Journal of Clinical and Translational Hepatology **2025** vol. 13(12) | 1036–1045
DOI: 10.14218/JCTH.2025.00152

Original Article

Fecal Microbiome and Bile Acid Profiles Differ in Preterm Infants with Parenteral Nutrition-associated Cholestasis

Ellen S. Wagner, Kaitlyn Oliphant, Mark D'Souza, Wilfredo Cruz-Ayala, Ruba K. Azzam, Bree Andrews and Erika C. Claud*

Department of Pediatrics, University of Chicago, Chicago, IL, USA

Received: April 08, 2025 | **Revised:** June 15, 2025 | **Accepted:** August 05, 2025 | **Published online:** November 05, 2025

Abstract

Background and Aims: Parenteral nutrition (PN)-associated cholestasis (PNAC) is frequently diagnosed in premature infants; however, not all PN-exposed infants develop PNAC. We propose that, in premature infants receiving PN and varying amounts of enteral feeds, differences in the gut microbiome and fecal bile acid content are associated with PNAC development. This study aimed to examine the fecal microbiome and bile acid content of premature infants on PN to determine if there is a relationship with the development of PNAC. ***Methods:*** Twenty-two preterm infants had serial bilirubin measurements and fecal samples collected during their neonatal intensive care unit admission. Fecal samples underwent 16S rRNA gene sequencing and bile acid analysis. Binomial regression, adjusting for postmenstrual age with feed amount as a moderator, was used to assess the impact of the fecal microbiome and bile acids on PNAC development. ***Results:*** Cholestatic patients (n = 11) had greater PN and antibiotic exposure (p = 0.020; p = 0.010) and longer neonatal intensive care unit stays (p = 0.0038) than non-cholestatic patients. Microbiome richness was higher in non-cholestatic infants (p < 2E-16), with no difference in β diversity (p = 1.0). Cholestatic infants had a significantly higher abundance of *Proteobacteria* and *Fusobacteriota* and a lower abundance of *Bacteroidota* (p < 2E-16). *Akkermansia* was abundant in all infants on low feeds; as feed volume increased, *Akkermansia* abundance significantly increased in non-cholestatic infants (p < 2E-16). Bile acid analysis demonstrated significantly lower deoxycholic acid concentrations in cholestatic infants (p < 2E-16). Metagenomic analysis revealed an increase in *Proteobacteria* requiring augmented stress responses in non-cholestatic infants. ***Conclusions:*** This is the first study to directly explore the relationship between PNAC susceptibility, the microbiome, and fecal bile acids in preterm infants. The microbiome and bile acid patterns identified here may inform the development of targeted therapeutics for this vulnerable population.

Citation of this article: Wagner ES, Oliphant K, D'Souza M, Cruz-Ayala W, Azzam RK, Andrews B, *et al*. Fecal Microbiome and Bile Acid Profiles Differ in Preterm Infants with Parenteral Nutrition-associated Cholestasis. J Clin Transl Hepatol 2025;13(12):1036–1045. doi: 10.14218/JCTH.2025.00152.

Introduction

Cholestasis is a decrease in bile formation or flow, causing bile retention, liver toxicity, and conjugated hyperbilirubinemia. Premature infants, defined as infants born before 37 weeks of gestation, have numerous risk factors for cholestasis, including immaturity of metabolic pathways, small-for-gestational-age status, severe illness, and other factors limiting enteral nutrition, thus exposing them to more parenteral nutrition (PN), which is a known hepatotoxin.[1] Parenteral nutrition-associated cholestasis (PNAC) is defined as a conjugated bilirubin level ≥ 2 mg/dL with PN exposure for 14 or more days without another underlying etiology. PNAC is a very common entity in the neonatal intensive care unit (NICU): in one cohort of infants less than 30 weeks gestational age, the incidence of cholestasis was 10.7% within the first 28 days of life, with greater PN exposure correlating to a significantly increased risk.[2] Left untreated, alongside ongoing PN exposure, PNAC can result in significant hepatic fibrosis and is an indication for liver transplant.[1]

The human microbiome is a set of microorganisms that inhabit and interact with the human body, contributing to both health and disease.[3] The preterm infant gut microbiome has been connected to conditions such as necrotizing enterocolitis (NEC) and late-onset sepsis, both of which are also associated with an increased risk of cholestasis.[1,4,5] Research has associated both PN use and cholestasis with microbiome changes in term and near-term cohorts.[6–8] To date, there has been little research into the gut microbiome and PNAC in preterm infants. By further clarifying microbiome patterns associated with PNAC, opportunities for both diagnostic and therapeutic interventions may emerge for this vulnerable patient population.

Bile acids, a major component of bile, are synthesized in the liver through cholesterol metabolism. Released into the gut, they serve as detergents to facilitate nutrient absorption and are recirculated back to the liver through the portal system in a phenomenon known as enterohepatic circulation. Interruptions in enterohepatic circulation and alterations in bile acid homeostasis are implicated in both nutrient malabsorption and liver disease pathogenesis.[9] Bile acid

Keywords: Cholestasis; Premature infants; Parenteral nutrition; Enteral nutrition; Gut microbiome; Bile acids; Metagenomics.

***Correspondence to**: Erika C. Claud, Department of Pediatrics, Biological Sciences Division, University of Chicago, 5841 S. Maryland Ave, MC6060, Chicago, IL 60637, USA. ORCID: https://orcid.org/0000-0002-2408-2114. Tel: +1-773-702-6210, Fax: +1-773-702-0764, E-mail: Eclaud@bsd.uchicago.edu.

metabolism is dependent on and regulated by the gut microbiome.[10] There is minimal published research comparing the bile acid profiles of preterm infants to those of term infants and adults. Based on a small study of nine healthy infants from 32–36 weeks of gestation, preterm infants were found to have a relatively small bile acid pool in comparison to term infants.[11] The infant bile acid profile transitions to a more "adult-like" composition during the second year of life, on a similar timeframe to the transition to a mature gut microbiome.[12] There is no published research to date investigating the role of the gut microbiome in bile acid metabolism in preterm infants on PN.

Given the complex and interrelated nature of cholestasis, bile acid metabolism, PN, and the microbiome, there is a significant opportunity for research into these pathologies. The purpose of this study was to examine the fecal microbiome and bile acid content of premature infants on PN to determine if there is a relationship with the development of PNAC. We hypothesize that in preterm infants on PN and varying amounts of enteral nutrition, features of the early gut microbiome and differences in fecal bile acid content are associated with the development of PNAC.

Methods

Study design

Study subjects' clinical data and biospecimens were examined retrospectively from the Microbiome in Neonatal Development (MIND) clinical cohort of preterm infants at the University of Chicago Comer Children's Hospital, which was established to determine the relationship between the preterm infant microbiome and neurodevelopment. The inclusion criteria of the MIND clinical cohort were preterm infants (born at less than 37 weeks gestational age) without any genetic syndromes or severe congenital anomalies that were deemed viable after birth by the attending physician. Thus, this clinical cohort had the characteristics necessary to address the study research questions without any inherent restraints, as the enrollment criteria were inclusive, the clinical data collection had a broad mandate, and the biospecimen acquisition was microbiome-focused. An additional inclusion criterion for the specific goals of this study was applied to select a subset of patients who received PN, for the purpose of comparing preterm infant patients who did and did not develop cholestasis while on PN, and cholestasis could be accurately diagnosed, as serial bilirubin measurements are taken from infants while on PN per hospital protocol. The Duchossois Family Institute (DFI) was utilized for preterm infant fecal sample analysis; the DFI was established at the University of Chicago for the specific purpose of investigating the microbiome to improve human health, and therefore houses facilities containing state-of-the-art methods for analyzing the microbiome. The methods employed in this study include 16S rRNA gene sequencing and deep shotgun metagenomics sequencing, which are the gold standard in the microbiome research field for understanding microbiome composition and functional capacity, respectively, and a comprehensive bile acid panel, which included the 49 most common and abundant primary, secondary, and glyco-/tauro-conjugated subclasses of bile acids. Together, these measurements allowed for a detailed evaluation of whether and how the fecal microbiome and bile acid content are associated with the development of cholestasis in preterm infants while on PN.

Cohort identification

Participants were drawn from the MIND study, which is approved by the University of Chicago Institutional Review Board (IRB16-1431) and is conducted in accordance with both the Declarations of Helsinki and Istanbul. After receiving written informed consent from a parent, patients were enrolled in the NICU at the University of Chicago Comer Children's Hospital between November 2010 and July 2018. Inclusion criteria were infants with a gestational age of less than 37 weeks. Infants with genetic or severe congenital anomalies (including major congenital heart disease, kidney, lung, or brain malformations) and infants deemed not to be viable by the attending physician were excluded. For the present study, all infants in the MIND cohort with exposure to PN for at least four weeks, with stool samples and bilirubin measurements obtained during the time period of PN exposure, were included.

Clinical data and sample collection

Infant diapers were collected weekly by nursing staff, and samples were stored at −80°C in order to enable assessment of the fecal microbiome. As per hospital protocol, bilirubin levels were measured at least once weekly for infants receiving PN. Additional potentially contributing clinical variables were gathered from patient charts by extracting information from the electronic medical record system at the University of Chicago Comer Children's Hospital. Data collected included gestational age at birth, sex, birth weight, information on nutrition regimens (percent feeds human milk, average received enteral nutrition in mL per kg body weight, average received PN in mL per kg body weight, and average received lipid in mL per kg body weight), total number of days of antibiotics received, length of NICU stay, and corrected gestational age at discharge. Chart review also captured additional diagnoses, such as NEC and sepsis, and additional therapies utilized, such as Ursodiol and SMOFlipid (Fresenius Kabi; Bad Homburg, Germany). For purposes of this study, NEC was defined by modified Bell's criteria, with disease classified if meeting at least Bell's stage 2.[13] Sepsis consisted of both those with early (<72 h) and late (>72 h) blood culture positivity.

Illumina 16S rRNA gene sequencing and data processing

Genomic DNA extraction and Illumina 16S rRNA gene sequencing of patient fecal samples were conducted by the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory (Lemont, IL, USA).[14,15] The raw data were subsequently processed using the DADA2 pipeline[16] implemented into QIIME2 version 2019.7[17] and classified to the genus level using the IDTAXA method[18] included in R package DECIPHER version 2.14.0 and the Genome Taxonomy Database version 8.[19] An additional classification step for determining species-like groups (i.e., the highest taxonomic resolution possible by 16S rRNA gene sequencing alone) was employed using the online National Center for Biotechnology Information Nucleotide Basic Local Alignment Search Tool (4,5)[20] with a ≥97% identity threshold, and very low abundant species-like groups of <0.1% mean abundance across all samples were culled. Low quality samples that had a sequence read depth of <1,000 were also removed prior to analysis. For the calculation of α-diversity, the R package iNEXT version 2.0.20 was utilized to obtain the richness, Simpson, and Shannon diversity metrics from the genus-level data.[21] In terms of β-diversity analysis, the taxonomic levels of phylum, family, and genus were individually studied. For the determination of overall β-diversity, community typing analysis was conducted using the R package DirichletMultinomial version 1.36.0 with 1 to n-1 community

types considered and the optimum solution selected by the minimum Laplace value.[22] For examining individual taxa, the data were center-log ratio transformed via the R package ALDEx2 version 1.18.0 to account for compositionality prior to statistical analysis.[23]

Fecal bile acid analysis

Bile acid analysis was performed on available samples of infants who were on PN at the time of stool sample acquisition (n = 15) through the University of Chicago DFI. In brief, bile acids were extracted with organic solvent, dried down, and resuspended for direct analysis. The bile acids had previously been validated by the DFI through retention time and fragmentation comparison to standards and available databases. Using negative mode liquid chromatography–electrospray ionization–quadrupole time-of-flight MS ((-)LC-ESI-QTOF-MS, Agilent, 6546), 49 bile acids from the primary, secondary, and glyco-/tauro-conjugated subclasses were analyzed.[24] In addition to retention time validation, the standard intact and fragment masses are routinely detected with differences < 5 ppm compared to calculated values. Metabolite-normalized peak abundances were recorded in the supplementary materials (Supplementary Table 1; qualitative). Quantitative values were also determined for 17 of the most common and abundant bile acids in human fecal samples; they are recorded in the supplementary materials (Supplementary Table 1; quantitative).

Shotgun metagenomics sequencing and data processing

Shotgun metagenomics sequencing was additionally conducted at the DFI on the same set of stool samples sent for bile acid analysis (n = 15), which were collected while the infants were on PN. The DFI completed genomic DNA extraction (QIAamp PowerFecal Pro DNA Kit, Qiagen 51804), library generation (QIAseq FX Library Kit, Qiagen 180479), and shotgun metagenomics sequencing on the Illumina NovaSeq 6000 platform using the 2× 150 paired-end read cassette. Data obtained from the DFI were processed using the bioBakery whole metagenome shotgun workflow version 3.0.0.,[25] which includes the KneadData tool for quality control and removal of host reads, and the HUMAnN tool for generation of microbial gene abundance profiles stratified by contributing microbial taxa utilizing the Kyoto Encyclopedia of Genes and Genomes (hereinafter referred to as KEGG) database.[26] The compositional data produced from this workflow were center-log ratio transformed, with zeroes imputed by the nonparametric multiplicative simple method through the R package zCompositions version 1.4.0.1.

Statistical analysis

All analyses were conducted in R statistical software version 4.2.1, and plots were generated using the R packages ggplot2 version 3.4.4 and patchwork version 4.2.3. The outcome (dependent variable, y) of this study was binary: patients with cholestasis versus no cholestasis. The microbiome metrics of interest included α-diversity, overall β-diversity, individual abundances of taxa, and KEGG Orthology abundances. This study aimed to determine the associations between these microbiome metrics and the amounts of bile acids (as individual independent variables, x) with the outcome, cholestasis. To accomplish this task, mixed effect binomial regression models were built after variable standardization using R packages lme4 version 1.1.34 and standardize version 0.2.2, with adjustment for postmenstrual age (completed weeks), enteral feeding amount (mL/kg body weight), enteral feeding amount × microbiome metric/bile acid interaction, and patient (as a random effect) via the following formula: y ~ x + postmenstrual age + enteral feeding amount + enteral feeding amount*x + (1/patient). Model fit was evaluated using the R package DHARMA version 0.4.6, along with calculation of the variance inflation factor through the R package car version 3.1.2 to assess collinearity (maximum < 5 considered not collinear). After fitting, *p*-values were determined from the Wald chi-square test implemented in the R package car and were subsequently adjusted by the Benjamini-Hochberg method. If the microbiome metric/bile acid *p*-value was <0.05 and adjustment indicated a false-positive rate of <1%, the microbiome metric/bile acid was next examined for substantial presence among the studied cohort (microbiome metric/bile acid needed to be present in ≥ three patients in at least one of the study groups) before being considered significant. Results and assumption tests of all models (significant or non-significant) are included in the supplementary materials (Supplementary Table 2). The associations between the significant microbiome metrics/bile acids (as the outcome, y) and the number of days of antibiotics or diagnosis of NEC (as individual independent variables, x) were also examined with adjustment for postmenstrual age (completed weeks) and patient (as a random effect) using linear mixed effect models via the same strategy as above: y ~ x + postmenstrual age + (1/patient). If the number of days of antibiotics and/or diagnosis of NEC were found to be significantly associated with the microbiome metrics/bile acids, the number of days of antibiotics and/or diagnosis of NEC was then added to the above binomial mixed effect models as additional independent variables. Only if the microbiome metrics/bile acids remained significant after this additional adjustment were they considered to be associated with cholestasis. These regression results for evaluation of the number of days of antibiotics and the diagnosis of NEC as potential confounding variables are included in the supplementary materials (Supplementary Table 3).

Results

Cohort definition

Twenty-two infants met inclusion criteria; eleven patients had PNAC, and eleven were classified as non-cholestatic. Cohort clinical characteristics are summarized in Table 1. The cohorts were well-matched in terms of gestational age at birth, with a mean of 25.8 weeks, ranging from 24 to 30 weeks. Mode of delivery, sex, and birth weight were also similar between groups. There were no significant differences in the percentage of human milk feeds nor in the diagnosis of sepsis, which had low prevalence in this cohort (one case). There were significant differences in average total enteral feeds per day, with the non-cholestatic cohort taking more on average than the cholestatic. In turn, the cholestatic group was exposed to significantly more PN and lipid per day over their length of stay compared to the non-cholestatic cohort. The cholestatic group also received significantly more days of antibiotics than the non-cholestatic group, specifically penicillins, gentamicin, vancomycin, and metronidazole. There were significantly more diagnoses of NEC in the cholestatic cohort, as well as significantly higher utilization of SMOFlipid (Fresenius Kabi; Bad Homburg, Germany) and ursodiol, each of which is used to treat PNAC. The cholestatic cohort had a significantly increased length of NICU stay in days, as well as a greater corrected gestational age at discharge in completed weeks.

Table 1. Cohort clinical characteristics

Clinical characteristic	Cholestatic (n = 11)	Non-Cholestatic (n = 11)	*p*-value
Gestational age at birth, completed weeks	25.3 ± 1.6	26.4 ± 1.7	0.11
Mode of delivery, vaginal	18.2% (2)	18.2% (2)	1.0
Sex, male	54.5% (6)	63.6% (7)	0.66
Birth weight, kg	0.76 ± 0.12	0.80 ± 0.25	0.63
% Feeds human milk	26.8 ± 27.8	40.9 ± 34.3	0.27
Average total enteral feeds per length of NICU stay (mL/kg bodyweight)	57.0 ± 28.5	85.2 ± 23.2	0.014
Average PN received per length of NICU stay (mL/kg bodyweight)	34.8 ± 9.9	24.1 ± 10.9	0.020
Average lipid received per length of NICU stay (g/kg bodyweight)	0.81 ± 0.16	0.58 ± 0.24	0.010
Days of antibiotics	36.1 ± 21.4	16.5 ± 12.6	0.010
Days of penicillins	24.4 ± 20.8	6.5 ± 3.1	0.00032
Days of gentamicin	14.9 ± 7.6	9.3 ± 5.3	0.042
Days of vancomycin	7.9 ± 5.8	3.5 ± 2.9	0.023
Days of cephalosporins	10.8 ± 10.8	3.3 ± 6.9	0.052
Days of erythromycin	1.8 ± 6.0	6.0 ± 11.3	0.25
Days of antifungals	2.1 ± 4.3	1.7 ± 4.1	0.83
Days of clindamycin	0.8 ± 1.8	0.3 ± 0.9	0.35
Days of metronidazole	6.6 ± 9.8	0.3 ± 0.9	0.020
Sepsis	9.1% (1)	0.0% (0)	0.23
NEC	27.3% (3)	0.0% (0)	0.031
Use of SMOFlipid	27.3% (3)	0.0% (0)	0.031
Use of ursodiol	36.4% (4)	0.0% (0)	0.011
Length of NICU stay, days	142.0 ± 46.0	95.5 ± 19.2	0.0038
Corrected gestational age at discharge, weeks	45.1 ± 5.6	39.6 ± 2.6	0.0054

SMOFlipid (Fresenius Kabi): an intravenous fat emulsion made with 30% soybean oil, 30% medium-chain triglycerides, 25% olive oil, and 15% fish oil. Numeric variables are depicted by the mean and standard deviation, and binary variables are depicted by the percentage and number of patients. *p*-values were determined by binomial regression and the Wald chi-square test. PN, parenteral nutrition; NICU, neonatal intensive care unit; NEC, necrotizing enterocolitis.

Differences in α diversity but not overall β diversity were observed between cohorts

In order to interrogate the role of the fecal microbiome in PNAC, we first analyzed overall microbiome diversity. Microbiome richness was significantly higher in the non-cholestatic cohort (n = 11 patients, n = 29 samples) compared to the cholestatic cohort (n = 11 patients, n = 34 samples) when controlling for postmenstrual age and amount of enteral feeds (Fig. 1A; $p < 2E\text{-}16$). There were no differences in overall microbiome β diversity between cohorts (Fig. 1B; $p = 1.0$).

Taxa-specific analysis showed differences between cholestatic and non-cholestatic cohorts

After identifying a reduction in microbiome richness in cholestatic patients, we aimed to determine which taxa were depleted in these patients. There was a noted expansion of the phylum *Proteobacteria* both in cholestatic infants when accounting for postmenstrual age (Fig. 2A; direct coefficient 0.79; $p < 2E\text{-}16$), with a 23% mean difference from 28–30 weeks postmenstrual age, as well as in cholestatic infants on lower amounts of enteral nutrition (Fig. 2B; interaction coefficient −0.72; $p < 2E\text{-}16$), with a 20% mean difference from 0–50 mL/kg enteral feeds. *Bacteroidota* displayed a protective pattern: regardless of postmenstrual age, a significantly greater abundance of *Bacteroidota* was associated with lack of cholestasis (Fig. 2C; direct coefficient −33.53; $p < 2E\text{-}16$), with a 4% overall mean difference. With regard to enteral feed amount, greater *Bacteroidota* abundance was similarly associated with lack of cholestasis, and this was more pronounced in infants on greater amounts of enteral nutrition (Fig. 2D; interaction coefficient −32.80; $p < 2E\text{-}16$), with a 3% mean difference at >50 mL/kg enteral feeds. There was a notable increase in the incidence of cholestasis in infants with a greater abundance of *Fusobacteriota*, particularly at low enteral nutrition (Fig. 2E; interaction coefficient −3.56; $p < 2E\text{-}16$), with a 2% mean difference from 0–50 mL/kg enteral feeds. No *Fusobacteriota* were detected in the non-cholestatic group.

At the family level, significant patterns emerged with *Bifidobacteriaceae*, *Bacteroidaceae*, *Dialisteraceae, Ruminococcaceae, Akkermansiaceae,* and *Erysipelatoclostridiaceae* ($p < 2E\text{-}16$). At the genus level, *Akkermansia* were found to be present in high amounts in both non-cholestatic and cholestatic infants on low enteral nutrition; however, as feeds increased, a significantly greater abundance of *Akkermansia* was associated with the non-cholestatic state (Fig. 2F; interaction coefficient −28.30; $p < 2E\text{-}16$), with a 0.004% mean difference at >50 mL/kg enteral feeds, and no *Akkermansia* were detected in the cholestatic group.

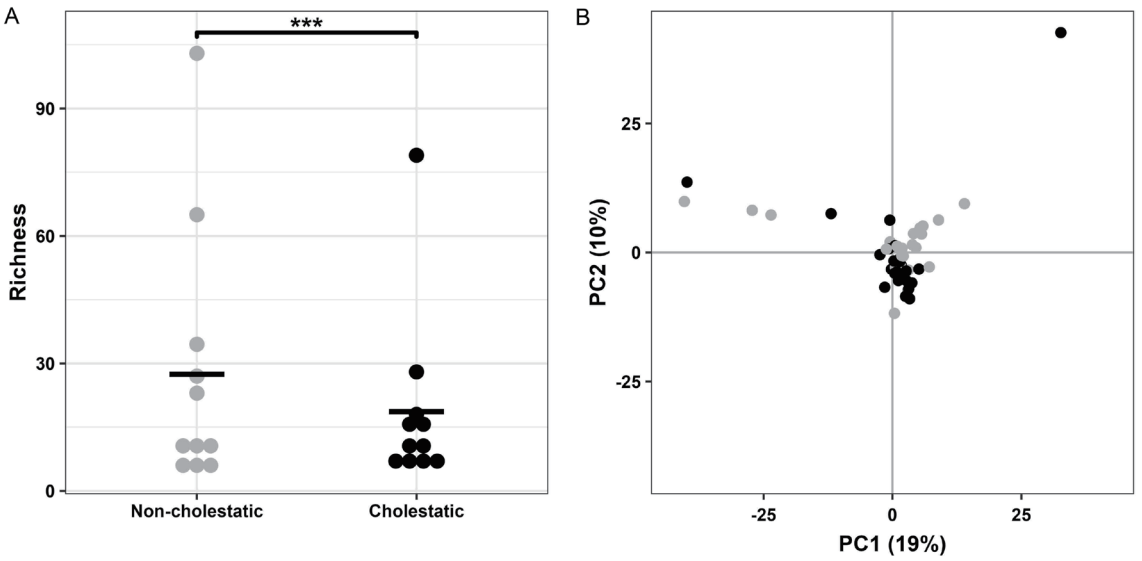

Fig. 1. Cohort α and β diversity. Richness was higher in non-cholestatic (gray) than in cholestatic patients (black) (Panel A; p < 2E-16). Each point represents the mean richness across time for each patient (n = 22), and richness was calculated per sample as the total number of observed microbial genera. The PCoA plot shows no significant difference in overall β diversity between non-cholestatic (gray) and cholestatic (black) samples (Panel B; p = 1.0). Each point represents one sample (n = 63). $^{***}p$ < 0.001.

Overall bile acid content was greater in non-cholestatic infants, with a significant increase in deoxycholic acid (DCA) specifically

Bile acid analysis was subsequently conducted in order to examine any potential differences in fecal bile acid content between cohorts. A smaller number of infants (n = 8 cholestatic, n = 7 non-cholestatic) had samples collected while on PN available for bile acid analysis. Among those analyzed, it was noted that non-cholestatic infants had significantly greater total bile acid content in their stool than cholestatic infants (Fig. 3A; direct coefficient −7.86; p = 0.0073). Cholestatic infants had significantly greater amounts of conjugated cholic acid than chenodeoxycholic acid (Fig. 3B; direct coefficient = 1.56; p = 0.034). A significant pattern also emerged with DCA; although the overall prevalence of DCA was low among both cholestatic and non-cholestatic infants, cholestatic patients had significantly lower levels of DCA than non-cholestatic infants when controlling for postmenstrual age and amount of enteral feeds (Fig. 3C; direct coefficient −171.58; p = 0.035).

Metagenomics analysis revealed functional differences in chemotaxis, carbohydrate metabolism, stress response, and zinc transporters

In order to evaluate specific functional differences between the microbiomes of cholestatic and non-cholestatic infants, metagenomics analysis was completed on the same fifteen samples as bile acid analysis (n = 8 cholestatic, n = 7 non-cholestatic). The *Proteobacteria* present in preterm infants without cholestasis had a feed-independent increase in chemotaxis-related proteins, amino acid transporters, stress response proteins, and microcin C permease. Further, these *Proteobacteria* had a feed-dependent increase in arginine, thiamine, glutathione, and sugar and sugar alcohol transporters, in addition to increased capabilities in polysaccharide degradation such as pectinase and monosaccharide utilization such as L-fuculose and D-allose kinases. Together, these findings suggest that the *Proteobacteria* are under more stress in the gut environment of non-cholestatic compared to cholestatic preterm infants from both nutrient competition and microbiota-produced antimicrobials including microcin C.[27] These *Proteobacteria* are thus required to maintain a diversity of nutrient sensing, searching, uptake, and consumption proteins, in addition to stress response mechanisms. In contrast, preterm infants with cholestasis were found to have *Proteobacteria* and *Streptococcus* with a feed-dependent increase in zinc transporters. Full metagenomic analysis results are reported in Supplementary Table 4.

Discussion

PN is both a known hepatotoxin and an essential component of care for premature infants who are unable to tolerate adequate enteral nutrition for growth. Although not every infant on PN develops PNAC, those who do may go on to develop significant liver damage with ongoing PN exposure. This study is the first to compare the gut microbiome and fecal bile acid content in premature infants with PNAC to non-cholestatic peers. We hypothesized that in premature infants on PN and varying amounts of enteral feeds, features of the early gut microbiome, as well as differences in fecal bile acid content, are associated with the development of PNAC. In this cohort of 22 preterm infants exposed to PN for four or more weeks, there is significantly greater microbiome richness in non-cholestatic infants compared to cholestatic; however, no significant differences were observed in overall β-diversity. At the phylum level, cholestatic infants were found to have significantly more *Proteobacteria* and *Fusobacteriota* than non-cholestatic peers, a pattern that was more pronounced at low enteral nutrition. Regardless of enteral feed amount, non-cholestatic infants had a significantly greater abundance of *Bacteroidota* than cholestatic infants. The genus *Akkermansia* was found to be abundant in both cholestatic and non-cholestatic infants on low enteral feeding volumes; however, as enteral feed amount increased, it was noted that non-cholestatic infants had a significantly increased abundance of *Akkermansia* compared to cholestatic infants. A sub-analysis of fecal bile acid content in fifteen infants showed overall

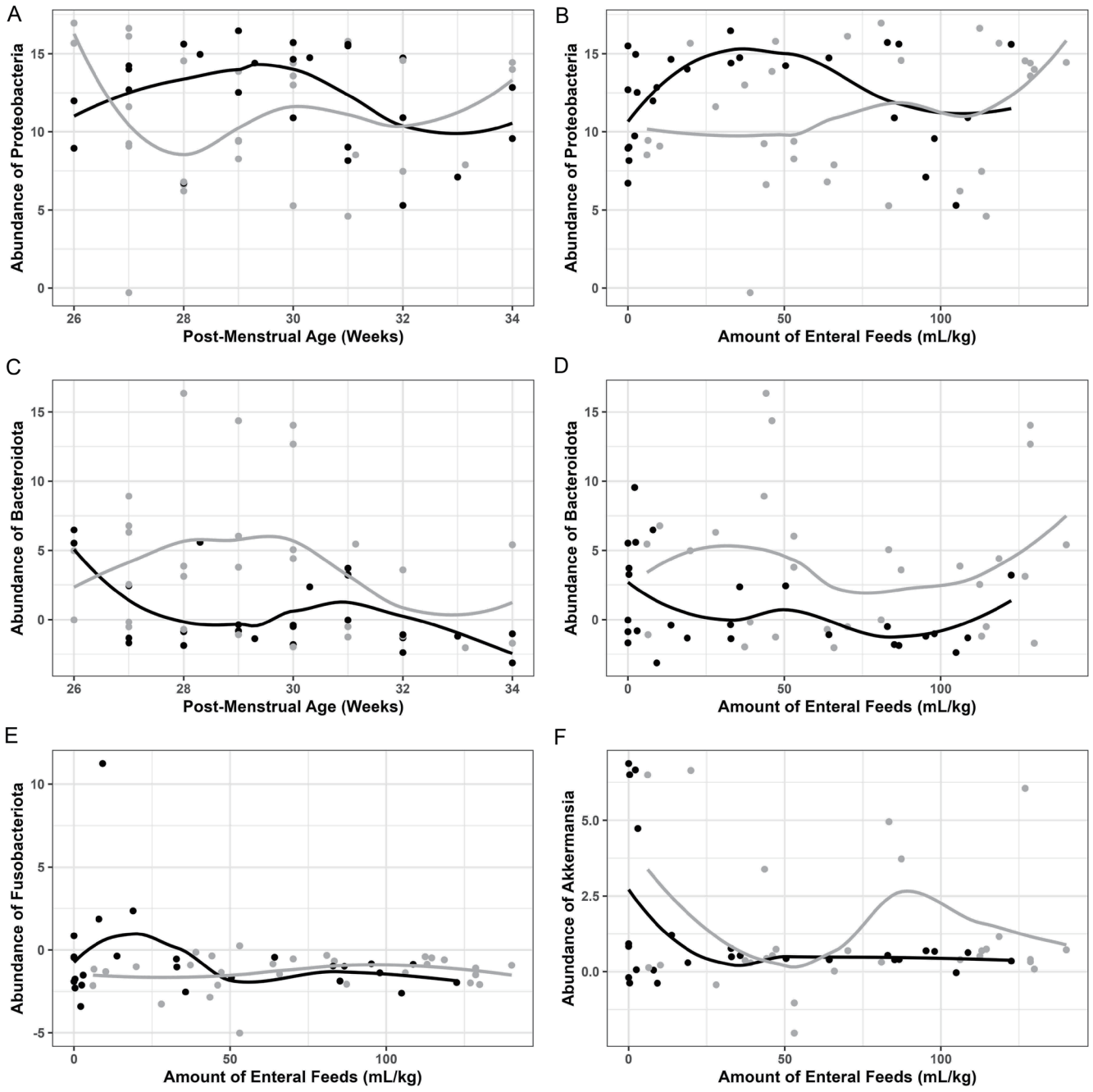

Fig. 2. Trends in taxa-specific analysis. Taxa-specific analysis results, with each point representing one sample (non-cholestatic gray, n = 29 samples; cholestatic black, n = 34 samples). The Y-axis represents the abundance of a bacterial phylum or genus and is center-log ratio transformed. At the phylum level, *Proteobacteria* are significantly expanded in cholestatic infants compared to non-cholestatic across different postmenstrual ages (Panel A; direct coefficient 0.79, $p < 2E\text{-}16$). When controlling for enteral feed amount, *Proteobacteria* have significantly greater abundance in cholestatic infants compared to non-cholestatic, a relationship more pronounced at low enteral nutrition (Panel B; interaction coefficient −0.72, $p < 2E\text{-}16$). At the phylum level, increased *Bacteroidota* abundance was found in non-cholestatic infants regardless of postmenstrual age (Panel C; direct coefficient −33.53, $p < 2E\text{-}16$) or enteral feed amount (Panel D; interaction coefficient −32.80, $p < 2E\text{-}16$). At the phylum level, *Fusobacteriota* are significantly expanded in cholestatic infants compared to non-cholestatic when controlling for enteral feed amount (Panel E; interaction coefficient −3.56; $p < 2E\text{-}16$). This relationship diminishes as enteral feeding increases. The genus *Akkermansia* has increased abundance in both non-cholestatic and cholestatic samples at low enteral nutrition. At high enteral nutrition, a significantly greater abundance is found in non-cholestatic samples (Panel F; interaction coefficient −28.30, $p < 2E\text{-}16$).

low concentrations of DCA among all patients, with significantly lower levels in cholestatic patients when controlling for postmenstrual age and enteral feed amount. Metagenomic analysis showed an increase of *Proteobacteria* requiring an augmented stress response in non-cholestatic infants and an increase in zinc transporter genes in *Proteobacteria* and *Streptococcus* in cholestatic infants.

The phylum *Proteobacteria*, which includes genera such as *Escherichia*, *Klebsiella*, and *Enterobacter*, has been shown by Dahlgren *et al*. to be expanded in premature infants on

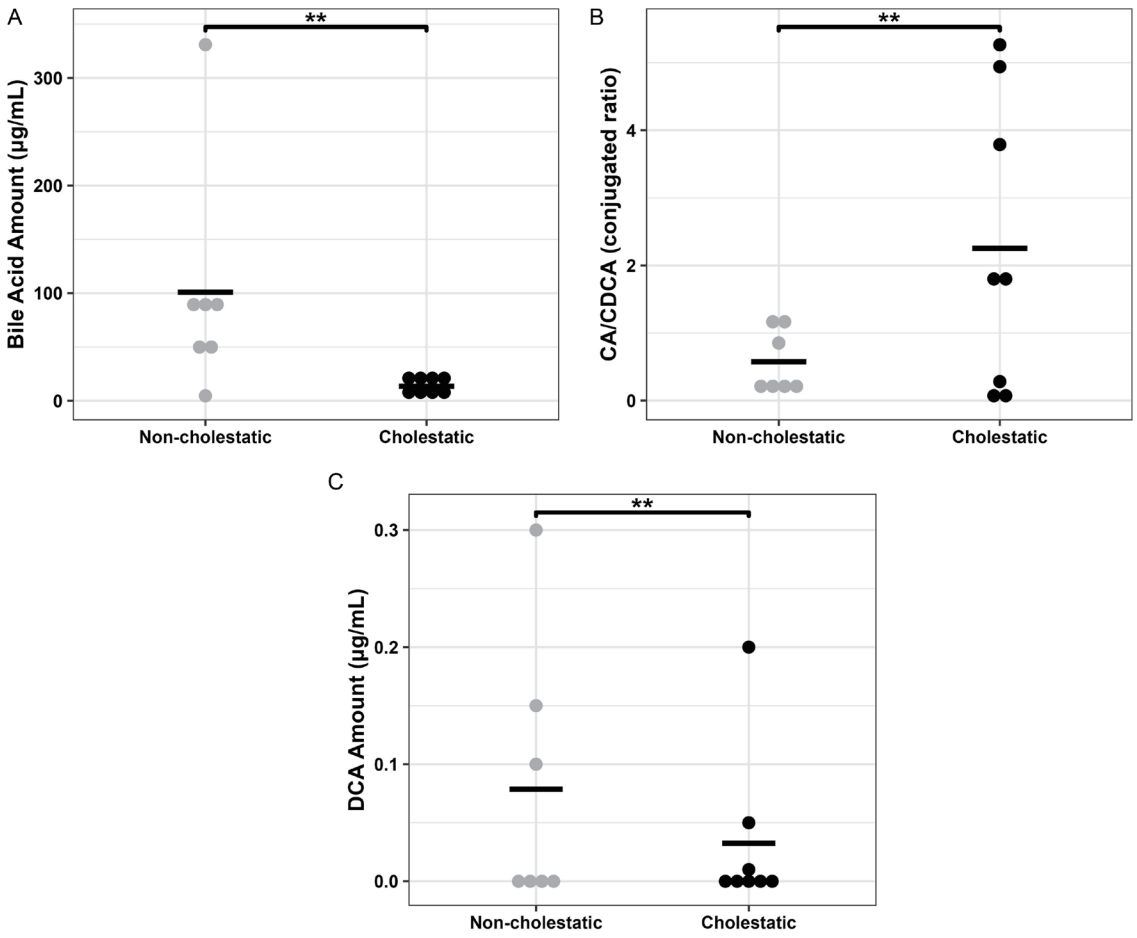

Fig. 3. Bile acid analysis results. Bile acid analysis was performed on 15 total samples, n = 8 in cholestatic (black) and n = 7 in non-cholestatic (gray) infants. Overall, total bile acid amount was higher in non-cholestatic than in cholestatic infants (Panel A; direct coefficient −7.86, $p = 0.007$). The ratio of CA to CDCA was significantly higher in cholestatic infants than in non-cholestatic regardless of enteral feed amount (Panel B; direct coefficient 1.56, $p = 0.03$). There was significantly lower deoxycholic acid (DCA) in cholestatic infants than in non-cholestatic infants when controlling for both enteral feed amount and postmenstrual age (Panel C; direct coefficient −171.58, $p = 0.03$). $^{**}p < 0.01$. CA, cholic acid; CDCA, chenodeoxycholic acid.

PN with cholestasis, but not reaching statistical significance due to cohort size.[6] Mokha *et al*. in 2019 compared the fecal microbiomes of premature infants on PN who did not become cholestatic to those who did. Interestingly, in that small study, *Proteobacteria* were found to be elevated in the control group, with longitudinal samples from the pre-cholestasis, cholestasis, and post-cholestasis infants showing expansion of the phylum over time.[28] Although this result differs from our cohort, we did not have enough representative samples from the pre-cholestasis time point to perform an analysis. Other studies in premature infants have shown a predominance of *Proteobacteria* in those who would go on to develop NEC, as well as those who display poor tolerance to enteral feeds compared to peers.[29,30] These findings, in conjunction with our results, which show higher *Proteobacteria* in cholestatic premature infants compared to non-cholestatic, particularly at low enteral nutrition, suggest that *Proteobacteria* can be associated with health outcomes. In adults, *Proteobacteria* have been associated with relative enteral starvation, which, when coupled with inflammation, contributes to their proliferation.[31] The findings for phylum *Fusobacteriota* were similar in that cholestatic infants had higher concentrations at lower enteral nutrition. Although there is some adult literature to suggest specific species of *Fusobacterium* contribute to disease states (*F. nucleatum* is associated with colorectal cancer, inflammatory bowel disease, and appendicitis), this finding in premature infants with cholestasis is novel.[32]

Bacteroidota are common gastrointestinal tract colonizers, known to be passed on to infants through vaginal birth.[33] In the present study, regardless of enteral feed amount, *Bacteroidota* were found in higher concentrations in non-cholestatic than in cholestatic infants. This echoes the findings of Dahlgren *et al*., who found lower *Bacteroidota* in premature infants on PN, as well as Guo *et al*., who reported lower *Bacteroidota* in cholestatic term infants.[6,7] *Bacteroidota* are involved in bile acid metabolism at multiple levels, including bile salt hydrolase (BSH) activity.[10,34] Although direct research into BSH activity in preterm infants is limited, Lynch *et al*. found BSH activity to be profoundly decreased in preterm infants who developed cholestasis, with correlation to a relative depletion of *C. perfringens* compared to controls.[35] In the present cohort, there were no significant findings relative to *C. perfringens* nor with signaling for BSH pathways in metagenomics analysis, which may reflect lower availability of fecal material for analysis. Regardless, our findings suggest that premature infants without cholestasis are able to more efficiently metabolize bile acids due to the abundance of *Bacteroidota* in comparison to their cholestatic peers.

At the genus level, *Akkermansia* were noted to be more abundant in both cholestatic and non-cholestatic infants on low enteral feeding volumes. *Akkermansia* is a mucin-degrading genus that has a competitive advantage during states of low enteral nutrition due to its ability to use mucus as its sole carbon and nitrogen source. The presence of *Akkermansia* has been considered a biomarker of a healthy, protective, and anti-inflammatory mucous layer: low *Akkermansia* abundance has been correlated with disease states such as Crohn's disease, ulcerative colitis, and appendicitis in human subjects.[36] This is because, while *Akkermansia* paradoxically degrades mucin, it simultaneously increases both the number of goblet cells and the total mucus secretion by goblet cells through cross-talk with the host intestinal epithelium, with the net result being a thicker mucosal layer and stronger intestinal barrier.[37] Although there has been no direct research implicating *Akkermansia* in cholestatic liver disease, one study utilizing a mouse model for alcoholic liver disease demonstrated that therapeutic *A. muciniphila* administration ameliorated hepatic injury.[38] The mechanism proposed by the authors was that the thicker mucus layer, and thus improved intestinal barrier induced by *A. muciniphila*, reduced the systemic endotoxin load (i.e., serum lipopolysaccharide concentrations), which ameliorated liver injury through decreased inflammation. In our cohort, as enteral feeding increased, *Akkermansia* were found in higher abundance in non-cholestatic patients, which is in line with prior research, reflecting its contribution to a protective mucous layer. Studies in both humans and mice also suggest that the presence of DCA is correlated with increased growth of *Akkermansia spp.* (specifically *A. muciniphila*).[39,40] Our results, which show both high *Akkermansia* and high DCA in non-cholestatic infants, align with these conclusions and are a novel finding in preterm infants.

Bile acid analysis was performed on a subset of samples and showed overall low bile acid levels but significantly decreased abundance of DCA in cholestatic compared to non-cholestatic infants. DCA is a secondary bile acid produced by 7α-dehydroxylation. Its presence in the stool is reflective of BSH activity, which can be induced by *Bacteroidota*.[10,41] DCA is also a known farnesoid X receptor (FXR) agonist. Disrupted signaling of FXR, which has an important role in overall bile acid homeostasis, has been proposed as a mechanism for PNAC.[42,43] Findings in the present study suggest that non-cholestatic infants have a more balanced bile acid profile, which may be attributed to a higher abundance of *Bacteroidota* in their microbiome.

Metagenomics analysis showed a pattern of *Proteobacteria* in the microbiome of non-cholestatic infants with increases in stress management, protein misfold correction, and chemotaxis capabilities, as well as amino acid and carbohydrate metabolism. This, along with the noted increase in *Proteobacteria* in the cholestatic cohort, suggests that competition from a microbiome with more richness in non-cholestatic infants may place selective pressure on the survival of *Proteobacteria* with a greater ability for adaptation to stressful conditions, in which bacterial mobility and metabolism are emphasized. This may further reflect that non-cholestatic infants possess a more "resilient" microbiome that is better able to prevent uncontrolled proliferation of the phylum.

Preterm infants with cholestasis were noted to have feed-dependent increases of both *Proteobacteria* and *Streptococcus* with zinc transporters. Zinc metabolism has been a topic of research in premature infants, as they are at risk for deficiency given that the majority of accretion occurs during the third trimester of pregnancy.[44] Abnormalities in zinc metabolism are described in chronic liver disease, given that bile is an excretory route for zinc.[45] While one study of extrahepatic biliary atresia patients shows low hepatic zinc, which is in line with other chronic liver diseases, there have also been some reports of increased hepatic zinc content in a group of consanguineous older children with cholestatic liver disease, suggesting a potential genetic mechanism.[45,46] Existing literature on zinc and the human microbiome demonstrates an association between zinc deficiency and reduced gut microbiota biodiversity.[47] Our study is the first to demonstrate a relationship between zinc metabolism and the microbiome in preterm infants with cholestasis, which suggests a potential selective pressure on the gut microbiome either to promote increased need for zinc absorption in a cholestatic state or as a reflection of a selective advantage for microbes that are able to metabolize zinc given the presence of excess.

This study is the first of its kind to directly explore the relationship between the susceptibility of certain preterm infants to PNAC and the fecal microbiome, as well as fecal bile acid content. Limitations include the small cohort size, with twenty-two infants meeting criteria for study inclusion. Patients in the study ranged in gestational age from 24 to 30 weeks. Although the analysis controlled for gestational age, the authors additionally note that patients may have had inherent differences in baseline liver maturity and fecal microbiome content based on gestational age alone.

Analysis was also limited by the amount of fecal matter available for analysis, particularly for bile acid sampling, which is a common problem in research from preterm infants on PN who are prone to infrequent defecation due to low enteral feed volumes and ongoing critical illness. Although the results reached statistical significance, group differences for relatively rare bile acids in this patient population should be interpreted with caution. In particular, given the limited data on extremely preterm and term infant fecal bile acid content, validation with larger cohorts is recommended. Additionally, the presence of bile acids and/or microbes in the stool may not be reflective of metabolic utility, as they are eliminated in the stool. Regardless, the analysis of our small cohort showing statistically significant results demonstrates the large effect size of these specific associations. Additionally, replicable findings with different populations are promising and suggest a more global effect. Nonetheless, future studies in this important patient population will benefit from enrollment of larger and more diverse cohorts.

Although our patients were well-matched in terms of gestational age at birth, mode of delivery, sex, birth weight, and diagnosis of sepsis, infants with cholestasis had higher rates of antibiotic exposure and more diagnoses of NEC than non-cholestatic peers. NEC can contribute to cholestasis directly by impacting portal blood supply as well as through bacterial translocation into portal blood.[5] Antibiotic administration has also been implicated in cholestasis through alterations in bile acid metabolism via disruption in gut microbiota, although these associations are stronger for enteral antibiotics than parenteral.[48] Of the antibiotics with statistically significant differences—penicillins, gentamicin, vancomycin, and metronidazole—there is no significant literature published to date attributing their parenteral administration to neonatal cholestasis. Regardless, the significant differences in antibiotic exposure between groups may limit the external applicability of these findings.

Conclusions

This study makes an important connection between PNAC, the gut microbiome, fecal bile acid content, and fecal

metagenomic differences in premature infants. Specifically, the increase in *Bacteroidota* correlating with a relative increase in DCA in non-cholestatic infants may reflect better regulation of FXR signaling in these patients, which may in turn protect them from PNAC. Additionally, the increase of zinc transporters in cholestatic infant gut microbes represents an interesting area for future study into zinc metabolism in preterm infants.

Future studies will expand on this topic by investigating a larger cohort, including serum bile acid testing in conjunction with stool bile acid content. Expanded investigation into this arena may lay the foundation for microbiome or bile acid therapeutics in this vulnerable patient population. As microbiome analysis technology improves, there may be an opportunity for real-time microbiome analysis to predict PNAC risk in premature infants, thus facilitating earlier identification and treatment.

Acknowledgments

The authors are grateful for the contributions of the University of Chicago Duchossois Family Institute in specimen analysis.

Funding

This research is funded by grants from the National Institutes of Health (R01 HD105234) and the University of Chicago Science of Early Trajectories (SET) Center.

Conflict of interest

ECC has served as a consultant to legal firms and to Mead Johnson Nutrition. BA is an equity partner in Preeme+You, a B Corporation aimed at improving health equity in the neonatal intensive care unit via parent engagement. The other authors have no conflicts of interest related to this publication.

Author contributions

Study concept and design (ECC, KO, ESW, BA, RKA), acquisition of data (ESW, KO, MD, WCA), analysis and interpretation of data (ESW, KO, MD, BA, ECC), drafting of the manuscript (ESW, KO), critical revision of the manuscript for important intellectual content (KO, RKA, BA, ECC), administrative, technical, or material support (MD, WCA, KO), and study supervision (ECC, BA, KO). All authors have made significant contributions to this body of work and have approved the final manuscript.

Ethical statement

Participants were drawn from the MIND study, which is approved by the University of Chicago Institutional Review Board (IRB16-1431) and is conducted in accordance with both the Declarations of Helsinki and Istanbul (as revised in 2024). Written informed consent has been obtained.

Data sharing statement

The bile acid data used in support of the findings of this study are included within the supplementary information files accompanying this publication in the *Journal of Clinical and Translational Hepatology*. The raw sequencing data and de-identified metadata used in support of the findings of this study are available from the corresponding author at Eclaud@bsd.uchicago.edu upon request.

References

[1] Frazer LC, Gura KM, Bines JE, Puder M, Martin CR. Prevention and Management of Parenteral Nutrition-Associated Cholestasis and Intestinal Failure-Associated Liver Disease in the Critically Ill Infant. World Rev Nutr Diet 2021;122:379–399. doi:10.1159/000514763, PMID:34352771.
[2] Steinbach M, Clark RH, Kelleher AS, Flores C, White R, Chace DH, *et al*. Demographic and nutritional factors associated with prolonged cholestatic jaundice in the premature infant. J Perinatol 2008;28(2):129–135. doi:10.1038/sj.jp.7211889, PMID:18059467.
[3] Ogunrinola GA, Oyewale JO, Oshamika OO, Olasehinde GI. The Human Microbiome and Its Impacts on Health. Int J Microbiol 2020;2020:8045646. doi:10.1155/2020/8045646, PMID:32612660.
[4] Healy DB, Ryan CA, Ross RP, Stanton C, Dempsey EM. Clinical implications of preterm infant gut microbiome development. Nat Microbiol 2022;7(1):22–33. doi:10.1038/s41564-021-01025-4, PMID:34949830.
[5] Potter CJ. Cholestasis in the Premature Infant. Clin Perinatol 2020;47(2):341–354. doi:10.1016/j.clp.2020.02.009, PMID:32439115.
[6] Dahlgren AF, Pan A, Lam V, Gouthro KC, Simpson PM, Salzman NH, *et al*. Longitudinal changes in the gut microbiome of infants on total parenteral nutrition. Pediatr Res 2019;86(1):107–114. doi:10.1038/s41390-019-0391-y, PMID:30965357.
[7] Guo C, Li Y, Wang P, Li Y, Qiu C, Li M, *et al*. Alterations of Gut Microbiota in Cholestatic Infants and Their Correlation With Hepatic Function. Front Microbiol 2018;9:2682. doi:10.3389/fmicb.2018.02682, PMID:30483228.
[8] Zhou S, Wang Z, He F, Qiu H, Wang Y, Wang H, *et al*. Association of serum bilirubin in newborns affected by jaundice with gut microbiota dysbiosis. J Nutr Biochem 2019;63:54–61. doi:10.1016/j.jnutbio.2018.09.016, PMID:30342317.
[9] Chiang JYL, Ferrell JM. Bile Acid Metabolism in Liver Pathobiology. Gene Expr 2018;18(2):71–87. doi:10.3727/105221618X15156018385515, PMID:29325602.
[10] Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metab 2016;24(1):41–50. doi:10.1016/j.cmet.2016.05.005, PMID:27320064.
[11] Watkins JB, Szczepanik P, Gould JB, Klein P, Lester R. Bile salt metabolism in the human premature infant. Preliminary observations of pool size and synthesis rate following prenatal administration of dexamethasone and phenobarbital. Gastroenterology 1975;69(3):706–713. PMID:1158088.
[12] Tessier MEM, Shneider BL, Petrosino JF, Preidis GA. Bile acid and microbiome interactions in the developing child. J Pediatr Gastroenterol Nutr 2025;80(5):832–839. doi:10.1002/jpn3.70014, PMID:39959949.
[13] Neu J. Necrotizing enterocolitis: the search for a unifying pathogenic theory leading to prevention. Pediatr Clin North Am 1996;43(2):409–432. doi:10.1016/s0031-3955(05)70413-2, PMID:8614608.
[14] Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, *et al*. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems 2016;1(1):e00009-15. doi:10.1128/mSystems.00009-15, PMID:27822518.
[15] Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, *et al*. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6(8):1621–1624. doi:10.1038/ismej.2012.8, PMID:22402401.
[16] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13(7):581–583. doi:10.1038/nmeth.3869, PMID:27214047.
[17] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–857. doi:10.1038/s41587-019-0209-9, PMID:31341288.
[18] Murali A, Bhargava A, Wright ES. IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. Microbiome 2018;6(1):140. doi:10.1186/s40168-018-0521-5, PMID:30092815.
[19] Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, *et al*. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 2018;36(10):996–1004. doi:10.1038/nbt.4229, PMID:30148503.
[20] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215(3):403–410. doi:10.1016/S0022-2836(05)80360-2, PMID:2231712.
[21] Hsieh TC, Ma KH, Chao A. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol Evol 2016;7(12):1451–1456. doi:10.1111/2041-210X.12613.
[22] Holmes I, Harris K, Quince C. Dirichlet multinomial mixtures: generative models for microbial metagenomics. PLoS One 2012;7(2):e30126. doi:10.1371/journal.pone.0030126, PMID:22319561.
[23] Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2014;2:15. doi:10.1186/2049-2618-2-15, PMID:24910773.
[24] Gómez C, Stücheli S, Kratschmar DV, Bouitbir J, Odermatt A. Development and Validation of a Highly Sensitive LC-MS/MS Method for the Analysis of Bile Acids in Serum, Plasma, and Liver Tissue Samples. Metabolites 2020;10(7):282. doi:10.3390/metabo10070282, PMID:32660078.
[25] McIver LJ, Abu-Ali G, Franzosa EA, Schwager R, Morgan XC, Waldron L, *et al*. bioBakery: a meta'omic analysis environment. Bioinformatics 2018;34(7):1235–1237. doi:10.1093/bioinformatics/btx754, PMID:29194469.
[26] Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes.

Nucleic Acids Res 2000;28(1):27–30. doi:10.1093/nar/28.1.27, PMID:10592173.
[27] Novikova M, Metlitskaya A, Datsenko K, Kazakov T, Kazakov A, Wanner B, *et al*. The Escherichia coli Yej transporter is required for the uptake of translation inhibitor microcin C. J Bacteriol 2007;189(22):8361–8365. doi:10.1128/JB.01028-07, PMID:17873039.
[28] Mokha JS, Davidovics ZH, Maas K, Caimano MJ, Matson A. Fecal Microbiomes in Premature Infants With and Without Parenteral Nutrition-Associated Cholestasis. J Pediatr Gastroenterol Nutr 2019;69(2):224–230. doi:10.1097/MPG.0000000000002352, PMID:31058777.
[29] Pammi M, Cope J, Tarr PI, Warner BB, Morrow AL, Mai V, *et al*. Intestinal dysbiosis in preterm infants preceding necrotizing enterocolitis: a systematic review and meta-analysis. Microbiome 2017;5(1):31. doi:10.1186/s40168-017-0248-8, PMID:28274256.
[30] Liu L, Ao D, Cai X, Huang P, Cai N, Lin S, *et al*. Early gut microbiota in very low and extremely low birth weight preterm infants with feeding intolerance: a prospective case-control study. J Microbiol 2022;60(10):1021–1031. doi:10.1007/s12275-022-2180-2, PMID:35984614.
[31] Litvak Y, Byndloss MX, Tsolis RM, Bäumler AJ. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr Opin Microbiol 2017;39:1–6. doi:10.1016/j.mib.2017.07.003, PMID:28783509.
[32] Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol 2015;23:141–147. doi:10.1016/j.mib.2014.11.013, PMID:25576662.
[33] Gregory KE, LaPlante RD, Shan G, Kumar DV, Gregas M. Mode of Birth Influences Preterm Infant Intestinal Colonization With Bacteroides Over the Early Neonatal Period. Adv Neonatal Care 2015;15(6):386–393. doi:10.1097/ANC.0000000000000237, PMID:26551793.
[34] Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 2013;3(1):14–24. doi:10.3390/pathogens3010014, PMID:25437605.
[35] Lynch LE, Hair AB, Soni KG, Yang H, Gollins LA, Narvaez-Rivas M, *et al*. Cholestasis impairs gut microbiota development and bile salt hydrolase activity in preterm neonates. Gut Microbes 2023;15(1):2183690. doi:10.1080/19490976.2023.2183690, PMID:36843227.
[36] Belzer C, de Vos WM. Microbes inside—from diversity to function: the case of Akkermansia. ISME J 2012;6(8):1449–1458. doi:10.1038/ismej.2012.6, PMID:22437156.
[37] Mo C, Lou X, Xue J, Shi Z, Zhao Y, Wang F, *et al*. The influence of Akkermansia muciniphila on intestinal barrier function. Gut Pathog 2024;16(1):41. doi:10.1186/s13099-024-00635-7, PMID:39097746.
[38] Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, *et al*. Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. Gut 2018;67(5):891–901. doi:10.1136/gutjnl-2016-313432, PMID:28550049.
[39] Hagi T, Geerlings SY, Nijsse B, Belzer C. The effect of bile acids on the growth and global gene expression profiles in Akkermansia muciniphila. Appl Microbiol Biotechnol 2020;104(24):10641–10653. doi:10.1007/s00253-020-10976-3, PMID:33159542.
[40] Hagi T, Belzer C. The interaction of Akkermansia muciniphila with host-derived substances, bacteria and diets. Appl Microbiol Biotechnol 2021;105(12):4833–4841. doi:10.1007/s00253-021-11362-3, PMID:34125276.
[41] Yu L, Liu Y, Wang S, Zhang Q, Zhao J, Zhang H, *et al*. Cholestasis: exploring the triangular relationship of gut microbiota-bile acid-cholestasis and the potential probiotic strategies. Gut Microbes 2023;15(1):2181930. doi:10.1080/19490976.2023.2181930, PMID:36864554.
[42] Guzior DV, Quinn RA. Review: microbial transformations of human bile acids. Microbiome 2021;9(1):140. doi:10.1186/s40168-021-01101-1, PMID:34127070.
[43] Manithody CS, Van Nispen J, Murali V, Jain S, Samaddar A, Armstrong A, *et al*. Role of Bile Acids and Gut Microbiota in Parenteral Nutrition Associated Injury. J Hum Nutr (Carson City) 2020;4(1):286. doi:10.36959/487/286, PMID:32705087.
[44] Ackland ML, Michalczyk AA. Zinc and infant nutrition. Arch Biochem Biophys 2016;611:51–57. doi:10.1016/j.abb.2016.06.011, PMID:27317042.
[45] Bayliss EA, Hambidge KM, Sokol RJ, Stewart B, Lilly JR. Hepatic concentrations of zinc, copper and manganese in infants with extrahepatic biliary atresia. J Trace Elem Med Biol 1995;9(1):40–43. doi:10.1016/S0946-672X(11)80007-6, PMID:8846156.
[46] Phillips MJ, Ackerley CA, Superina RA, Roberts EA, Filler RM, Levy GA. Excess zinc associated with severe progressive cholestasis in Cree and Ojibwa-Cree children. Lancet 1996;347(9005):866–868. doi:10.1016/s0140-6736(96)91347-1, PMID:8622393.
[47] Skalny AV, Aschner M, Lei XG, Gritsenko VA, Santamaria A, Alekseenko SI, *et al*. Gut Microbiota as a Mediator of Essential and Toxic Effects of Zinc in the Intestines and Other Tissues. Int J Mol Sci 2021;22(23):13074. doi:10.3390/ijms222313074, PMID:34884881.
[48] Xiao Y, Zhou K, Lu Y, Yan W, Cai W, Wang Y. Administration of antibiotics contributes to cholestasis in pediatric patients with intestinal failure via the alteration of FXR signaling. Exp Mol Med 2018;50(12):1–14. doi:10.1038/s12276-018-0181-3, PMID:30504803.