FEMS Microbiology Letters, 368, 2021, fnab038

doi: 10.1093/femsle/fnab038
Advance Access Publication Date: 9 April 2021
Research Letter

RESEARCH LETTER – Environmental Microbiology

Short-chain fatty acid and fecal microbiota profiles are linked to fibrosis in primary biliary cholangitis

Craig Lammert[1], Andrea S. Shin[1,*,†], Huiping Xu[2], Christopher Hemmerich[3], Thomas M. O'Connell[4] and Naga Chalasani[1]

[1]Division of Gastroenterology and Hepatology, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, USA, [2]Department of Biostatistics, Indiana University School of Medicine, Indianapolis, IN 46202, USA, [3]Center for Genomics and Bioinformatics, Indiana University Bloomington, Bloomington, IN 47405, USA and [4]Department of Otolaryngology—Head and Neck Surgery, Indiana University School of Medicine, Indianapolis, IN 46202, USA

*Corresponding author: Indiana University School of Medicine, 702 Rotary Circle, Suite 225, Indianapolis, IN 46202, USA. Tel: +1-317-274-3505; E-mail: ashin@iu.edu

One sentence summary: Fecal microbiota and short-chain fatty acids correlate with severity of fibrosis in primary biliary cholangitis; fecal short-chain fatty acids may represent a novel microbial biomarker in this disease.

Editor: Akihito Endo

†Andrea S. Shin, http://orcid.org/0000-0001-7284-3398

ABSTRACT

The gut microbiota and metabolome could play a role in primary biliary cholangitis (PBC) progression. We aimed to assess fecal microbiota and fecal short-chain fatty acids (SCFAs) in PBC according to fibrosis. In a cross-sectional study of 23 PBC patients, fecal microbiota and SCFAs were determined using 16S rRNA sequencing and nuclear magnetic resonance spectroscopy, respectively. Fecal acetate and SCFAs were higher in advanced fibrosis. Advanced fibrosis microbiota exhibited decreased alpha diversity, increased *Weisella* and a distinct community composition. SCFAs correlated with individual taxa in non-advanced fibrosis. Fecal microbiota and SCFAs correspond to fibrosis in PBC.

Keywords: microbiome; metabolome; acetate; propionate; butyrate; primary biliary cirrhosis

INTRODUCTION

Primary biliary cholangitis (PBC), a chronic cholestatic autoimmune disease that results in the inflammatory destruction of bile ducts, can lead to progressive liver fibrosis. Although it is thought that PBC results from environmental stressors in genetically susceptible individuals (Selmi *et al.* 2011), knowledge on the specific mechanisms that drive PBC disease onset and progression remains incomplete. Therefore, examination of novel disease mechanisms is critical to expand our understanding of disease pathogenesis and identify future treatment strategies. Recent modifications to the etiological understanding of PBC have incorporated aspects of gut–liver communications involving the intestinal microbiota (Tripathi *et al.* 2018).

Gut microbiota play a pivotal role in regulating immune homeostasis, a key aspect of PBC pathogenesis, and appear altered in several chronic liver disorders (Jiang and Schnabl 2020). There are recent data suggesting that the microbiota and microbial metabolites may serve as modifiable PBC treatment targets (Tang *et al.* 2018); however, only a handful of published studies have examined the composition of the gut microbiota in PBC. Several groups described reduced microbial diversity (Furukawa *et al.* 2020) or richness (Tang *et al.* 2018) in PBC compared with healthy controls, while others have reported alterations in specific bacterial taxa compared with healthy controls (Lv *et al.* 2016) or changes in bacterial profiles characterized by increased taurine-metabolizing bacteria (Chen *et al.* 2020) after

Received: 25 September 2020; **Accepted:** 7 April 2021

treatment with ursodeoxycholic acid (UDCA). Among most noteworthy are findings reported by Tang *et al.* (2018) who observed that microbial dysbiosis in PBC could be partially reversed by UDCA. Yet, most studies have compared PBC patients with healthy controls; whether or not changes in gut microbiota composition correlate with disease severity or disease progression in established PBC patients is unknown. Efforts to define the role of the microbiome in PBC pathophysiology may be aided by complementary assessment of microbial products that further interact with the intestinal epithelium and immune responses.

Metabolomics has proved a promising tool in advancing our knowledge of pathophysiology in numerous diseases. It is a tool that has recently been applied for biomarker discovery or for the study of pathological or treatment-related changes in metabolic functions in PBC (Lv *et al.* 2016; Hao *et al.* 2017). Metabolomic profiling studies (Amathieu *et al.* 2016) have identified various metabolites or metabolic fingerprints associated with specific disease states, but detailed analysis of specific metabolic responses may be useful in defining the clinical utility and biological relevance of the metabolome. Studies by Lv *et al.* (2016) and Furukawa *et al.* (2020) both reported depletion of bacterial taxa associated with butyrate production. Short-chain fatty acids (SCFAs) such as butyrate exhibit diverse physiologic effects and are arguably among the most important microbial metabolites that mediate the interaction between the colonic microbiome and the gut (Macfarlane and Macfarlane 2012). In addition to serving as an important nutrient source for colonic epithelial cells, SCFAs induce colonic regulatory T cells and regulate host–defense mechanisms. Thus, characterization of SCFAs together with the intestinal microbiota in patients with PBC represents an important area of investigation. The aim of our study was to analyze the fecal microbiota and SCFAs, among PBC patients with and without advanced fibrosis.

MATERIALS AND METHODS

From November 2015 to January 2018, we conducted a cross-sectional single-center study of the fecal microbiota and SCFAs in 15 PBC patients with non-advanced fibrosis (≤F2 fibrosis) and 8 PBC patients with advanced fibrosis (≥F3) at Indiana University.

Participants and study procedures

Adults ≥18 years of age with at least two of three accepted PBC diagnostic criteria (antimitochondrial antibody, elevated alkaline phosphatase and/or liver biopsy consistent with PBC) and compliant with dose-appropriate (13–15 mg/kg/day) UDCA were eligible. Patients with a diagnosis of other chronic liver diseases (viral, alcoholic, non-alcoholic, genetic causes of hepatitis, autoimmune hepatitis), a history of liver transplantation, active alcohol use, and pregnant females were excluded. Informed consent was obtained from all participants after study approval by the Indiana University Institutional Review Board. Fibrosis stage was determined on liver biopsy at diagnosis. Demographic and clinical data were collected upon enrollment to limit information bias. Fecal samples were collected once from all participants and immediately frozen at −80°C until analysis.

Analysis of fecal microbiota

DNA was extracted from fecal samples using the QIAamp® PowerFecal® DNA kit (Life Technologies Corporation, Carlsbad, California, USA). DNA quality and concentration were measured using automated electrophoresis (Agilent TapeStation, Agilent Technologies, Santa Clara, California, USA) and fluorescent double stranded DNA assay on a Qubit device (Life Technologies Corporation, Carlsbad, California, USA). The 16S ribosomal RNA (rRNA) gene V4 region was amplified using polymerase chain reaction (NEXTflex® 16S V4 Amplicon-Seq Kit; Bioo Scientific, Austin, TX). Amplicon libraries were pooled and sequenced using an Illumina MiSeq (Illumina, San Diego, CA). Sequence variants were generated using DADA2 within QIIME2 2018.8 and assigned taxonomic classification using release 132 of the SILVA database. Analyses were limited to taxa with a relative abundance >1% to remove poorly measured genera.

SCFA quantification

Fecal samples were homogenized by mixing ∼100 mg of feces in 400 μL of phosphate-buffered saline (pH 7.4) and vortexing for ∼2 min. The slurry was filtered through a 0.22-μm filter to remove cellular debris. The supernatant was mixed with additional buffer to a final volume of 540 μL. To provide a chemical shift and quantitation reference, 60 μL of Chenomx reference solution containing 5.0 mM 2,2-dimethyl-2-silapentane-5-sulfonate-d_6 (DSS-d6) was added.

Nuclear magnetic resonance (NMR) data were acquired on a Bruker Avance III 700 MHz NMR spectrometer with a TXI triple resonance probe operating at 25°C. Spectra were collected with a 1D NOESY pulse sequence covering 12 ppm. The spectra were digitized with 32 768 points during a 3.9 s acquisition time. The mixing time was set to 100 ms and the relaxation delay between scans was set to 2.0 s.

The data were processed using Advanced Chemistry Development Spectrus Processor (version 2016.1; Toronto, Canada). The spectra were zero filled to 65 536 points, apodized using a 0.3 Hz decaying exponential function and fast Fourier transformed. Automated phase correction and third-order polynomial baseline correction were applied to all samples. Metabolite concentrations were quantified using the Chenomx NMR Suite (version 8.2; Edmonton, Canada).

Statistical analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC) and R (https://www.R-project.org/). Univariate associations of fibrosis with fecal SCFAs and microbiota profiles (alpha diversity, individual taxa) were analyzed using the two-sample *t*-test and Wilcoxon rank-sum test. Permutational multivariate analysis of variance was used to evaluate associations of microbiota beta diversity (weighted and unweighted UniFrac distances) with fibrosis and SCFAs. Relationships between SCFAs and microbial taxa were assessed with Spearman correlation. False discovery rate (FDR) control was performed using the Benjamini–Hochberg method (Benjamini and Hochberg 1995). Participants with missing data were excluded from the analyses.

RESULTS

Participant characteristics

A total of 24 patients were enrolled (Table 1). Sequencing data were not available for 1 participant, leaving 23 PBC patients (8 with advanced fibrosis, 15 with non-advanced fibrosis) who were included in the final analyses. A majority of the patients with advanced fibrosis had evidence of decompensation (7/8, 87.5%), defined as presence of at least one of the following at

Table 1. Patient characteristics.

	Advanced fibrosis[a] (N = 8)	Non-advanced fibrosis[b] (N = 15)	P-value
Age (years)	61 (57.75, 65.75)	62.5 (53.5, 66.75)	0.82
Sex (% female)	100%	93.3%	0.46
Race (% Caucasian)	100%	88%	0.3
Body mass index (kg/m^2)	28.9 (24.6, 33.7)	31.5 (25.7, 32.5)	0.66
Disease duration (months)	42 (12, 134)	48 (21, 102)	0.83
Alkaline phosphatase (ALP; U/L)	132 (94, 435)	163 (100, 257)	0.98
Total bilirubin (mg/dL)	1.0 (0.5, 2)	0.5 (0.4, 0.7)	0.05
Albumin (g/dL)	3.9 (3.7, 4.4)	4.1 (4, 4.2)	0.29
Aspartate aminotransferase (U/L)	29 (23, 67)	27 (22, 40)	0.53
Alanine aminotransferase (U/L)	25 (16, 74)	25 (18, 46)	0.98
International normalized ratio	1.1 (1.0, 1.2)	1.0 (0.95, 1.1)	0.08
Biochemical response per Toronto criteria[c]	63%	67%	0.76
UDCA per day (mg)	1000 (900, 1000)	1000 (900, 1150)	0.74

Data show median (interquartile range, IQR) unless specified.
[a]Among advanced fibrosis: N = 6 with F4, N = 1 with F3/4 and N = 1 with F2/F3 fibrosis.
[b]Among non-advanced fibrosis: N = 5 with F0, N = 3 with F0/F1, N = 3 with F1, N = 3 with F1/F2 and N = 1 with F2 fibrosis.
[c]Alkaline phosphatase (ALP) <1.67 times upper limit at 1 year; the upper limit of normal for ALP was used to standardize results of ALP testing over different years as reference values changed intermittently during the study period.

Table 2. Fecal SCFA levels among all participants.

	Advanced fibrosis[a] (N = 8)	Non-advanced fibrosis[b] (N = 15)	P-value
Total fecal SCFA (mM)	9 (6–11.4)	3.3 (1.9–7.2)	**0.03**
Acetate (mM)	6 (4.1–7.9)	2.7 (1.1–4.9)	**0.03**
Butyrate (mM)	0.9 (0.5–1.3)	0.4 (0.2–1.1)	0.23
Propionate (mM)	1.8 (0.8–2.8)	0.7 (0.3–1.7)	0.06
Valerate (mM)	0.2 (0.1–0.4)	0.1 (0–0.3)	ns
Isobutyrate (mM)	0.1 (0.1–0.2)	0.1 (0–0.1)	ns
Isovalerate (mM)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	ns

Data show median (IQR) unless specified. Significant p-values < 0.05 are bolded. Non-significant p-values are shown as 'ns.'
[a]Among advanced fibrosis: N = 6 with F4, N = 1 with F3/4 and N = 1 with F2/F3 fibrosis.
[b]Among non-advanced fibrosis: N = 5 with F0, N = 3 with F0/F1, N = 3 with F1, N = 3 with F1/F2 and N = 1 with F2 fibrosis.
Bolded p-values are those that met our threshold for significance (p < 0.05); ns = non-significant.

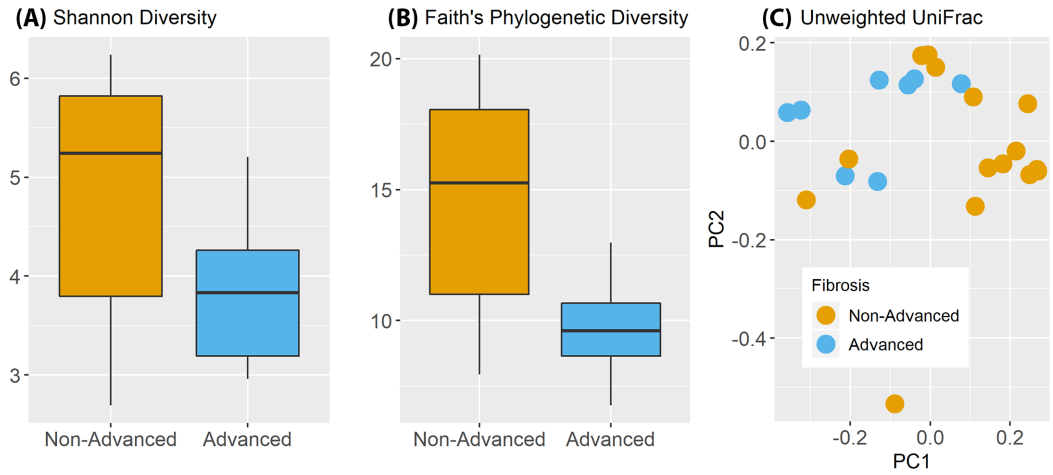

Figure 1. Fecal microbiota **(A)** alpha diversity with Shannon diversity index, **(B)** alpha diversity with Faith's phylogenetic diversity index and **(C)** beta diversity using unweighted UniFrac distances are associated with fibrosis group.

Table 3. Relative abundance (%) of bacterial genera that demonstrated the strongest associations with fibrosis group.

Bacteria	Advanced fibrosis[a] (*N* = 8)	Non-advanced fibrosis[b] (*N* = 15)	*P*-value	FDR-adjusted *P*-value
Weissella	0.71 (±1.42)	5.36E − 05 (±2.08E−04)	<0.05	0.045
Veillonella	1.78 (±3.95)	0.45 (±1.70)	<0.05	ns
Ruminococcaceae UCG-005	0.02 (±0.04)	0.28 (±0.45)	<0.05	ns
Christensenellaceae group R-7	0.03 (±0.04)	1.05 (±1.60)	<0.05	ns
Lachnospiraceae group NC2004	0.03 (±0.06)	0.29 (±0.39)	<0.05	ns
Ruminococcaceae UCG-010	7.09E − 04 (±2.00E−03)	0.12 (±0.29)	<0.05	ns
Ruminococcaceae UCG-004	0.073 (±0.11)	0.47 (±0.49)	<0.05	ns
Ruminococcaceae group NK4A214	0.06 (±0.11)	0.48 (±0.71)	<0.05	ns
Ruminococcaceae UCG-014	0.01 (±0.03)	0.38 (±0.61)	0.07	ns
Faecalitalea	0.02 (±0.05)	0.24 (±0.39)	0.08	ns

Data show mean (±standard deviations). Non-significant p-values are shown as 'ns.'
[a]Among advanced fibrosis: *N* = 6 with F4, *N* = 1 with F3/4 and *N* = 1 with F2/F3 fibrosis.
[b]Among non-advanced fibrosis: *N* = 5 with F0, *N* = 3 with F0/F1, *N* = 3 with F1, *N* = 3 with F1/F2 and *N* = 1 with F2 fibrosis.
ns = non-significant.

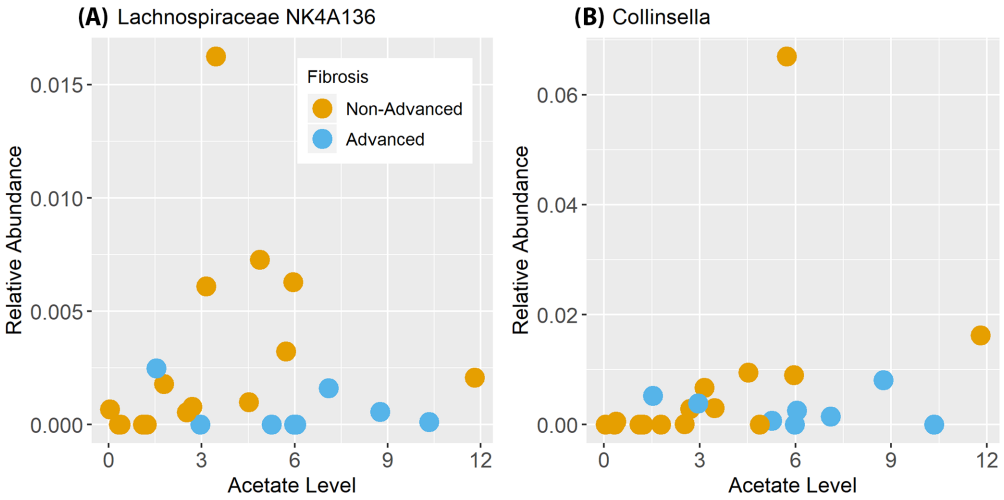

Figure 2. Correlations between fecal acetate and relative abundance of **(A)** *Lachnospiraceae* group NK4A136 and **(B)** *Collinsella*.

recruitment: esophageal varices, ascites, hepatic encephalopathy, jaundice or total bilirubin >2.5, Child–Pugh score >6 or MELD score >14. Among these patients, esophageal varices were most common (6/7, 85.7%), followed by hepatic encephalopathy (2/7, 28.6%) and total bilirubin >2.5 (2/7, 28.6%). There were no significant differences in baseline serologic characteristics except total bilirubin ($P = 0.05$) between fibrosis groups.

Fecal SCFAs

Total fecal SCFAs ($P = 0.031$) and acetate ($P = 0.026$) were significantly higher in advanced fibrosis (Table 2) compared with non-advanced fibrosis. There were no differences in fecal butyrate or fecal propionate between groups.

Assessment of fecal microbiota

Fecal microbiota alpha diversity was significantly reduced (Faith's phylogenetic diversity, $P = 0.001$; Shannon index, $P = 0.045$) in advanced fibrosis (Fig. 1). Analysis of fecal microbiota beta diversity revealed a significant separation (Fig. 1) between fibrosis groups with unweighted ($P = 0.005$), but not weighted UniFrac distances ($P = 0.24$). Analysis of taxonomic level datasets revealed increased relative abundance of the genus *Weissella* in advanced fibrosis ($P = 0.0.045$) after correcting for FDR. *Weissella* was identified in six of eight (75%) participants with advanced fibrosis, but in no participants with non-advanced fibrosis. Several additional genera (Table 3) were associated with fibrosis before but not after correcting for FDR.

Associations of fecal SCFAs and microbiota composition

Correlation analyses revealed no significant associations between total or individual SCFAs and alpha diversity within all participants or separately within fibrosis groups. Beta diversity by weighted UniFrac distances was significantly associated with butyrate within advanced fibrosis ($P = 0.024$), but not within all participants or within participants with non-advanced fibrosis. Examination of associations of fecal SCFAs with individual taxa demonstrated positive correlations between total SCFAs and relative abundance of *Collinsella* ($r_s = 0.84$; $P = 0.007$) within non-advanced fibrosis after correcting for FDR, but not within advanced fibrosis or all participants. Fecal acetate was positively correlated (Fig. 2) with relative abundance of *Lachnospiraceae* group NK4A136 ($r_s = 0.77$; $P = 0.044$) and *Collinsella* ($r_s = 0.76$; $P = 0.044$) within non-advanced fibrosis after correcting for FDR, but not within advanced fibrosis or all participants. There were

no significant correlations between fecal butyrate or propionate and individual taxa within all participants or within groups.

DISCUSSION

In this pilot study, fecal microbiota alpha diversity was reduced in PBC patients with advanced fibrosis, suggesting diminished microbial richness. Fibrosis was also associated with fecal microbiota beta diversity to suggest that changes in microbiota community composition are associated with PBC progression. Tang *et al.* (2018) reported similar findings of reduced microbial diversity in treatment-naïve PBC patients compared with controls. In contrast, Lv *et al.* (2016) reported no significant difference in alpha diversity between healthy controls and PBC patients. However, patients in the study by Lv *et al.* were in the early stages of PBC, which may explain some of these differences and further support the notion that decreased microbial diversity is associated with disease progression. We also observed increased *Weissella*, a common inhabitant of the gut identified by others as a potential probiotic based on its ability to produce lactate and acetate via oligosaccharide utilization, in advanced fibrosis (Manberger *et al.* 2020). Although the clinical implications of increased *Weissella* are not yet fully understood, it is interesting to note that a corresponding increase in fecal acetate was observed in our participants with advanced fibrosis.

We examined fecal SCFAs to explore microbial mechanisms in PBC. As stated, we observed increased fecal acetate and total SCFAs in advanced fibrosis. Others have reported decreases in butyrate-producing bacteria (Abe *et al.* 2018; Furukawa *et al.* 2020), *Clostridium* subcluster XIVa in PBC and autoimmune liver disease. Though SCFAs are generally considered beneficial, recent studies suggest SCFA effects may be disease dependent; elevated acetate and SCFAs were associated with pro-inflammatory responses and disease progression in non-alcoholic fatty liver disease and hepatocellular carcinoma (Rau *et al.* 2018; Singh *et al.* 2018). Our data suggest that fecal acetate and SCFAs could also be relevant in PBC fibrosis progression. Acetate achieves the highest concentrations within the intestinal lumen, is the net fermentation product of most anaerobic bacteria (Flint *et al.* 2015) within the gut and also serves as an important nutrition source for other microbial organisms that produce propionate and butyrate (Manberger *et al.* 2020). Thus, its biological significance should not be overlooked.

Correlation analyses further revealed an association between microbiota community composition and fecal butyrate within advanced fibrosis. *Collinsella* and *Lachnospiraceae* group NK4A136 were associated with fecal acetate and total SCFAs within non-advanced fibrosis, but not within all participants or advanced fibrosis. *Lachnospiraceae* belong to clostridial cluster XIVa, which as previously mentioned are prominent SCFA producers (Flint *et al.* 2015). Increased *Lachnospiraceae* have been reported in primary sclerosing cholangitis (Torres *et al.* 2016; Quraishi *et al.* 2017); however, the exact role of *Lachnospiraceae* group NK4A136 is unclear. *Collinsella* are considered lactate-producing pathobionts that have been associated with insulin resistance in pregnancy (Gomez-Arango *et al.* 2018). Our observations, while largely descriptive, suggest fecal SCFAs may be shaped by specific microbial features depending on disease severity.

Study weaknesses include the potential for tertiary referral bias and small sample size, which may limit generalizability, and the inherent limitations of 16S profiling of the V4 hypervariable region, which estimates relative rather than absolute abundance, provides limited resolution at the species level and restricts our ability to assess the metabolic capacity of identified taxa. We did not control for diet or medications (yet all included patients had no history of bile acid binding medications) that may influence assessment of the fecal microbiota and SCFAs. However, this pilot study is hypothesis generating and may be used to guide sample size calculations and methodological approaches for future follow-up studies. Strengths include strictly standardized study procedures in carefully phenotyped PBC patients. This is the first study to evaluate relationships between fecal microbiota and fecal SCFAs in PBC. Findings suggest that SCFAs could be novel microbial biomarkers in PBC and will require further validation in larger prospective studies.

DISCLOSURES

CL, ASS, HX, CH and TMOC have no disclosures. NC has no financial disclosures relevant for this study. For full disclosure, NC has ongoing consulting agreements with Abbvie, Madrigal, Zydus, Galectin, Allergan, Axcella, Foresite and La Jolla in last 12 months. He has received research support from Galectin, DSM and Exact Sciences.

FUNDING

This work was supported, in part, by Intercept Pharmaceuticals. CL was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant: K23DK11456. AS was supported by NIDDK K23DK122015.

REFERENCES

Abe K, Takahashi A, Fujita M *et al.* Dysbiosis of oral microbiota and its association with salivary immunological biomarkers in autoimmune liver disease. *PLoS One* 2018;**13**:e0198757.

Amathieu R, Triba MN, Goossens C *et al.* Nuclear magnetic resonance based metabolomics and liver diseases: recent advances and future clinical applications. *World J Gastroenterol* 2016;**22**:417–26.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995;**57**:289–300.

Chen W, Wei Y, Xiong A *et al.* Comprehensive analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. *Clin Rev Allergy Immunol* 2020;**58**:25–38.

Flint HJ, Duncan SH, Scott KP *et al.* Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* 2015;**74**:13–22.

Furukawa M, Moriya K, Nakayama J *et al.* Gut dysbiosis associated with clinical prognosis of patients with primary biliary cholangitis. *Hepatol Res* 2020;**50**:840–52.

Gomez-Arango LF, Barrett HL, Wilkinson SA *et al.* Low dietary fiber intake increases *Collinsella* abundance in the gut microbiota of overweight and obese pregnant women. *Gut Microbes* 2018;**9**:189–201.

Hao J, Yang T, Zhou Y *et al.* Serum metabolomics analysis reveals a distinct metabolic profile of patients with primary biliary cholangitis. *Sci Rep* 2017;**7**:784.

Jiang L, Schnabl B. Gut microbiota in liver disease: what do we know and what do we not know? *Physiology (Bethesda)* 2020;**35**:261–74.

Lv LX, Fang DQ, Shi D *et al.* Alterations and correlations of the gut microbiome, metabolism and immunity in patients

with primary biliary cirrhosis. *Environ Microbiol* 2016;**18**: 2272–86.

Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int* 2012;**95**:50–60.

Manberger A, Verbrugghe P, Guethmundsdottir EE *et al*. Taxogenomic assessment and genomic characterisation of *Weissella cibaria* strain 92 able to metabolise oligosaccharides derived from dietary fibres. *Sci Rep* 2020;**10**:5853.

Quraishi MN, Sergeant M, Kay G *et al*. The gut-adherent microbiota of PSC-IBD is distinct to that of IBD. *Gut* 2017;**66**:386–8.

Rau M, Rehman A, Dittrich M *et al*. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J* 2018;**6**:1496–507.

Selmi C, Bowlus CL, Gershwin ME *et al*. Primary biliary cirrhosis. *Lancet* 2011;**377**:1600–9.

Singh V, Yeoh BS, Chassaing B *et al*. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell* 2018;**175**:679–94.e22.

Tang R, Wei Y, Li Y *et al*. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. *Gut* 2018;**67**:534–41.

Torres J, Bao X, Goel A *et al*. The features of mucosa-associated microbiota in primary sclerosing cholangitis. *Aliment Pharmacol Ther* 2016;**43**:790–801.

Tripathi A, Debelius J, Brenner DA *et al*. The gut–liver axis and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018;**15**:397–411.