



REVIEW

Cholestasis in Alcohol-Associated Liver Disease

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Alcohol-associated liver disease (ALD) is a leading cause of liver-related morbidity and mortality. ALD covers a spectrum of diseases, ranging from mild and reversible hepatic steatosis to the development of fibrosis, cirrhosis, and alcohol-associated hepatitis (AH). AH is marked by a rapid onset of jaundice and elevated serum levels of aspartate aminotransferase in individuals with heavy alcohol use. It can progress to acute-on-chronic liver failure, with a mortality rate of approximately 30% within the first month. Unfortunately, treatment options for AH are still limited. Cholestasis refers to an impairment in bile formation or flow, leading to clinical symptoms, such as fatigue, pruritus, and jaundice. Cholestasis and biliary dysfunction are commonly seen in patients with AH and can significantly worsen the prognosis. However, the mechanisms and roles of cholestasis in ALD are not yet fully understood. In this review, we will summarize recent findings and explore the potential roles and mechanisms of cholestasis in the progression of ALD. (*Am J Pathol* 2026, 196: 35–49; <https://doi.org/10.1016/j.ajpath.2025.04.015>)

As a leading cause of liver-related morbidity and mortality, alcohol-associated liver disease (ALD) covers a spectrum of diseases, ranging from mild and reversible hepatic steatosis to fibrosis, cirrhosis, and alcohol-associated hepatitis (AH).^{1–3} AH is characterized by a rapid onset of jaundice and elevated levels of serum aspartate aminotransferase in patients with heavy alcohol use.⁴ AH can progress to acute-on-chronic liver failure, resulting in both hepatic and extrahepatic organ failure, with a mortality rate of approximately 30% within the first month. Despite this severity, therapeutic options for AH remain limited. Cholestasis refers to an impairment in bile formation or flow, leading to clinical symptoms, such as fatigue, pruritus, and jaundice.⁵ Cholestasis and biliary dysfunction are commonly observed in patients with AH and can significantly worsen the prognosis.^{6–8} In this review, we will summarize recent evidence and explore the potential roles and mechanisms of cholestasis in the progression of ALD.

Bile Acid Homeostasis and Human Diseases

Bile Acid Homeostasis

Bile acids (BAs) are physiological detergents that facilitate the absorption and transport of lipids and vitamins in the intestine.⁹ In humans, BA synthesis is the main metabolic pathway for the catabolism of cholesterol. Cholesterol is converted to two primary BAs in humans, cholic acid (CA) and chenodeoxycholic acid (CDCA), the *de novo* synthesis

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of which requires 14 enzymes in the liver.⁹ Two major BA synthesis pathways have been identified in the liver, including a classic (neutral) BA biosynthetic pathway initiated by cytochrome P450 7A1 (CYP7A1) and an alternative (acidic) pathway initiated by CYP27A1.⁹ Most BAs are conjugated to glycine or taurine in the liver, a process that decreases their toxicity and increases their solubility for secretion into bile. This conjugation is facilitated by bile acid: CoA synthase and bile acid:amino acid transferase are involved.⁹ *De novo* BA synthesis is regulated by BA-activated farnesoid X receptor (FXR) in the liver and the ileum in a negative feedback manner.¹⁰ In the liver, activated FXR induces small heterodimer partner and represses CYP7A1 expression. While in the intestine, FXR activation induces the expression of fibroblast growth factor (FGF) 15/19, which inhibits CYP7A1 expression in the liver.

The gut microbiome (GM) plays a central role in the homeostasis of BA metabolism. Conjugated bile acids are deconjugated by bile salt hydrolases produced by GM. Intriguingly, recent studies show that bile salt hydrolases reshape the pool of BAs and are responsible for a mechanism of BA metabolism by the GM involving amino acid conjugation to the acyl site of BAs, thus producing a diverse suite of microbially conjugated BAs.^{11–13} These microbially conjugated BA products have antimicrobial properties, indicating the potential of these underappreciated metabolites to affect the microbiome and human health. Primary BAs, CA and CDCA, are dehydroxylated at the 7 α position and converted into secondary BAs, deoxycholic acid (DCA) and lithocholic acid, respectively, by microbial 7-dehydroxylation.^{14,15} CA, CDCA, and DCA are reabsorbed in the intestine and transported back to the liver to inhibit bile acid synthesis, whereas most of the lithocholic acid is excreted in feces.⁹ BAs, in turn, can reshape GM by their antimicrobial effects and indirect effects through FXR-induced antimicrobial peptides.^{16–19} A decrease in BA levels in the gut favors the growth of Gram-negative bacteria, which can include potential pathogens and cause the production of potent lipopolysaccharide (LPS).¹⁷ An increase of BA levels in the gut favors the growth of Gram-positive members of the *Firmicutes*, which include bacteria with 7-dehydroxylation capability, and leads to a conversion of primary BAs to secondary BAs.¹⁷

The enterohepatic circulation of BAs is critical for maintaining BA homeostasis. In humans, BAs are synthesized in the liver. Conjugated BAs are secreted into bile and stored in the gallbladder.⁹ The contraction of the gallbladder empties BAs into the intestine. Most BAs are reabsorbed in the ileum when passing through the intestinal tract. BAs are then transported back to the sinusoid of hepatocytes through portal blood circulation. In addition to the intestine, some BAs are reabsorbed by the renal tubules in the kidney and by the cholangiocytes.⁹ BA transporters are critical for maintaining the enterohepatic circulation of BAs.^{20,21} In the liver, BAs are transported across the sinusoidal membrane

primarily by the sodium-taurocholate cotransporting polypeptide, with assistance from members of the organic anion transporting polypeptide family. BAs are then secreted across the canalicular membrane in an ATP-dependent process via the bile salt export pump.^{20,21} In the ileum, the uptake of BAs from the intestinal lumen is mediated by the apical sodium-dependent bile acid transporter (ASBT/SLC10A2) located on the brush border of enterocytes.^{20,21} Inhibitors of ASBT have been developed as promising hypocholesterolemic or hepatoprotective agents, as they block BA reabsorption, reducing cholesterol levels and liver damage.²²

Bile Acids in the Development of Diseases

BAs are critical as metabolic regulators, nutrient sensors, and mediators of inflammation and cellular stress responses in the liver.^{23–26} The cytotoxicity of BAs is determined by their hydrophobicity.^{27,28} The bile acid pool in humans is more hydrophobic than in mice, which can lead to differential biological functions of BAs in humans and mice.²⁸ As the major organ that synthesizes and secretes BAs, the liver may be subject to damage following a dysfunction in BA homeostasis. In the clinic, cholestasis manifests with fatigue, pruritus, and jaundice caused by the impairment in bile formation or flow,⁵ which we will further discuss later. Interestingly, BAs have also been implicated in the development of thrombotic diseases,^{29,30} carcinogenesis,^{31,32} metabolic disorders,³³ cardiometabolic conditions,^{34,35} inflammatory bowel diseases,^{36,37} brain injuries in extremely premature infants,³⁸ bone destruction diseases,³⁹ and skeletal muscle function.⁴⁰ Furthermore, BAs and their metabolites play a key role in modulating host immune responses, contributing to the development of several of these diseases.^{41,42}

Cholestasis in Liver Disease

Cholestatic Liver Diseases

Cholestasis clinically presents with symptoms such as fatigue, pruritus, and jaundice, which result from impaired bile formation or flow.⁵ Cholestatic liver diseases arise in various clinical settings, with etiologies differing based on the anatomic location of the defect and the acuity of the presentation.⁵ Cholestasis is typically indicated by elevated serum alkaline phosphatase and γ -glutamyl transpeptidase levels, along with the development of hyperbilirubinemia.⁵ Acute cholestasis is primarily caused by conditions like common bile duct stenosis, cholangitis, drug-induced liver injury, sepsis, pregnancy, and total parenteral nutrition. Chronic cholestasis, on the other hand, is associated with disorders like primary sclerosing cholangitis, primary biliary cholangitis, secondary sclerosing cholangitis, biliary atresia, and various genetic conditions. Disruption of BA metabolism and intrahepatic circulation promotes the progression

of cholestatic liver diseases.⁴³ Importantly, acute cholestasis can sometimes progress to a chronic state, whereas chronic cholestasis can occasionally present with acute flare-ups.^{5,44}

Although cholestasis is a common feature in many liver diseases, therapeutic options remain limited and generally ineffective. Treatments for cholestatic liver diseases caused by hereditary transporter defects include ursodeoxycholic acid, primary bile acid replacement, biliary diversion, and liver transplantation.⁴⁵ A study demonstrated that synthetic human ATP-binding cassette B4 (also known as MDR2) mRNA could effectively rescue cholestatic liver disease caused by *Mdr2* deficiency in mice, highlighting gene therapy as a potential treatment for cholestatic liver disease.⁴⁶ Several drugs have shown potential in improving primary biliary cholangitis, including ursodeoxycholic acid, obeticholic acid, and certain immunosuppressive agents, with ursodeoxycholic acid being the most effective treatment.^{47,48} In contrast, treatments for primary sclerosing cholangitis have shown limited effectiveness.^{47,48}

Cholestasis in ALD

Impaired bile acid homeostasis and cholestasis are observed in both metabolic dysfunction–associated steatotic liver disease and ALD, two common chronic liver diseases.^{49,50} However, unlike in metabolic dysfunction–associated steatotic liver disease, mechanisms and roles of cholestasis in ALD remain understudied. Here, we will discuss potential mechanisms of cholestasis in the progression of ALD.

Cholestasis and biliary dysfunction are common in patients with AH⁶ and can worsen AH prognosis.^{7,8} When compared with control patients and patients with alcohol use disorder, patients with AH show a significant increase of both total and conjugated BAs in the serum, which is positively correlated with disease severity.⁵¹ The BA composition analysis shows significantly elevated levels of glycocholic acid, taurocholic acid, and taurochenodeoxycholic acid in patients with AH compared with those with alcohol use disorder and controls. Serum levels of BAs, including CDCA, DCA, glycodeoxycholic acid, and CA, are lower in patients with AH compared with those with alcohol use disorder and controls. Additionally, serum levels of certain BAs, such as hyodeoxycholic acid, taurodeoxycholic acid, and tauroolithocholic acid, are lower in patients with AH compared with control patients. The proportion of conjugated BAs is further analyzed by combining different conjugation types. Interestingly, serum levels of conjugated CA are significantly increased in patients with AH compared with those with alcohol use disorder and controls. In contrast, lower levels of conjugated DCA are observed in patients with AH compared with those with alcohol use disorder and controls. Serum levels of conjugated CDCA are increased in patients with AH compared with those in control patients. To examine the correlation between BA levels and disease severity, a univariate regression analysis is performed on the most abundant BAs

with the model for end-stage liver disease scores in patients with AH. Serum levels of total BAs, absolute conjugated BAs, and proportional conjugated BAs are positively correlated with the model for end-stage liver disease scores. In contrast, serum levels of unconjugated BAs (CDCA, CA, and DCA), as well as conjugated DCA, are negatively correlated with the model for end-stage liver disease scores. Given the critical role of GM in converting primary BAs to secondary BAs, the increased levels of conjugated primary BAs and the decreased levels of secondary BAs suggest a dysfunction in the gut-liver interaction of BA metabolism in patients with AH, which may be related to the severity of ALD.⁵¹

Similar findings have been reported in another study that investigated the effects of heavy alcohol consumption on BAs and the related microbiome.⁵² In this study, plasma and stool samples were collected from healthy individuals, heavy-drinking controls without overt liver disease, and patients with AH, to examine alcohol-induced changes in BAs and the microbiome, and to define their relationship to disease severity. An increased proportion of primary BAs and a decreased proportion of secondary BAs were observed in plasma samples from patients with AH. However, BA profile changes did not distinguish between moderate and severe AH in this study. Plasma levels of primary BAs (including glycocholic acid, taurocholic acid, taurochenodeoxycholic acid, and glychenodeoxycholic acid) were significantly elevated in patients with AH. Additionally, an increase in glucuronidated glychenodeoxycholic acid and sulfated glychenodeoxycholic acid was found in plasma samples from patients with AH. Consistent with the previously mentioned study,⁵¹ plasma levels of secondary BAs (including DCA, taurodeoxycholic acid, and glycodeoxycholic acid) were decreased in patients with AH. Interestingly, some other secondary BAs were increased in plasma samples from patients with AH, such as glycochenolic acid sulfate, taurochenolic acid sulfate, glycolithocholic acid, glycolithocholic acid 3-sulfate, tauroolithocholic acid 3-sulfate, and taurooursodeoxycholic acid. This study also indicated a decrease in isoursodeoxycholic acid levels in plasma from patients with AH.

Alterations in BA profiles, similar to those reported in previous studies, are also observed in patients with alcohol-associated cirrhosis and hepatitis compared with patients with alcohol-associated cirrhosis without severe hepatitis.⁵³ The BA profile alterations from these three studies focusing on BAs in patients with AH are summarized in Table 1. Although some discrepancies exist regarding changes in individual BAs, several BAs follow the same alteration patterns observed in the other two studies (Table 1). Patients with alcohol-associated cirrhosis and hepatitis exhibit a higher relative proportion of primary BAs, taurochenodeoxycholic acid, and taurooursodeoxycholic acid in plasma compared with patients with alcohol-associated cirrhosis without severe hepatitis. They also have a lower relative proportion of total secondary BAs, including DCA,

Table 1 Alteration of BA Profiles in Patients with AH

BA	Serum or plasma			Stool	
	AH/AUD ^{*51}	Cir_sAH/Cir_noAH ⁵³	AH/AUD ^{†52}	Cir_sAH/Cir_noAH ⁵³	AH/AUD ^{†52}
Total BA (abs.)	High	High	High	Low	N.S.
Conjugated BA (abs.)	High	High	High	N.S.	N.S.
Unconjugated BA (abs.)	N.S.	N.S.	N.S.	Low	Low
Conjugated BA	High	N.S.	High	N.S.	High
Unconjugated BA	Low	N.S.	Low	N.S.	Low
Taurine-conjugated BA	High	High	—	N.S.	—
Glycine-conjugated BA	High	N.S.	—	N.S.	—
Primary BA (abs.)	—	N.S.	High	N.S.	N.S.
Secondary BA (abs.)	—	N.S.	N.S.	Low	Low
Primary BA	—	High	High	High	High
Secondary BA	—	Low	Low	Low	Low
CA	Low	N.S.	N.S. (abs.)	High	N.S. (abs.)
CA-3-S	—	—	—	N.S.	—
T-CA	High	N.S.	High (abs.)	N.S.	N.S. (abs.)
G-CA	High	N.S.	High (abs.)	N.S.	N.S. (abs.)
G-CA-3-G	—	—	—	—	N.S. (abs.)
G-CA-3-S	—	—	High (abs.)	—	N.S. (abs.)
CDCA	Low	N.S.	N.S. (abs.)	High	N.S. (abs.)
CDCA-3-S	—	Low	—	N.S.	—
G-CDCA	—	N.S.	High (abs.)	N.S.	N.S. (abs.)
G-CDCA-3-G	—	—	High (abs.)	—	High (abs.)
G-CDCA-3-S	—	—	High (abs.)	—	High (abs.)
T-CDCA	High	High	High (abs.)	High	High (abs.)
DCA	Low	Low	N.S. (abs.)	Low	Low (abs.)
DCA-3-S	—	N.S.	—	N.S.	—
G-DCA	Low	Low	Low (abs.)	Low	Low (abs.)
G-DCA-3-S	—	—	N.S.	—	N.S. (abs.)
T-DCA	N.S.	Low	Low (abs.)	Low	Low (abs.)
LCA	—	Low	—	N.S.	Low (abs.)
LCA-3-S	—	Low	—	N.S.	—
G-LCA	—	Low	High (abs.)	N.S.	N.S. (abs.)
G-LCA-3-S	—	Low	High (abs.)	N.S.	N.S. (abs.)
T-LCA	N.S.	N.S.	—	N.S.	—
T-LCA-3-S	—	Low	High (abs.)	—	N.S. (abs.)
DH-LCA	—	—	—	—	Low (abs.)
UCA	—	—	—	—	N.S. (abs.)
UDCA	—	N.S.	N.S. (abs.)	N.S.	Low (abs.)
UDCA-3-S	—	N.S.	—	N.S.	—
Iso-UDCA	—	—	Low (abs.)	—	Low (abs.)
G-UDCA	—	N.S.	N.S. (abs.)	N.S.	N.S. (abs.)
G-UDCA-3-S	—	N.S.	—	N.S.	—
T-UDCA	—	High	High (abs.)	N.S.	N.S. (abs.)
G-C-3-S	—	—	High (abs.)	—	High (abs.)
T-C-3-S	—	—	High (abs.)	—	High (abs.)
HDCA	N.S.	—	—	—	—
C4	Low (abs.)	—	—	—	—
7-HOCA	—	—	High (abs.)	—	—

*Comparison of the proportion of total bile acids, except where noted as absolute values.

†Comparison of each individual bile acid is based on absolute values.⁵²

—, Not analyzed in the original article; 7-HOCA, 7 α -hydroxy-3-oxo-4-cholestenoic acid; abs., absolute; AH, patients with alcohol-associated hepatitis; AUD, patients with alcohol use disorder; BA, bile acid; C4, 7 α -hydroxy-4-cholesten-3-one; CA, cholic acid; CA-3-S, cholic acid 3-sulfate; CDCA, chenodeoxycholic acid; CDCA-3-S, chenodeoxycholic acid 3-sulfate; Cir_noAH, patients with alcohol-associated cirrhosis without severe hepatitis; Cir_sAH, patients with alcohol-associated cirrhosis and hepatitis; DCA, deoxycholic acid; DCA-3-S, deoxycholic acid 3-sulfate; DH-LCA, dehydrolithocholic acid; G-C-3-S, glycochenolic acid sulfate; G-CA, glycocholic acid; G-CA-3-G, glycocholic acid 3-glucuronide; G-CA-3-S, glycocholic acid 3-sulfate; G-CDCA, glycochenodeoxycholic acid; G-CDCA-3-G, glycochenodeoxycholic acid 3-glucuronide; G-CDCA-3-S, glycochenodeoxycholic acid 3-sulfate; G-DCA, glycodeoxycholic acid; G-DCA-3-S, glycodeoxycholic acid 3-sulfate; G-LCA, glycolithocholic acid; G-LCA-3-S, glycolithocholic acid 3-sulfate; G-UDCA, glyoursodeoxycholic acid; G-UDCA-3-S, glyoursodeoxycholic acid 3-sulfate; HDCA, hyodeoxycholic acid; Iso-UDCA, isoursodeoxycholic acid; LCA, lithocholic acid; LCA-3-S, lithocholic acid 3-sulfate; N.S., not significant; T-C-3-S, taurochenolic acid sulfate; T-CA, taurocholic acid; T-CDCA, taurochenodeoxycholic acid; T-DCA, taurodeoxycholic acid; T-LCA, tauroolithocholic acid; T-LCA-3-S, tauroolithocholic acid 3-sulfate; T-UDCA, taoursodeoxycholic acid; UCA, ursocholic acid; UDCA, ursodeoxycholic acid; UDCA-3-S, ursodeoxycholic acid 3-sulfate.

glycodeoxycholic acid, and taurodeoxycholic acid, than patients with alcohol-associated cirrhosis without severe hepatitis. The discrepancies observed in this study occurred mainly due to the absence of healthy controls in this cohort. Indeed, one study has shown that serum BA levels are elevated in patients with alcohol-associated cirrhosis,⁵⁴ suggesting that BA homeostasis is altered in patients with alcohol-associated cirrhosis and can be further modified by AH.

In summary, serum and plasma BA levels are generally comparable between healthy controls and patients with alcohol use disorder in most studies. However, increased BA levels in the blood are a distinctive change observed in patients with AH. Therefore, modulating BA homeostasis or signaling could be a promising therapeutic approach for these patients.

Gut-Liver Axis in ALD

The gut-liver axis significantly contributes to the pathophysiology of ALD (Figure 1).^{55–57} GM is responsible for BA deconjugation and the conversion of primary BAs to secondary BAs.⁴⁸ Alcohol consumption causes gut dysbiosis and the dysfunction of BA metabolism in both mouse models⁵⁸ and patients with AH.^{51,59,60} Recent findings show that patients with AH have increased fecal numbers of *Enterococcus faecalis*, and the presence of cytolyisin-positive (cytolytic) *E. faecalis* is correlated with

the severity of liver disease and mortality in patients with AH.⁶⁰ Mice harboring the GM from a patient with severe AH develop more severe liver inflammation, higher liver necrosis, and greater intestinal permeability than mice harboring the GM from a patient with ALD without AH.⁶¹ GM-based therapeutic methods have shown encouraging effects on ALD.^{62–64} Increased intestinal permeability can cause endotoxemia, thereby contributing to alcohol-associated liver damage.^{65,66} Ductular reaction with inflammatory profile and infiltrated neutrophils is commonly observed in liver histology from patients with AH.^{67–70} Hepatocyte dedifferentiation and impaired hepatocyte regeneration may also contribute to hepatocellular failure in patients with AH.^{71,72} Endotoxemia in patients with AH is associated with the dysfunction of the type 3 inositol trisphosphate receptor in cholangiocytes,⁷³ suggesting a potential role for gut dysbiosis in alcohol-associated cholangiopathy. Although evidence has suggested a link between gut dysbiosis and AH, how alcohol-induced gut dysbiosis contributes to cholestasis and AH remains unclear. Recent evidence supports a link between hepatic immune response and damage to the intrahepatic bile duct. Although alterations in macrophages^{74,75} and neutrophils⁶⁹ can contribute to the development of biliary injury, the extent to which dysbiosis has a role in hepatic immune response, thereby causing damage to the intrahepatic bile duct in the setting of ALD, remains unclear and requires further investigation.

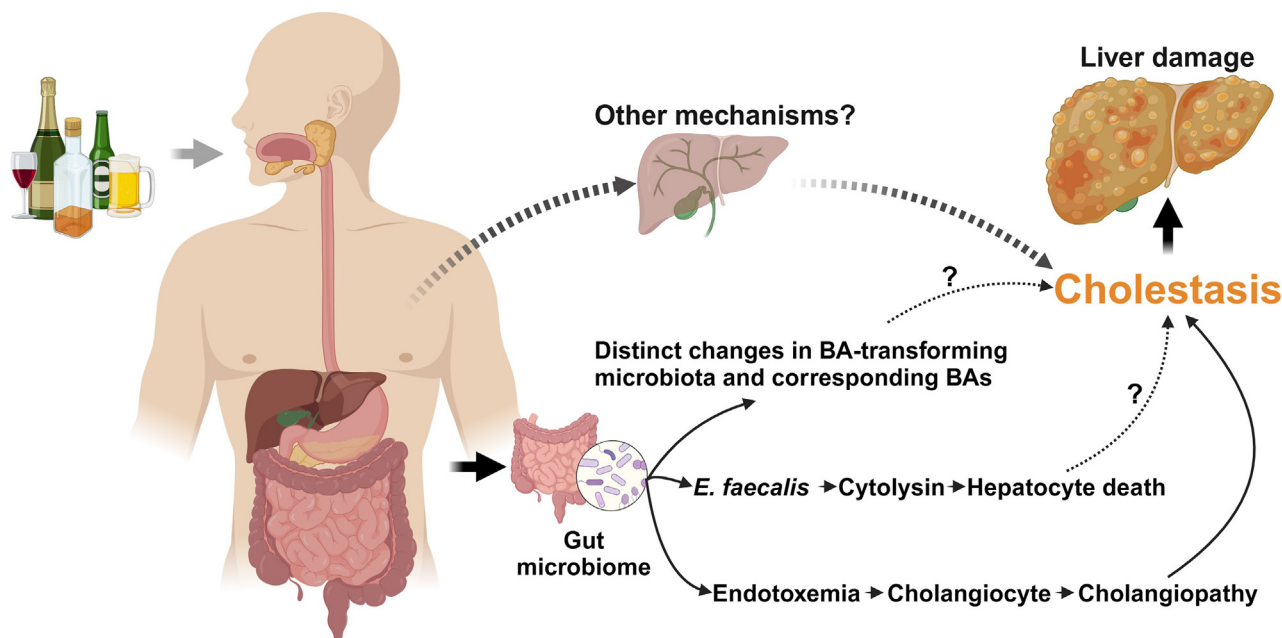


Figure 1 Cholestasis and alcohol-associated liver disease (ALD). Cholestasis is frequently observed in patients with ALD, particularly those with alcohol-associated hepatitis. However, the underlying mechanisms of cholestatic liver injury in ALD remain underexplored. Studies suggest that an altered gut microbiome may disrupt bile acid (BA) homeostasis, and bacterial products can directly cause hepatocyte death and cholangiocyte dysfunction, indicating that the gut microbiome is critical in the development of severe ALD. Nonetheless, further investigations are required to elucidate the mechanisms and role of cholestasis in ALD. This figure was generated in BioRender.com (Toronto, ON, Canada). *E. faecalis*, *Enterococcus faecalis*.

Given the critical role of the gut-liver interaction in BA homeostasis, BAs in stool samples from patients were analyzed in previously reported studies.^{52,53} An increased proportion of primary BAs (particularly taurochenodeoxycholic acid) and a decreased proportion of secondary BAs (particularly DCA, glycodeoxycholic acid, and taurodeoxycholic acid) were observed in stool samples from patients with AH (Table 1),^{52,53} suggesting that the GM plays a critical role in BA alterations in patients with AH. Indeed, all differentially abundant BA-metabolizing taxa were decreased in alcohol-associated hepatitis compared with heavy-drinking controls, except *Fusobacteriaceae Fusobacterium*, which was increased.⁵² Concordant with the reduction in DCA, there was a reduction in *Clostridium* cluster XIVa, including the species *Clostridium hylemonae* and *Clostridium scindens*, which are known to be critically involved in the 7 α -dehydroxylation of CA to DCA.

Preclinical Models regarding Cholestatic Liver Injury in ALD

The most widely used murine models in ALD research have limited features of AH. Several pathologic changes in livers from patients with AH, such as Mallory-Denk bodies, bilirubinostasis, and ductular reaction, are rarely recapitulated in murine models for ALD.^{6,76} These limitations have significantly curbed research related to AH. The species difference between humans and rodents in the hydrophobicity of BAs may contribute to the differential response to alcohol and the development of AH in rodent ALD models. Recent findings have demonstrated that CYP2C70 contributes to species differences in BA metabolism between mice and humans.⁷⁷ Deletion of *Cyp2c70* in mice causes increased BA hydrophobicity and biliary injury,^{78,79} suggesting humanized BA composition can cause liver pathologic changes in mice. Given the rising concerns of cholestasis during the progression of ALD, several studies have reported murine models for alcohol-associated cholestasis. One previous study showed that mice primed with a diet containing 3,5-diethoxycarbonyl-1,4-dihydrocollidine (0.05% w/w) for 8 weeks and then fed with a high-fat liquid diet containing ethanol via a gastric feeding tube⁸⁰ exhibited various pathologic changes of human AH, including fibrosis, neutrophil infiltration, and ductular reaction. However, the duration of the treatment (12 weeks in total) and the need for permanent intragastric intubation significantly limit the application of this model in most research labs. Another study challenged mice who underwent bile duct ligation with one acute ethanol binge and found that ethanol induced acute-on-chronic liver failure in bile duct ligation mice with features including liver damage and systemic inflammation.⁸¹ This study suggested that alcohol can promote existing cholestatic liver disease, yet the model itself may not help to understand the underlying mechanisms of cholestasis in patients with AH. Cholestatic

features are also observed in ethanol-fed mice given fructose and multiple doses of LPS.⁶⁹ Although intriguing, a limitation of the study was that results from pair-fed mice with LPS treatment were not published, raising concerns about the effects of LPS. Several genetic murine models have phenotypes similar to human AH following ethanol treatment, such as mice with intestine-specific FXR deficiency.⁸² However, because these mice harbor gene mutations, they have limited application for the screening of therapeutic targets for AH.

To study the interaction of cholestasis and alcohol, chronically ethanol-fed mice were challenged with a subtoxic dose of α -naphthylisothiocyanate (ANIT), a well-studied intrahepatic cholestasis inducer.⁸³ Intriguingly, ANIT synergistically enhanced liver injury following a 4-week ethanol feeding with several features of AH, including increased levels of alanine aminotransferase, aspartate aminotransferase, and total bile acids in the serum, and cholestasis, neutrophil infiltration, and accumulation of neutrophil extracellular traps in the liver. In another set of studies, following a 10-day ethanol feeding, only female mice showed more severe liver injury after additional ANIT challenge, indicating that a longer term of ethanol feeding is critical for biliary injury and cholestasis in mice. The expression of multiple genes was uniquely altered in the livers of ethanol/ANIT-treated mice, as shown by RNA-sequencing results. Functions of these differentially expressed genes showed an enrichment of genes related to inflammatory response. Indeed, depletion of neutrophils by anti-lymphocyte antigen 6 complex locus G6D antibody reduced liver injury in ethanol/ANIT-treated mice, suggesting that neutrophil-related inflammation is critical for alcohol-associated cholestatic liver injury in mice. Furthermore, dissolving neutrophil extracellular traps with DNase I also reduced liver damage in mice, indicating that extracellular DNA may play a role in hepatocellular cell death in alcohol-associated cholestatic liver injury in mice. Taken together, these data showed that cholestatic insult triggers alcohol-associated hepatitis in mice. The experimental evidence from this study also supports the hypothesis that cholestasis may be critical for the progression of AH. Although this treatment scheme has limitations in fully replicating acute-on-chronic liver damage in AH, the combined administration of ethanol and ANIT in mice exhibits biochemical, histologic, and molecular characteristics that closely resemble those observed in patients with AH. This suggests that this model may be valuable for understanding the pathogenesis of alcohol-associated cholestasis and hepatitis.

Potential Therapeutic Approaches for AH by Targeting Cholestasis

Bile Acid—Mediated Signaling Pathways

BAs can directly activate several nuclear receptors, including FXR, pregnane X receptor (PXR), constitutive

androstane receptor (CAR), and vitamin D receptor (VDR), as well as G-protein–coupled receptors, including G protein bile acid receptor-1 (TGR5) and sphingosin-1-phosphate receptor 2 (S1PR2).^{10,26,84,85} FXR and TGR5 are two major BA receptors.^{85,86} Functionally, TGR5 mediates the regulation of intermediary metabolism by BAs, whereas FXR is critical for the synthesis and homeostasis of BAs.^{87,88} Studies have shown that BA-mediated signaling can be involved in the development of ALD. In this section, we will discuss the main BA-mediated signaling pathways as potential therapeutic targets for ALD.

FXR

FXR is critical for BA homeostasis. The role of FXR in ALD has been well recognized both in transgenic mice^{89,90} or in mice treated with FXR agonists.^{58,91} Evidence from transgenic mice has shown that the roles of FXR in ALD are organ-specific. Either constitutive knockout or intestine-specific knockout of FXR in mice results in enhanced liver damage following ethanol feeding.^{82,92,93} Constitutive FXR knockout promotes alcohol-induced liver damage by increasing hepatic steatosis, changing BA profiles, altering ethanol metabolism, and increasing oxidative stress in a chronic-plus-binge ALD mouse model.^{92,93} Overexpression of FXR can prevent alcohol-induced steatosis in mouse livers.⁹⁴ Interestingly, liver-specific deletion of FXR has limited effects on alcohol-induced liver damage, suggesting that FXR in the liver is dispensable in the progression of ALD.⁹⁵ Intestinal FXR is critical for BA homeostasis. Indeed, deletion of FXR in the intestine increases gut permeability and significantly enhances alcohol-induced liver damage.⁸² The protective role of FXR in ALD is also verified by BA derivative obeticholic acid⁹¹ or intestine-restricted FXR agonist fexaramine.⁵⁸ Mechanistic studies suggest that FXR-induced FGF15/19 may be critical, which will be discussed later. Although the underlying mechanisms remain unclear, evidence from transgenic mice suggests that the gut-liver axis contributed by intestinal FXR plays a protective role in the development of ALD, which can be an intriguing target for ALD therapies.

TGR5

TGR5 is a bile acid receptor expressed in multiple organs and nonparenchymal liver cells but not expressed in hepatocytes.⁹⁶ TGR5 plays critical roles in modulating metabolic homeostasis and inflammatory response through intracellular cAMP-activated pathways and suppression of NF- κ B signaling, respectively.⁹⁶ TGR5 agonists protect mice from liver injury, steatosis, and inflammation in two models (3-day binge model and prolonged Lieber-DeCarli diet for 12 days) of early ALD,⁹¹ suggesting TGR5 activation can be beneficial to ALD. However, discrepant evidence has been reported in *Tgr5*-knockout mice following ethanol treatments. *Tgr5*-knockout mice following 10-day ethanol feeding plus one binge show less liver damage and metabolic alterations,^{96,97} which may be related to enhanced

leptin and FGF21 signaling as well as BA-mediated activation of brown adipose tissues. However, in another study TGR5 deficiency enhanced ALD in mice following a 2-week ethanol feeding without binge.⁹⁸ This study also showed that gut dysbiosis and altered BA profiles may contribute to ethanol-induced liver damage in *Tgr5*-knockout mice. Given the different treatments and backgrounds of *Tgr5*-knockout mice used in these studies, the role of TGR5 in ALD may vary from different ethanol treatments and genetic backgrounds of mice, which requires further investigation.

S1PR2

S1PR2 is responsible for the activation of extracellular signal-regulated kinases 1/2 and protein kinase B (also known as AKT) by conjugated BAs in primary rodent hepatocytes.⁹⁹ Deletion of S1PR2 or JTE-013 treatment, a specific antagonist of S1PR2, can reduce cholangiocyte proliferation and cholestatic injury in a bile duct ligation mouse model.¹⁰⁰ Activated S1PR2 is a key regulator of hepatic sphingosine kinase 2.¹⁰¹ Ethanol-fed sphingosine kinase 2-knockout mice exhibit more severe liver damage accompanied by an up-regulation of proinflammatory mediators and a more severe intestinal barrier dysfunction,¹⁰² suggesting that sphingosine lipid signaling may play a role in ALD. Interestingly, sphingosine-1-phosphate/S1PR1 signaling is required for the development of ALD mediated by gut type 17 helper T cells, and *in vivo*, intervention blocking of sphingosine-1-phosphate/S1PR1 signaling improves alcohol-induced liver inflammation, steatosis, and damage.¹⁰³ Overall, sphingosine lipid signaling may play distinct roles in a receptor-dependent manner in ALD, and further studies are required to develop novel therapeutic strategies by targeting sphingosine lipid signaling for ALD.

PXR

Predominantly expressed in the liver and intestine, PXR is known as a master regulator of xenobiotic and endobiotic metabolism, as well as having a regulatory effect on cholesterol and lipid homeostasis.¹⁰⁴ PXR modulates the expression of genes related to BA metabolism (eg, suppressing CYP7A1 expression) and BA transporters (eg, enhancing multidrug resistance-associated protein 2 and organic anion transporting polypeptide 2 expression).^{85,105} PXR also stimulates CYP3A expression and the formation of hydroxylated BAs, which are excreted from the body in the urine. Unlike FXR, PXR cannot be activated efficiently by CA, CDCA, or their conjugated derivatives. However, both the mouse and human PXR can be activated efficiently by LCA and its 3-keto metabolite.^{85,105} *Pxr*-knockout mice display more severe liver histology when subjected to various models of cholestasis and/or BA overload,¹⁰⁶ indicating a critical role of PXR in cholestatic liver injury.

Animal studies reveal sex differences in the role of PXR in ALD. Male mice present a significant increase of PXR expression in the liver following 8-week ethanol feeding.¹⁰⁷

Male PXR-deficient mice are protected from hepatic steatosis induced by either chronic ethanol feeding¹⁰⁷ or acute ethanol binge.¹⁰⁸ Another study using a chronic-plus-binge ALD model in male mice shows that the PXR agonist pregnenolone-16 α -carbonitrile can induce hepatic expression of fatty acid-binding protein 4 and exacerbate ALD in a PXR-dependent manner.¹⁰⁴ Treatment with andrographolide, a natural compound and dual inhibitor of PXR and fatty acid-binding protein 4, can improve ALD in male mice. These studies suggest that PXR may contribute to the development of ALD in male mice.

Controversially, Kupffer cells activated by gut-derived endotoxin contribute to the down-regulation of PXR and CYP3A11 expression in female ICR mouse livers following chronic ethanol feeding.¹⁰⁹ Female PXR-deficient mice are resistant to chronic ethanol-induced hepatotoxicity.¹¹⁰ In mice carrying human PXR, females are less susceptible to hepatotoxicity induced by an acute ethanol binge.¹¹¹ This may be related to the relative suppression of cellular stress and the enhanced expression of enzymes involved in both ethanol metabolism and hepatocyte proliferation and repair in female transgenic mice. Taken together, PXR may be a potential therapeutic target for ALD with potential limitations caused by sex differences. However, because current studies lack information regarding the impact of PXR on ALD-related BA homeostasis, the role of PXR in ALD-related cholestasis remains unclear.

CAR

CAR functions together with PXR as a sensor and detoxifier for endobiotics and xenobiotics.¹¹² Several BAs activate CAR, including CA, 6-keto-LCA, and 12-keto-LCA.⁸⁵ Although BA levels are not altered in *Car*-knockout mice compared with wild-type mice, these mice are more susceptible to cholestatic liver damage with altered expression of genes related to BA metabolism in the liver,^{113,114} indicating CAR can protect mice from cholestatic liver damage by altering BA metabolism. *Car*-knockout mice display more severe liver damage following chronic ethanol feeding, suggesting that the basal level of CAR activity is critical for protecting mice from ethanol-induced liver damage.¹¹² However, the same study also shows that pre-activation of CAR by a CAR agonist, TCPOBOP (1,4-bis [2-(3,5-dichloropyridyloxy)]benzene), promotes liver damage following either chronic or acute ethanol treatment in wild-type, but not in CAR-deficient, mice. Currently available evidence suggests that the role of CAR in ALD is complex, and CAR-activating chemicals may promote ALD. Additionally, whether and how CAR interacts with BAs in the context of ALD remains unclear.

VDR

VDR expression is low in hepatocytes but high in non-parenchymal cells, such as sinusoidal endothelial, Kupffer, and stellate cells.¹¹⁵ VDR can be activated by LCA and 3-keto-LCA,^{85,116} and may play a role in BA synthesis.¹¹⁷

Secondary BAs, like DCA and LCA, can increase bone marrow myelopoiesis possibly through VDR-dependent pathways,¹¹⁸ suggesting that VDR activation by BAs may modulate immune responses. Indeed, loss of VDR induces spontaneous liver inflammation in mice, but activation of VDR suppresses LPS- and obesity-induced inflammation in hepatic macrophages,¹¹⁹ suggesting that macrophage VDR plays a critical role in inflammation. Vitamin D deficiency is common in patients with alcohol-associated cirrhosis.¹²⁰ Decreased 25-hydroxyvitamin D levels are correlated to increased liver damage and mortality in ALD, indicating that vitamin D might be both a biomarker of severity and a potential therapeutic target in ALD.¹²¹ Indeed, one study shows that 1,25-dihydroxyvitamin D₃ treatment improves liver injury in rats following 8-week ethanol treatment in drinking water.¹²² Another study also shows that 1,25-dihydroxyvitamin D₃ treatment can protect mice from ethanol-induced intestinal barrier injury.¹²¹ These results suggest that VDR may be a potential therapeutic target for ALD. Interestingly, VDR genetic polymorphisms are significantly associated with the occurrence of hepatocellular carcinoma in alcoholic cirrhosis.¹²³ There is still a lack of direct evidence regarding the role of VDR in ALD and how BAs contribute to VDR-related mechanisms in ALD, which requires further study.

Modulation of Gut Signaling and Reabsorption of Bile Acids

Gut signaling and reabsorption of BAs are critical for maintaining the level of enterohepatic BAs. Several studies have revealed that modulation of FGF15/19 level and BA reabsorption can alter the pathology of ALD in animal models.

FGF15/19

Levels of serum FGF19 and bile acids are significantly increased in patients with AH, but the *de novo* BA synthesis is suppressed,^{51,124} suggesting that FGF19 expression and BA metabolism are altered in AH (Figure 2). Different from human studies, the expression of FGF15, a mouse FGF19 ortholog, is reduced in mice following 8-week ethanol feeding.⁵⁸ Overexpression of a nontumorigenic FGF19 variant using adeno-associated viruses provides beneficial metabolic effects and ameliorates steatohepatitis in mice when the viruses are given 3 weeks after ethanol feeding was started. The beneficial role of FGF15 in ALD is also shown in enterocyte-specific *Fgf15* knockout mice.¹²⁵ Following a chronic-plus-binge ethanol feeding, these mice exhibit higher levels of liver injury and steatosis, suggesting that FGF15 may prevent the development of ALD in mice. Interestingly, another study showed that overexpression of FGF19 enhances liver inflammation and gut dysbiosis in female mice following chronic-plus-binge ethanol feeding.¹²⁶ Evidence from animal models suggests that FGF19 may play distinct roles at different stages of ALD, which requires further investigation.

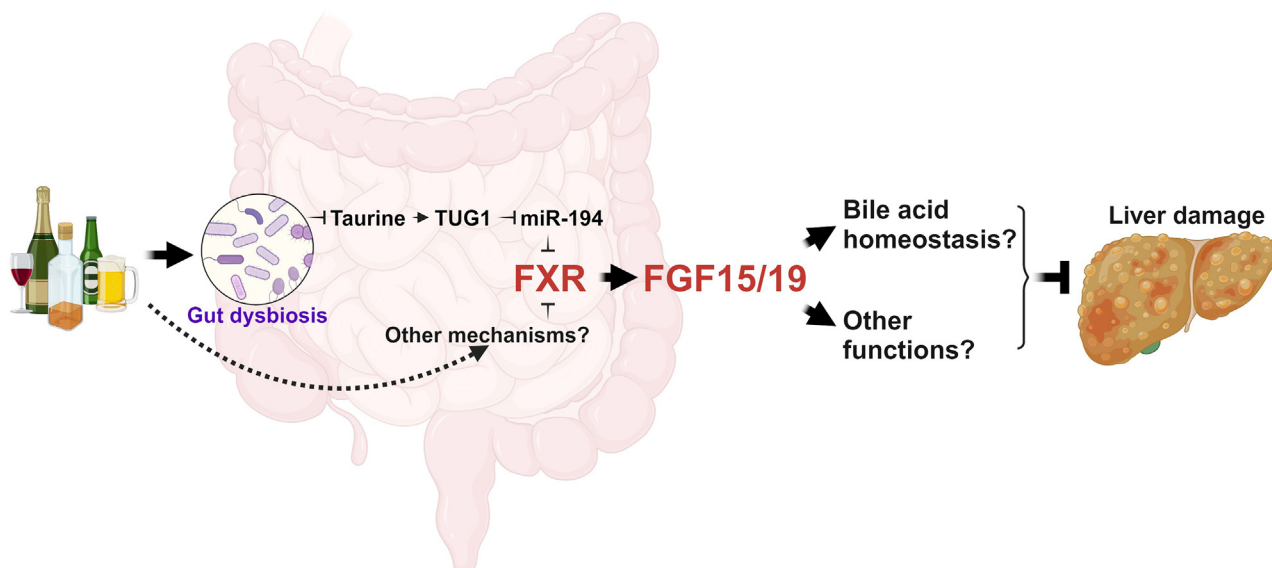


Figure 2 Intestinal farnesoid X receptor (FXR) serves as a crucial link between the gut and liver in alcohol-associated liver disease (ALD). The suppression of intestinal FXR activity has been extensively studied in animal models of ALD. Deletion of intestinal FXR or fibroblast growth factor 15 (FGF15) exacerbates ethanol-induced liver damage in mice, whereas activation of intestinal FXR or overexpression of FGF15 confers protection against such damage. Gut dysbiosis following alcohol consumption decreases taurine metabolism and the expression of taurine—up-regulated gene 1 (TUG1) in the intestine. TUG1 inhibits the expression of miR-194, which, in turn, suppresses FXR expression. Reduced TUG1 levels in ALD lead to increased miR-194 expression and a consequent reduction in the FXR-FGF15 signaling pathway. Although FGF15 protects mice from ethanol-induced liver damage, its human homolog, FGF19, is elevated in patients with alcohol-associated hepatitis. This discrepancy necessitates further investigation into the role of FGF15/19 in ALD. Moreover, the mechanisms by which alcohol consumption alters the FXR-FGF15/19 pathway and how FGF15/19 affects the pathogenesis of ALD are still largely unknown. This figure was generated in BioRender.com (Toronto, ON, Canada).

ASBT Inhibitors and Bile Acid Sequestrants

ASBT plays a critical role in the reabsorption of BAs from the gut. A gut-restricted ASBT inhibitor, GSK2330672, attenuates liver injury and steatosis in a chronic-plus-binge ALD mouse model.¹²⁷ Reducing BA reabsorption by BA sequestrants also shows beneficial impacts on ALD in animal models. Cholestyramine improves hepatic inflammation, cell death, endoplasmic reticulum stress, and autophagy dysfunction by reducing hepatic BA levels and lowering toxic BA levels in mice following 8-week ethanol feeding.¹²⁸ In another study, germ-free mice were colonized with feces from patients with AH and subjected to a chronic-plus-binge ethanol feeding model.¹²⁹ Colesevelam, another BA sequestrant, was given to mice. Interestingly, hepatic levels of triglycerides and cholesterol were reduced, but not liver injury and inflammation, in mice given colesevelam compared with non-treated mice. These studies suggest that modulation of the reabsorption of BAs from the gut may be beneficial to ALD.

Modulation of Gut Microbiome

Altered GM is observed in both heavy drinkers and patients with ALD.^{55–57} Studies have shown a reduction in *Bacteroidetes* and an elevation in *Proteobacteria* in heavy drinkers, regardless of ALD status, when compared with healthy controls. Patients with alcohol-associated cirrhosis

and AH are typically associated with an increase in *Enterococcus*, *Streptococcaceae*, *Enterobacteriaceae*, *Bifidobacterium*, and *Lactobacillus*, as well as a reduction in *Lachnospiraceae* and *Ruminococcaceae*. Emerging evidence has demonstrated the benefits of GM-based therapeutic methods in improving the pathogenesis of ALD.^{62–64,130,131} Here, we will discuss recent studies regarding the impacts of modulating GM on ALD in experimental models and patients.

Probiotics

Giving probiotics to patients with ALD for 7 days reduces liver injury markers compared with patients under standard treatments.¹³² Patients with ALD show a significantly reduced number of *Bifidobacterium*, *Lactobacillus*, and *Enterococcus*, as determined by colony-forming units per gram of stool. In patients receiving 5 days of *Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3, all these genera are restored. Recent studies in animal models have shown that this beneficial effect may be related to the activity of FXR. *Lactobacillus reuteri* treatment reduces liver injury and steatosis in a chronic-plus-binge ALD mouse model.¹³³ Transcriptomic analysis suggests that the beneficial effects of *L. reuteri* are likely related to activated FXR, and an FXR inhibitor, glycine- β -muricholic acid, can reduce the effect of *L. reuteri*. Supplementation of another *Lactobacillus*, *Lactobacillus rhamnosus* GG, increases intestinal FXR-FGF15 signaling pathway and prevents cholestatic

liver injury in mice.¹³⁴ In ALD, *L. rhamnosus* GG and *L. rhamnosus* GG-derived exosome-like nanoparticles protect mice from ethanol-induced liver injury and steatosis.^{62,135} One possible mechanism is that bacterial aryl hydrocarbon receptor ligand-enriched *L. rhamnosus* GG-derived exosome-like nanoparticles increase regenerating family member 3 and nuclear factor, erythroid derived 2, like 2 expression and improve barrier function in mice.⁶² Additionally, *L. rhamnosus* GG-derived exosome-like nanoparticles can also recover the intestinal FXR-FGF15 signaling pathway by reducing intestinal miR-194, the latter of which requires gut microbiota regulation of taurine metabolism and taurine-up-regulated gene 1 up-regulation (Figure 2).¹³⁵ Taken together, these studies suggest that probiotic supplementation has protective roles in ALD, likely through modulating BA homeostasis and gut-liver axis.

Prebiotics

Prebiotics are nondigestible food components that nourish beneficial microorganisms, helping to maintain healthy GM. Dietary fibers are the primary prebiotic components, and recent studies have shown that they may protect against the development of ALD. Pectin improves ALD and BA homeostasis in ethanol-fed mice transplanted with feces from patients with AH.¹³⁶ Pectin lowers BA levels and increases the proportion of hydrophilic BA in the plasma and liver, which are linked to altered GM and FXR signaling. Soluble fibers (inulin and oligofructose mixture) improve ALD in mice following chronic-plus-binge ethanol feeding.¹³⁷ Reduced liver damage and increased abundance of *Bacteroides acidifaciens* are observed in ethanol-fed mice given a diet rich in soluble dietary fibers when compared with ethanol-fed mice given a regular liquid diet or a diet rich in insoluble dietary fibers, suggesting that soluble dietary fibers can protect mice against ALD through GM. This study also shows that *B. acidifaciens* contributes to BA deconjugation, thereby activating intestinal FXR and inducing FGF15 expression. FGF15 promotes the expression of ornithine aminotransferase in hepatocytes and facilitates the metabolism of ornithine into glutamate in the liver. The increase of hepatic glutamate improves ammonia detoxification. Taken together, these studies suggest that the supplementation of prebiotics, like soluble dietary fibers, is protective against the development of ALD by GM and BA-mediated signaling pathways.

Bacteriophages

Phage therapy targeting GM can improve the pathology of gastrointestinal diseases in animal models.^{138,139} High alcohol-producing *Klebsiella pneumoniae* in the intestinal microbiome can be one of the causes of steatohepatitis.¹⁴⁰ Interestingly, phage therapy targeting high alcohol-producing *K. pneumoniae* can alleviate liver pathology and reduce the expression of cytokines and lipogenic genes caused by high alcohol-producing *K. pneumoniae* in male

mice. The presence of cytolysin-positive (cytolytic) *E. faecalis* is correlated with the severity of liver disease and mortality in these patients.⁶⁰ Germ-free mice colonized with feces from cytolysin-positive patients with AH show more severe liver damage following chronic-plus-binge ethanol feeding. Intriguingly, phage therapy against *E. faecalis* abolishes ethanol-induced liver pathology in gnotobiotic mice. Overall, these studies suggest that phage therapy targeting GM can be a potential approach for ALD treatment.

Fecal Microbiome Transplant

Fecal microbiome transplant (FMT) is the most widely studied treatment targeting GM.^{141–143} Several clinical trials have shown promising results that FMT from healthy donors improves short- and medium-term survival, leads to improvement in clinical severity scores, and is associated with favorable alteration of GM and short-term reduction in alcohol craving in patients with ALD.^{144–146} Although these clinical studies suggest that rebalancing GM by healthy donor FMT may be a solution for ALD treatment, larger clinical trials have not been reported yet to show the safety and efficacy of FMT. The underlying mechanisms of the beneficial effects of FMT on ALD in humans remain unclear.

Conclusions and Perspectives

Cholestasis and altered BA homeostasis are common in patients with ALD, particularly in patients with AH. Several BA-mediated pathways, particularly BA receptor FXR, play critical roles in the development of ALD and show potential as therapeutic targets. Modulation of gut signaling and levels of enterohepatic BAs also provide beneficial effects on ALD in animal models. GM plays a critical role in metabolizing BAs and regulating BA homeostasis. Evidence from animal models and clinical trials shows that targeting GM, particularly through FMT, is a promising therapeutic approach for ALD. Although GM and intestinal FXR are known to influence alcohol-associated cholestatic liver injury, further research is necessary to elucidate the hepatic mechanisms by which alcohol consumption leads to cholestasis. Additionally, understanding whether and how BAs contribute to liver damage in ALD and alcohol-associated liver cancers remains an important area for future investigation. Modulating BA-mediated pathways in different cell types may have varying roles in ALD, with some pathways exhibiting sex differences. This underlines the need for more pre-clinical studies to understand these underlying mechanisms. Finally, although modulating GM, particularly through FMT, has shown benefits in ALD, the number of patients in these clinical trials is limited. Larger clinical trials are necessary to assess the safety and efficacy of these interventions.

Author Contributions

S.Y. conceived of and designed the study and wrote the manuscript; and S.Y. and X.-M.Y. revised the manuscript.

Disclosure Statement

None declared.

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