICP: Intrahepatic Cholestasis of Pregnancy;

ALT: Alanine Aminotransferase;

AST: Aspartate Aminotransferase;

Tbil: Total Serum Bilirubin;

Dbil: Direct Bilirubin;

OTUs: Operational Taxonomic Units;

PCoA: Principal Coordinate Analysis;

LEfSe: Linear Discriminant Analysis Effect Size;

FDR: False Discovery Rate;

IQR: Interquartile Range;

SCFA: Short-Chain Fatty Acids;

DCA: Deoxycholic Acid.

Declarations

Ethics approval and consent to participate

Approval was also granted by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (No.201530). Written informed consent were collected from all participating patients.

Consent for publication

Not applicable.

Availability of data and materials

The data reported in this study have been deposited in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession code CNP0000403.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WJ and HB designed research; RL, XH, ZZ, collected samples and clinical data from patients, and conducted experiments; YC, CL, LF, LX, ZJ, JH analyzed the data; Sledzinski T analysed data; WJ, HB wrote the paper.

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Figures

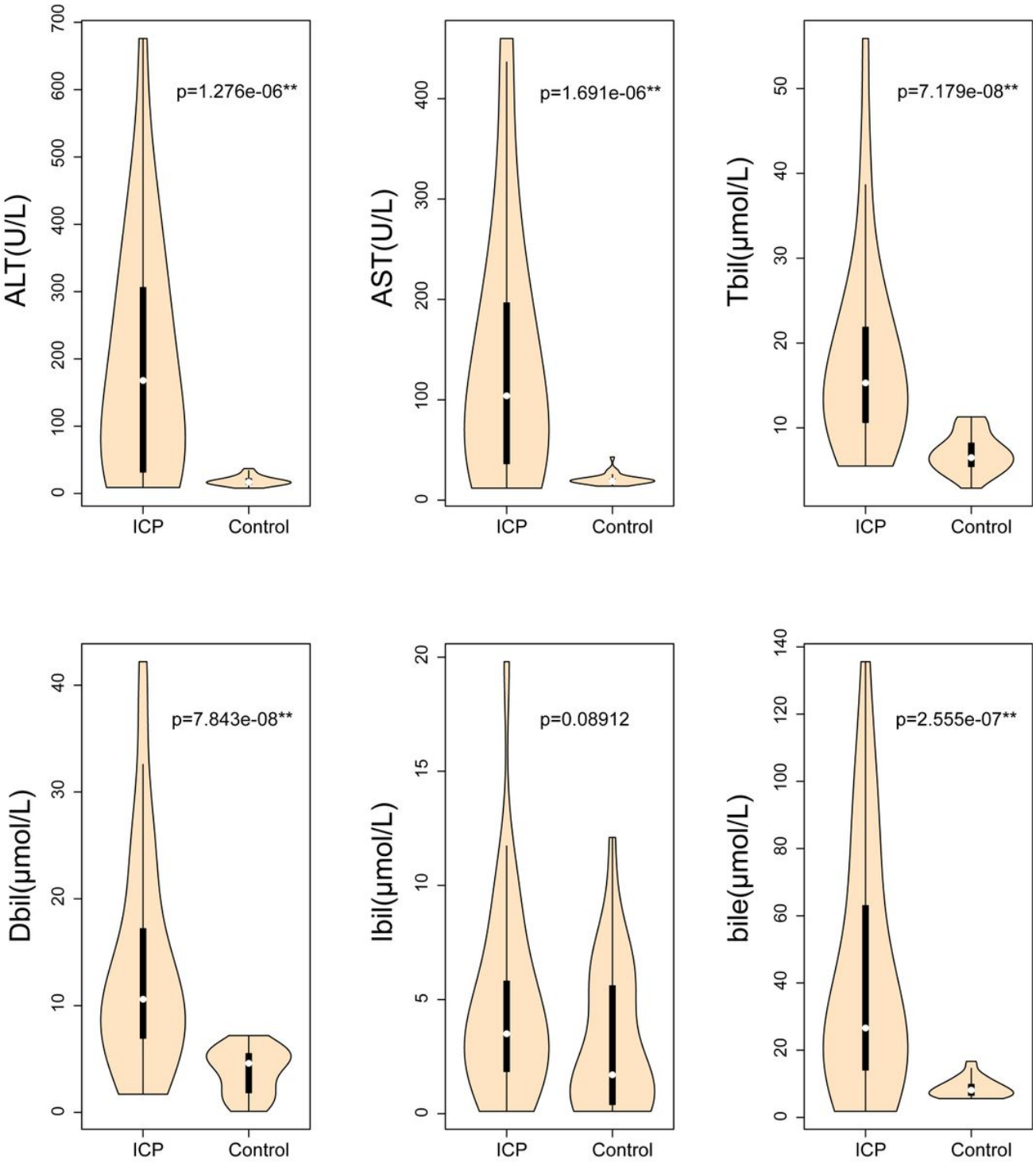

Figure 1

The distributions of ALT, AST, bile, Dbil, Ibil, and Tbil values in ICP and healthy control patients. AST: aspartate aminotransferase; ALT: alanine aminotransferase; Dbil: direct bilirubin; Ibil: indirect bilirubin; and Tbil: total serum bilirubin.

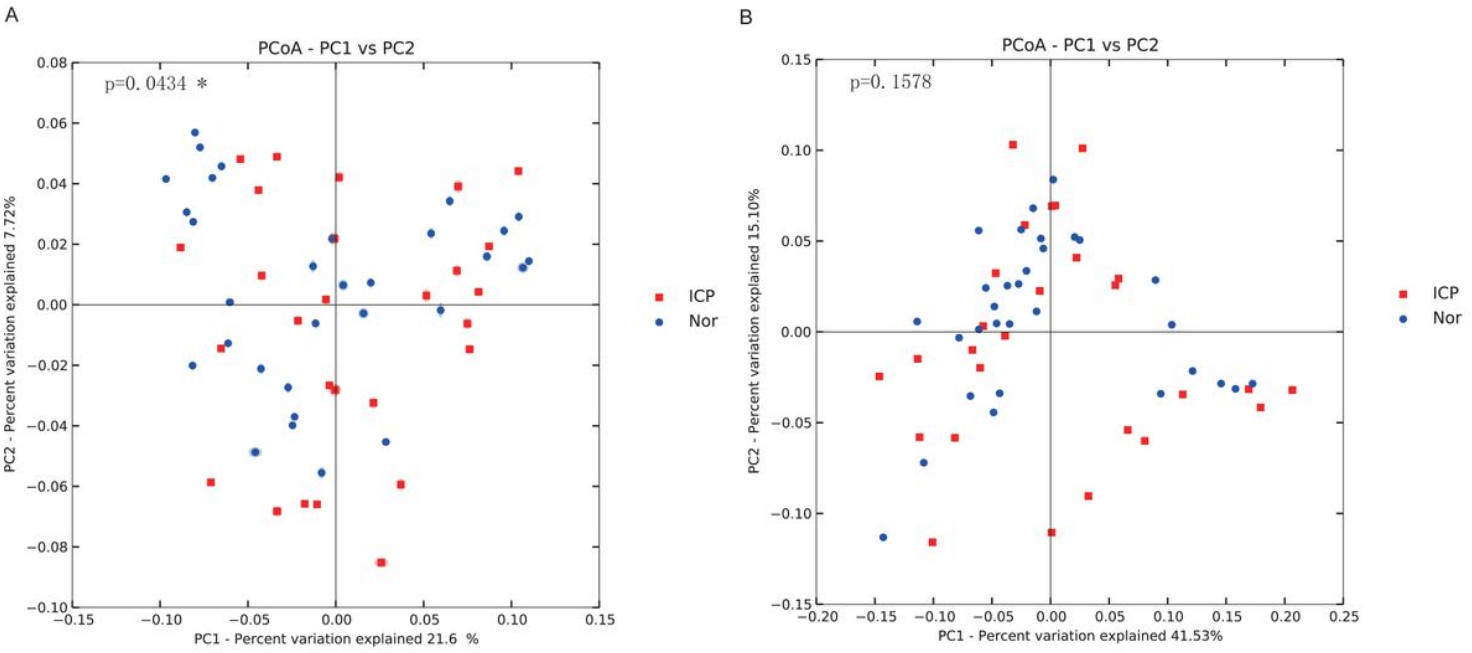

Figure 2

Principal coordinate analysis (PCoA) plots of stool samples from ICP and healthy control patients. PCoA plots of unweighted (A) and weighted (B) UniFrac distance matrices. Each dot represents the bacterial community composition of one individual stool sample. Axis titles indicate the percentage of variation explained.

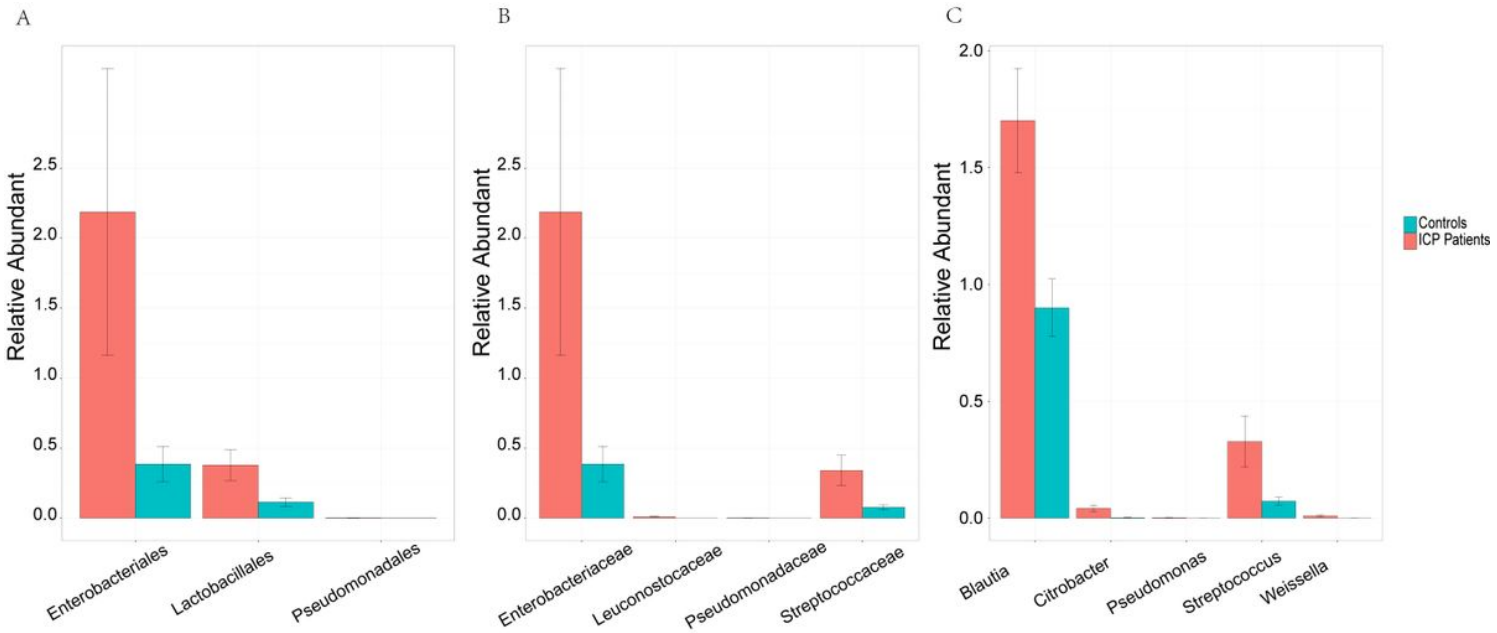

Figure 3

Differential relative abundance between ICP and healthy control patients. Assessed and organized according to taxonomic levels of Order (A), Family (B) or Genus (C).

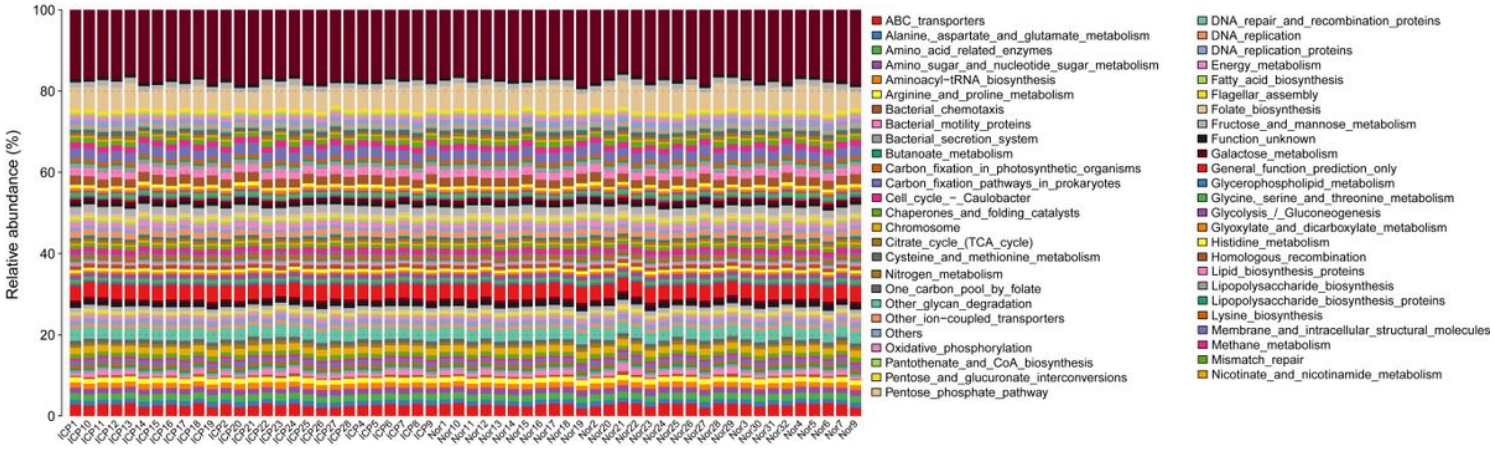

Figure 4

The relative abundance of stool samples on 51 metabolic pathways.

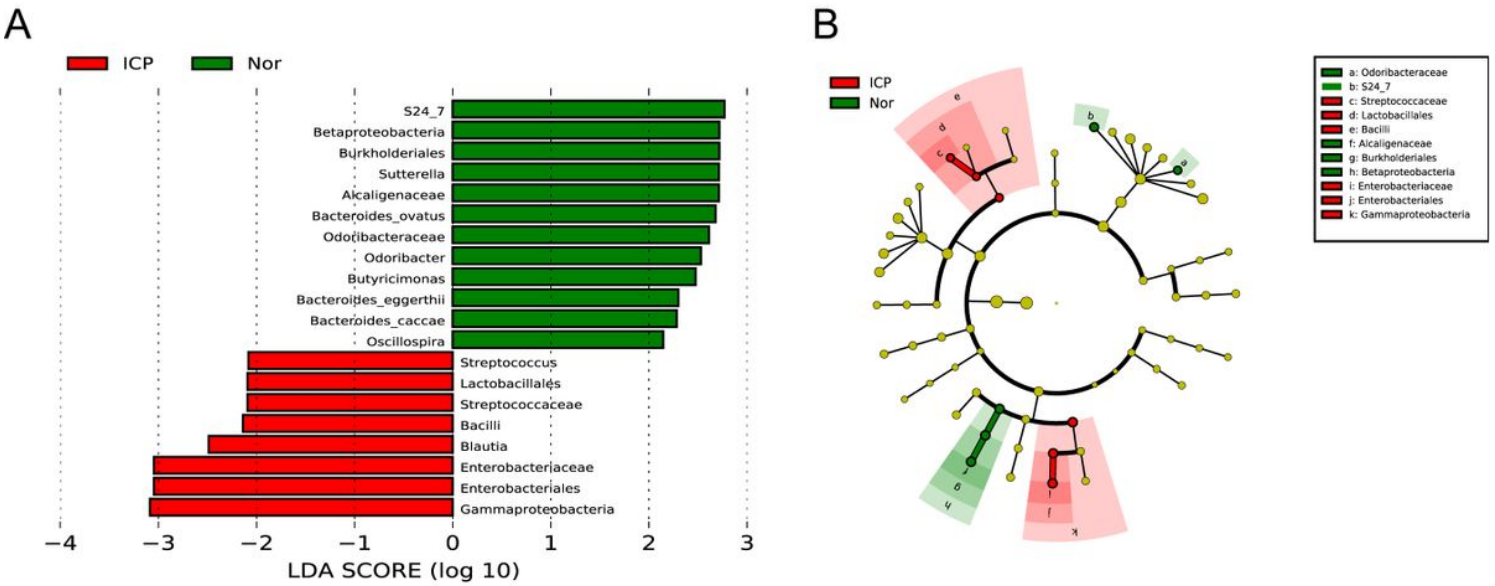

Figure 5

Analysis of LDA effect size of community differences between ICP and healthy control patients.

Supplementary Files

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