Alcohol, liver disease and the gut microbiota

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Abstract | Alcoholic liver disease, which ranges from mild disease to alcoholic hepatitis and cirrhosis, is a leading cause of morbidity and mortality worldwide. Alcohol intake can lead to changes in gut microbiota composition, even before liver disease development. These alterations worsen with advancing disease and could be complicit in disease progression. Microbial function, especially related to bile acid metabolism, can modulate alcohol-associated injury even in the presence of cirrhosis and alcoholic hepatitis. Microbiota changes might also alter brain function, and the gut–brain axis might be a potential target to reduce alcoholic relapse risk. Gut microbiota manipulation including probiotics, faecal microbial transplant and antibiotics has been studied in alcoholic liver disease with varying success. Further investigation of the modulation of the gut–liver axis is relevant, as most of these patients are not candidates for liver transplantation. This Review focuses on clinical studies involving the gut microbiota in patients with alcoholic liver disease across the spectrum from alcoholic fatty liver to cirrhosis and alcoholic hepatitis. Specific alterations in the gut–liver–brain axis that are complicit in the interactions between the gut microbiota and alcohol addiction are also reviewed.

Alcohol-related harm is one of the most common preventable sources of disease worldwide, with 3.3 million deaths or 6% of all global deaths attributable to alcohol[1,2]. Alcoholic injury is multi-systemic, which adversely affects quantity and quality of life in affected individuals and their family members[3]. Alcoholic liver disease (ALD) is a spectrum of disease ranging from asymptomatic liver steatosis to the development of fibrosis, cirrhosis and alcoholic hepatitis (FIG. 1). As a cause of death and disability, this condition ranks among the top modifiable factors in most regions, with alcohol being the fourth leading cause of preventable death in the USA[3,4]. In patients with alcohol use disorders (AUDs), defined as consumption of >3 drinks per day in men and >2 drinks per day in women or binge drinking (>5 drinks in males and >4 drinks in females consumed over a 2-hour period as per the National Institute of Alcoholism and Alcohol Abuse definition), ALD and cirrhosis are the most common causes of death[5–7]. Not captured in these numbers are the days of work missed and the substantial burden on families and the health-care system[6].

Alcohol causes damage to multiple end organs, predominantly the liver, the gut and the brain. However, the development of end-organ damage owing to alcohol or alcohol-related harm is very individualized and often unpredictable. Approximately 20–30% of patients who misuse alcohol develop liver damage, and even fewer will experience liver cirrhosis or alcoholic hepatitis[3]. The risk of progression from asymptomatic liver steatosis to more advanced ALD is modulated by the extent and duration of alcohol misuse, heritable factors and sex[8]. However, studies conducted in the past few years have started to demonstrate the role of potentially modifiable factors in this progression, particularly the gut microbiota[9,10]. Thus, the study of the gut microbial milieu in the progression and potential reversal of ALD progression is a relevant goal. This Review largely focuses on human studies of the interaction between alcohol, liver disease and the gut microbiota to evaluate translation of preclinical work into clinical studies in this field. Specifically, alterations in the gut–liver–brain axis that are complicit in the interactions between the gut microbiota and alcohol addiction are also reviewed.

Current therapies for ALD

Current treatments for ALD depend on the stage of ALD and treatment acceptability, safety and tolerability. Regardless of disease severity, the goal of therapy is to prevent the occurrence and progression of injury by ensuring lasting alcohol abstinence. Encouraging abstinence requires judicious use of psychosocial and pharmacological approaches, which have a success rate of between 20% and 40% for early remission[11,12].

In the more advanced stages of cirrhosis, the treatment strategies in addition to alcohol abstinence are largely those used for complications of cirrhosis, such

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Key points

- Alcohol affects many organ systems, but alcoholic liver disease develops in selected patients and ranges from simple steatosis to inflammation, cirrhosis and alcoholic hepatitis.
- Gut microbiota composition and function, especially bile acid physiology, are affected throughout the spectrum of alcohol use disorder, and these changes can improve after alcohol cessation in patients without alcoholic liver disease.
- In patients who have substantial liver fibrosis, gut microbial changes occur in parallel to liver injury, with an increase in endotoxin-producing and a reduction in autochthonous bacterial taxa, which continue through active drinking in cirrhosis until alcoholic hepatitis.
- Functional microbial changes, in particular, hepatic bile acid production and bacterial biotransformation, are altered in parallel with the disease stages and differ between actively drinking patients with cirrhosis and those with alcoholic hepatitis.
- Alcohol use disorder can also affect the gut–brain axis, which could potentiate further misuse and affective disorders and hasten the development of hepatic encephalopathy.
- Strategies that address both alcohol cessation and microbiota alteration are needed for meaningful improvement in the natural history of this multifaceted disorder.

as control of ascites, treatment and prevention of hepatic encephalopathy recurrence and variceal bleeding and monitoring for hepatocellular cancer[6]. The focus on abstinence is important, as patients with cirrhotic ALD have a higher risk of infections and brain complications than those without ALD[13,14]. In most settings, supervised alcohol abstinence is also required before liver transplant listing[15]. Despite the focus on abstinence, even in the later stages of ALD, a substantial proportion of patients remain who continue to suffer from AUDs. Without abstinence, prognosis is uniformly poor, which is most apparent in patients with alcoholic hepatitis. In these individuals, mortality in patients who continue to drink is almost 50% higher than for those who stop[16]. This excess mortality and morbidity stems from liver disease as well as psychosocial complications such as unintentional and violent injuries, motor vehicle accidents and inability to maintain employment and inter-personal relationships[1]. Therefore, approaches that can reduce the extent of alcohol-induced injury, potentially through targeting the gut microbial milieu, might be promising.

Gut microbiota composition and function

The human gut microbiota consists of bacteria, fungi, archaea and viruses[17]. Although most publications to date have focused on bacterial composition and function, interest in the non-bacterial microbiome has increased over the past few years in diseases related and unrelated to alcohol, including cirrhosis and IBD[18–20]. One term to describe changes in gut microbial composition is 'dysbiosis', loosely defined as an imbalance or alteration in the microbiota that can have an unfavourable effect on the host. However, the inaccuracy of this term and the ubiquity of its use have made it increasingly controversial[21]. The composition of the gut microbiota is reactive to many inputs and varies markedly from birth to old age[22]. Most microbial variation is related to environmental factors, such as diet and bowel movement frequency, rather than host genetics[23–26]. Given this wide variation that occurs in healthy humans and in response to environmental and disease-related factors, specific alterations in gut microbial composition that can be labelled dysbiosis vary on the basis of the individual. However, in general, the absence of commensal organisms that produce beneficial metabolites such as short-chain fatty acids is typically regarded as a healthy or 'eubiotic' state[27]. Alterations in gut microbiota have been described in a wide variety of diseases such as cirrhosis, IBD, Parkinson disease, autism and *Clostridium difficile* infection[28–31]. A more informative construct than microbial composition is gut microbial function, which has the potential to directly affect the host, the immune system and other gut microorganisms. The function of the gut microbiota can be analysed using metabolomics, proteomics and transcriptomics via a hypothesis-generating approach, or focused microbiome-associated products can be studied[32]. The primary bacterial products that have been studied in the context of human liver disease are lipopolysaccharides, also known as endotoxins, and bile acids[10,33].

Assessing the gut microbiota. The techniques for evaluating the gut microbiota have developed markedly over the preceding decade. Next-generation DNA sequencing has enabled in-depth sample analysis and culture-independent methods of composition assessment[34]. These approaches can determine community diversity and structure from the phylum to the species level. Sequencing of the 16S ribosomal RNA (rRNA) gene is widely used for providing a community census, after which the primary data are processed following DNA extraction from particular human or environmental samples and filtered on the basis of the sequence of the variable region chosen from one of the nine hypervariable regions (V1–V9) within the bacterial 16S gene[35]. Specific bacterial taxa and their relative abundances can then be assessed by referencing public databases, and the resulting data are ultimately depicted as similarity of communities, abundance curves, biodiversity plots and other statistical descriptors of the microbiota composition[17]. However, these techniques reach only the genus level and do not allow exploration of species-level or strain-level differences. Metagenomic shotgun approaches, which can sequence microbiota and human DNA, provide a greater depth of interrogation than 16S rRNA sequencing methods. This approach randomly shears DNA, sequences several short sequences and constructs them into a consensus sequence. This method yields all genes present in a given sample, which can then predict abundances of specific metabolic processes[36]. Although metagenomics has great depth, it cannot be used for biopsy samples in which most of the DNA is human. Thus, samples in which the majority of DNA is bacterial could be the subject of both techniques, whereas samples in which there is a mix of eukaryotic and prokaryotic DNA might make 16S rRNA sequencing a better option for analysis. Metagenomics is also more expensive than 16S rRNA sequencing.

Gut–liver axis and the intestinal barrier. The gut–liver axis is a major pathway for ALD development and progression. Through multiple functions, including bile acid production and the enterohepatic circulation, as well as responsiveness to gut bacterial end-products and

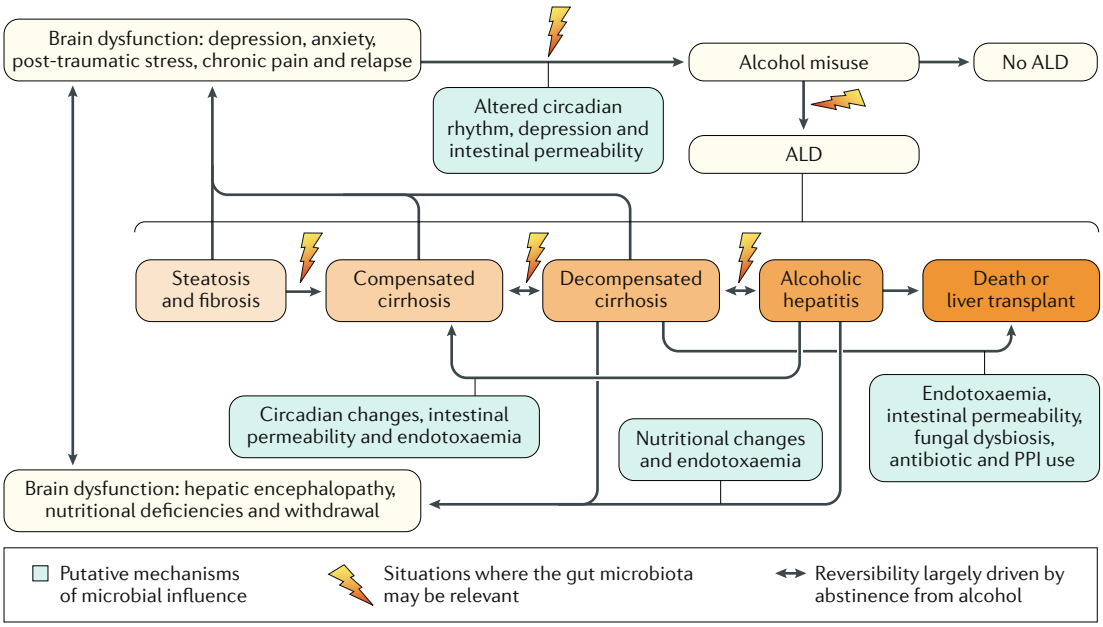

Fig. 1 | **The spectrum of ALD.** The spectrum of alcoholic liver disease (ALD) ranges from asymptomatic steatosis and fibrosis to the development of cirrhosis, associated decompensation and alcoholic hepatitis. The occurrence and progression of ALD are associated with multiple factors, including altered intestinal permeability, microbiota changes, genetic factors and circadian changes. Alcoholic hepatitis and decompensated cirrhosis are often complicated by infections and hepatic encephalopathy, which are also associated with dysbiosis. An altered gut–brain axis can modulate disease progression at each stage of the spectrum, with concomitant psychiatric disorders, nutritional deficiencies, circadian changes and hepatic encephalopathy depending on the stage studied. Despite the multiple factors associated with ALD, including dysbiosis, complete abstinence from alcohol is necessary to ensure meaningful reversal and continued improvement in the prognosis, even in the later stages of disease.

nutrients received via the portal vein, the liver is an essential player in the modulation of the gut microbiota and its effects. This gut–liver axis has implications for intestinal immune response, intestinal barrier function, hepatic and systemic inflammation and is severely disrupted during ALD[37] (TABLE 1). An integral part of the gut–liver axis is an intact intestinal barrier, which comprises several physical and immunological layers. Specifically, these include the mucous layer, containing commensal microorganisms, secretory immunoglobulin A (IgA) and antimicrobial peptides; the epithelial intestinal layer with tight junctions between adjacent cells; and finally the lamina propria with its resident population of innate and adaptive immune cells[38]. A gut–vascular barrier also controls the translocation of antigens and under normal circumstances prevents microbial translocation[39]. The gut is connected to the systemic circulation, brain and liver via the mesenteric lymph nodes, nerves (neuropods and vagal afferents) and the portal vein[38]. Bile acids, produced by the liver and released into the duodenum in conjugated form, undergo extensive microbial modification and participate in enterohepatic cycling through the farnesoid X receptor (FXR)–fibroblast growth factor 19 (FGF19) axis that also modulates the gut barrier and is an integral part of the gut–liver axis[40].

The intestinal barrier in ALD

The multiple layers of defence in the intestinal barrier, including physical, humoral and immunological components, can be affected by alcohol[41]. Acute alcohol intake or binges even without prior ALD can impair this barrier and increase serum levels of bacterial products in animal and human models[42,43]. However, the intestinal permeability change in patients with alcoholism who do not have cirrhosis is not uniform, is associated with depression and can reverse after alcohol abstinence[44]. In elegant rodent studies, alcohol-associated intestinal permeability changes are linked with gut microbial change through specific reductions in intestinal hypoxia-induced factor 1α activity and expression and are reversed by probiotic *Lactobacillus rhamnosus GG* therapy and by modulating saturated and unsaturated fat intake[45–49]. Hypoxia-induced factor 1α is also important in the development of alcohol-associated hepatic steatosis[50]. Alcohol is also associated with relative increases in abundance of endotoxin-producing Enterobacteriaceae and a reduction in taxa that produce short-chain fatty acids (SCFAs) such as Lachnospiraceae and Ruminococcaceae[51,52]. The higher concentration of secondary bile acids in active drinkers with ALD than in non-drinkers with ALD and abstinent patients with ALD could also promote intestinal permeability changes and create a vicious cycle in these patients[53]. Measuring intestinal permeability in humans is challenging, but the concept of a functional and structural impairment in this barrier is important to evaluate the full extent of ALD progression, and several assessment techniques have been developed[54]. The urinary recovery of administered non-metabolizable sugars (such as lactulose or rhamnose) is an important technique for small intestinal permeability, whereas ^{51}Cr-EDTA, polyethylene glycols

Table 1 | **Investigations into ALD and microbiota composition and function in humans**

Study	Design and participant details	Microbial findings and summary of results
ALD without cirrhosis		
Mutlu et al. 2012 (REF.[9])	• Cohort study of stool and colonic mucosal microbiota with healthy controls ($n = 18$) • Patients with ALD without cirrhosis ($n = 28$) • Patients with alcoholic cirrhosis ($n = 19$)	Dysbiosis with lower Bacteroidetes and higher Proteobacteria seen in selected individuals with alcoholism, which was linked with endotoxin
Leclercq et al. 2014 (REF.[58])	• Longitudinal study of stool microbiota before and after 3 weeks of abstinence • Patients with F0 or F1 liver disease ($n = 60$)	A selected group had increased intestinal permeability and dysbiosis associated with lower abundance of Ruminococcaceae taxa, which reversed after abstinence
Alcoholic cirrhosis with abstinence		
Chen et al. 2011 (REF.[104])	• Cross-sectional analysis of microbiota • Outpatients with cirrhosis of multiple aetiologies ($n = 12$ with ALD)	Dysbiosis was similar in different aetiologies
Bajaj et al. 2012 (REF.[65])	• Cross-sectional analysis of cognition and gut microbiota • Patients with cirrhosis and HE ($n = 17$) with ALD alone in 13 patients and ALD and with hepatitis C in 8 patients	HE rather than alcoholic aetiology was the determinant of dysbiosis and cognitive impairment
Bajaj et al. 2014 (REF.[52])	• Cross-sectional and longitudinal studies • Patients with cirrhosis ($n = 219$ in total, $n = 43$ with ALD and $n = 32$ with NASH)	Patients with ALD-related cirrhosis had worse dysbiosis than those without ALD despite abstinence and similar cirrhosis severity
Kakiyama et al. 2013 (REF.[105])	Cross-sectional study of microbiota and faecal bile acids in 47 patients with cirrhosis (7 with ALD-related cirrhosis)	Dysbiosis and bile acid profiles were similar in different aetiologies
Alcoholic cirrhosis with active drinking		
Kakiyama et al. 2014 (REF.[53])	• Analysis of secondary bile acids • Healthy controls ($n = 19$) • Actively drinking individuals without cirrhosis ($n = 6$) • Patients with cirrhosis ($n = 78$, of whom $n = 10$ were actively drinking, $n = 38$ were abstinent and $n = 30$ had cirrhosis unrelated to alcohol)	Regardless of cirrhosis status, secondary bile acid levels were higher in the serum and stool of active drinkers and lower in non-drinkers
Tuomisto et al. 2014 (REF.[68])	• Autopsy study of liver microbiota • Patients with alcoholism and cirrhosis ($n = 13$) • Patients with alcoholism without cirrhosis ($n = 15$) • Control individuals without alcoholism ($n = 14$) • A group of living individuals was also recruited • Healthy control individuals ($n = 7$) • Patients with alcoholism and cirrhosis ($n = 12$)	Sterility of the liver was lowest in patients with alcoholic cirrhosis
Dubinkina et al. 2017 (REF.[61])	• Cross-sectional metagenomic analysis of stool microbiota and functional capacity • Patients with alcohol dependence and cirrhosis ($n = 27$) • Patients with alcohol dependence without cirrhosis ($n = 72$)	Commensal bacteria were depleted in both groups but increases in *Lactobacillus* spp. and *Bifidobacterium* spp. and oral microbiota were seen in patients with cirrhosis. Functional microbiota pathways related to alcohol metabolism and inflammation were upregulated in those with cirrhosis
Bajaj et al. 2017 (REF.[51])	• Stool, duodenal, ileal and colonic microbiota analysis; duodenal and faecal bile acid profile and stool metabolomics • Healthy control individuals ($n = 34$) • Actively drinking patients with cirrhosis ($n = 37$) • Abstinent patients with cirrhosis ($n = 68$)	Dysbiosis was prevalent in all tissues studied in actively drinking patients with cirrhosis. Higher secondary bile acid levels were seen in both duodenal fluid and stool, with greater concentration of metabolites in the stool in actively drinking patients with cirrhosis
Alcoholic hepatitis		
Llopis et al. 2016 (REF.[69])	• Stool microbiota analysis • Patients without alcoholic hepatitis ($n = 16$) • Patients with non-severe alcoholic hepatitis ($n = 12$) • Patients with severe alcoholic hepatitis ($n = 10$)	Levels of streptococci, enterobacteria and bifidobacteria were higher in severe alcoholic hepatitis than in other patient groups. Bilirubin concentrations were positively linked with levels of enterobacteria and negatively linked with levels of clostridial taxa
Grander et al. 2017 (REF.[70])	• Analysis of *A. muciniphila* levels in stool samples • Healthy control individuals ($n = 16$) • Patients with non-severe alcoholic hepatitis ($n = 21$) • Patients with severe alcoholic hepatitis ($n = 15$)	*A. muciniphila* abundance reduced with increasing severity of ALD and was lowest in alcoholic hepatitis
Puri et al. 2017 (REF.[73])	• Analysis of circulating microbiota and inferred functionality • Non-drinking control individuals ($n = 20$) • Active drinkers without liver disease ($n = 19$) and patients with moderate alcoholic hepatitis ($n = 18$) • Patients with severe alcoholic hepatitis ($n = 19$)	Increased levels of circulating 16S rRNA in both alcoholic hepatitis groups. Patients with severe alcoholic hepatitis had the worst endotoxaemia. All alcohol drinking groups had enrichment for circulating Fusobacteria

Table 1 (cont.) | **Investigations into ALD and microbiota composition and function in humans**

Study	Design and participant details	Microbial findings and summary of results
Alcoholic hepatitis (cont.)		
Ciocan et al. 2018 (REF.[72])	• Analysis of gut microbiota composition and faecal and plasma bile acids • Patients without cirrhosis or alcoholic hepatitis ($n = 61$) • Patients without cirrhosis with alcoholic hepatitis ($n = 13$) • Patients with cirrhosis without alcoholic hepatitis ($n = 17$) • Patients with cirrhosis with alcoholic hepatitis ($n = 17$)	Patients with cirrhosis and alcoholic hepatitis had higher total plasma bile acid levels, whereas faecal levels of total and secondary bile acids were lower than other groups. These patients had higher relative abundance of Actinobacteria and lower relative abundance of Bacteroidetes

A. muciniphila, *Akkermansia muciniphila*; ALD, alcoholic liver disease; HE, hepatic encephalopathy; NASH, nonalcoholic steatohepatitis; rRNA, ribosomal RNA.

or sucralose can be used for entire gastrointestinal tract permeability analysis, as these molecules are not digested by members of the microbiota in the large intestine[38]. In addition, serum, plasma and faecal biomarkers such as calprotectin, fatty acid binding proteins, endotoxin and bacterial DNA have been used as corollaries to measure these[38]. Most of these techniques are difficult to perform in clinical practice, but non-metabolizable sugar urinary excretion is the approach most commonly employed in human research.

Gut–liver axis in ALD

Only approximately 15–20% of patients with AUDs develop ALD[5,55]. Although changes in the intestinal microbiota have been proposed to contribute to this heterogeneity in ALD risk, investigations have yielded mixed results (TABLE 1). However, there is emerging evidence that coexistent depression and other psychiatric conditions as well as changes in circadian rhythms can have a role in ALD progression, which could interact and confound the microbial data as well as unfavourably alter intestinal permeability[44,56]. Mutlu et al.[9] studied the stool and mucosa-associated colonic microbiota in 18 healthy individuals and 48 patients with AUDs with ALD ($n = 19$) and without ALD ($n = 28$). They found higher serum levels of endotoxin in both AUD groups than in the control group, but no difference was found between the groups with AUDs. In addition, the overall distribution of the microbial differences showed major overlaps between patients with AUDs who did or did not have ALD. When specific phyla were interrogated, the relative abundance of Bacteroidaceae was lowest in the AUD with ALD group and highest in the healthy control group over a continuum. However, overall, colonic mucosa dysbiosis in the alcohol-consuming groups was not perfectly correlated with ALD status. This lack of difference between patients with AUDs with or without ALD could be due to the predominant effect of alcohol rather than liver disease in the early stages of ALD and the low sample size. The same group also demonstrated changes in the metabolome of the faecal matter in patients with AUDs compared with healthy control individuals, including different levels of microbial metabolism products such as SCFAs and sulfides and a decrease in antioxidant fatty acids[57]. However, this study did not separate the patients with AUDs into those with and without ALD. From a microbial perspective, our current knowledge cannot reliably differentiate patients with and without early ALD or identify those patients whose ALD might worsen in the future. Leclercq et al. studied the potential reversibility of dysbiosis associated with alcohol in 60 patients with AUDs[58]. Interestingly, they found that only 40% of patients had dysbiosis, characterized by reduced Ruminococcaceae abundance and high intestinal permeability. Ruminococcaceae abundance increased after 3 weeks of successful alcohol abstinence, and the total levels of bacteria using quantitative PCR, which were lower at baseline with AUDs, returned to the levels of control individuals in this population. However, increased intestinal permeability in these patients was linked with higher levels of depression, anxiety and craving for alcohol even after alcohol withdrawal. These features portend a potential relapse if not adequately addressed, although future studies are needed to assess this association. Thus, in keeping with the multi-organ effects of alcohol, the gut–brain axis is important to characterize in patients with AUDs and ALD. The interfaces between the liver, gut and brain result from systemic metabolites, inflammatory cytokines and direct neural connections that can be bi-directional and have major contributions to the overall prognosis.

Gut–liver axis in cirrhosis and hepatitis

The spectrum of ALD is most severe with the development of cirrhosis and superimposed alcoholic hepatitis[59]. In addition to having very poor clinical outcomes including a several-fold increase in infection, portal hypertensive complications and acute-on-chronic liver failure, these patients also have demonstrable changes in their microbiota composition and function, which are associated with the occurrence and progression of liver injury.

Role in cirrhosis. In patients with alcoholic cirrhosis, there are three typical modes of clinical presentation: patients who are abstinent without alcoholic hepatitis, patients who are still drinking without alcoholic hepatitis and patients with alcoholic hepatitis, which assumes recent or current drinking (FIG. 1). An analysis of patients with alcoholic cirrhosis who had stopped drinking found that these individuals had worse gut dysbiosis and higher endotoxaemia than outpatients who did not have alcoholic cirrhosis and who were also not drinking[52]. The gut microbiota profile of those patients with alcoholic cirrhosis had increased relative abundance of Enterobacteriaceae and decreased relative abundance of Lachnospiraceae and Ruminococcaceae. These findings indicate that the alcohol-associated damage to the gut microbiota continues despite abstinence, which also extends to the gut–brain axis, as these patients also have persistent cognitive impairment[14].

Patients with cirrhosis who continue to drink alcohol have a worsened dysbiosis with lower levels of autochthonous taxa and functionality in microbiota in faeces and the colonic and duodenal mucosa[53]. These changes are accompanied by increased secondary bile acid formation and enterohepatic circulation of bile acids in actively drinking patients with cirrhosis compared with healthy control individuals or abstinent patients with cirrhosis[53] (FIGS 2,3). The increase in secondary bile acid levels occurs despite the full engagement of the FXR–FGF19 axis, which ordinarily represses bile acid synthesis, and could be related to CREBH (also known as CREB3L3) activation via direct binding to the promoters of bile acid synthesis genes in the liver[51] (FIGS 2,3). This observation is relevant because an increase in secondary bile acids can affect cell membrane stability, worsen the already impaired intestinal barrier and ultimately propagate alcohol-associated injury to the gut–liver axis[60]. Another study by Dubinkina et al. compared the composition and functional capacity of the gut microbiota in actively drinking patients with or without cirrhosis[61]. The paper reported that, irrespective of cirrhosis, commensal taxa were decreased with alcohol intake. Interestingly, there was a greater increase in levels of oral-origin microbiota and Lactobacillaceae in the stool of patients with cirrhosis than in those without cirrhosis. The increase in oral microbiota in the stool in those with cirrhosis, especially in patients with alcoholic cirrhosis, is probably an epiphenomenon given the high rate of periodontitis, change in salivary microbiota, proton pump inhibitor use and relatively low gastric acid levels in these patients[31,62–64]. The increase in Lactobacillaceae has been shown in prior studies of the gut microbiota in cirrhosis and could be related to therapeutic lactulose use[65]. The study team concluded that this increased abundance of Lactobacillaceae could be concerning for the use of this family as potential probiotics. However, human and animal trials in ALD and cirrhosis have suggested that probiotics that include Lactobacillaceae spp. can improve disease outcomes[45,66,67]. The metagenomics analysis in the study by Dubinkina et al. showed that expression of bacterial genes related to inflammation and alcohol metabolism was increased in actively drinking patients with cirrhosis as expected. The underlying difference between cirrhotic and non-cirrhotic conditions despite continued alcohol intake was underlined by an intriguing autopsy study that showed higher translocation into the ascites and liver in patients with cirrhosis than in those without cirrhosis[68].

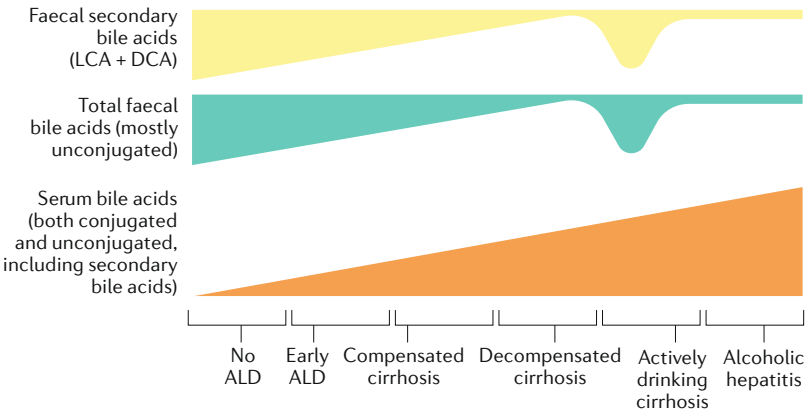

Fig. 2 | **Serum and faecal bile acid changes in ALD.** During worsening cirrhosis, levels of faecal bile acids decrease and levels of serum bile acids increase. The faecal bile acid pool has reduced levels of secondary bile acids in decompensated cirrhosis. In patients with cirrhosis who misuse alcohol but do not have alcoholic hepatitis, there is a paradoxical increase in the total and secondary faecal bile acid levels despite intact enterohepatic bile acid circulation. Once patients develop alcoholic hepatitis, cholestasis worsens, with increased levels of serum bile acids and reduced levels of faecal bile acids, including secondary bile acids. ALD, alcoholic liver disease; DCA, deoxycholic acid; LCA, lithocholic acid.

Role in alcoholic hepatitis. In alcoholic hepatitis, the majority of which occurs on the background of cirrhosis, gut microbiota composition and functionality are further altered. Llopis et al.[69] performed a translational analysis of patients with alcoholic hepatitis using 38 hospitalized patients (16 without alcoholic hepatitis, 12 patients with non-severe alcoholic hepatitis and 10 patients with severe alcoholic hepatitis). Increased abundance of the potentially pathogenic families of Enterobacteriaceae and Streptococcaceae was correlated with severity of alcoholic hepatitis[69]. Secondary bile acids were also increased in this study with increasing alcoholic hepatitis severity, similar to what was seen in actively drinking patients with cirrhosis without alcoholic hepatitis, as reported earlier. Given the importance of interactions between intestinal barrier dysfunction, secondary bile acids and the gut microbiota in ALD, Grander et al. examined the behaviour of a commensal bacterium that is associated with the intestinal mucous layer, *Akkermansia muciniphila*, in alcoholic hepatitis[70]. In clinical faecal samples, the relative abundance of this microorganism was lowest in patients with alcoholic hepatitis. In a mouse model of ALD, *A. muciniphila* administration improved alcohol-associated hepatic injury and gut barrier function, but human studies are required[70]. In another study in patients with alcoholic hepatitis, de novo liver bile acid production was reduced and serum bile acid levels were higher than in control individuals, but the microbiota and intestinal barrier function were not measured[71]. These findings were further corroborated by another study that found that patients with cirrhosis and alcoholic hepatitis had reduced levels of Bacteroidetes and increased Actinobacteria abundance as well as changes to predicted microbial pathways, including upregulated glutathione metabolism and downregulated biotin metabolism, compared with patients who were cirrhotic without alcoholic hepatitis[72].

Highlighting the pervasive nature of the impaired immune response in ALD, an interesting study demonstrated alterations in circulating microorganisms in patients with alcoholic hepatitis compared with patients with cirrhosis without alcoholic hepatitis and healthy control individuals[73]. Fusobacteria was higher in relative abundance in those with alcohol intake than in healthy individuals, but levels were lower in those with severe alcoholic hepatitis than in those with moderate or absent alcoholic hepatitis. Interestingly, these findings were reversed for the Bacteroidetes phylum, with highest relative abundance found in healthy individuals not consuming alcohol. As expected, patients with severe alcoholic hepatitis had the highest endotoxaemia.

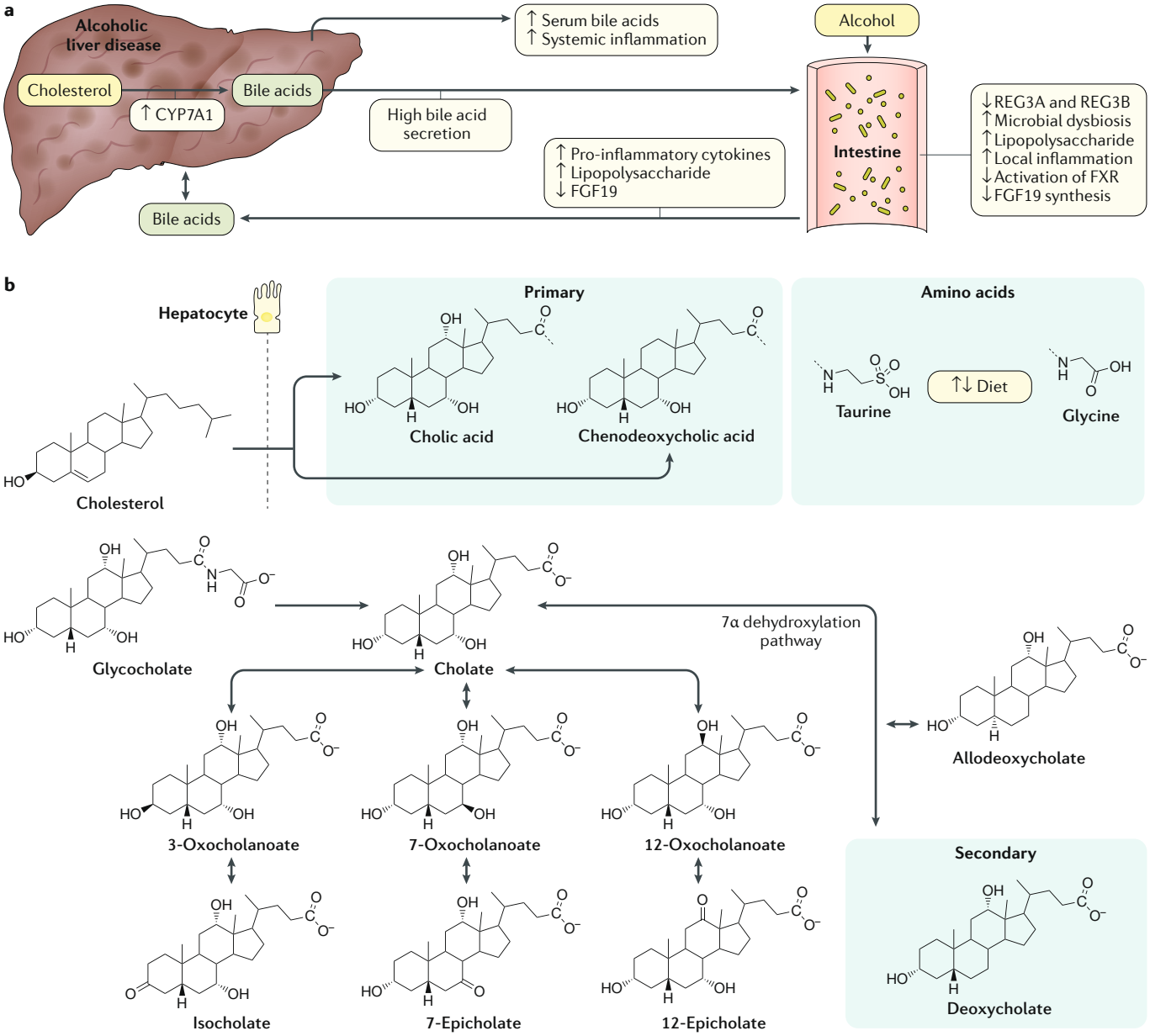

Fig. 3 | **Changes in the gut–liver axis before alcoholic hepatitis. a** | Alcohol intake directly affects the intestinal barrier and gut microbiota and facilitates an increased ingress of inflammatory cytokines and endotoxin to the liver. This process can potentiate further bile acid synthesis and systemic inflammation, which leads to a vicious circle. **b** | Primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized from cholesterol by the liver and then conjugated with either taurine or glycine before being secreted into the duodenal lumen. The ubiquitous bacterial bile salt hydrolase deconjugates these bile acids, which form the bulk of faecal bile acids. The deconjugated bile acids can then undergo 7α dehydroxylation by a selected group of Gram-positive bacteria into secondary bile acids (deoxycholic and lithocholic acid). In addition, the bile acids can undergo epimerization and form iso and oxo bile acids. Pathways pertaining to cholic acid are shown. Chenodeoxycholic acid undergoes bacterial transformation to form lithocholic acid. FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; REG3A, regenerating islet-derived protein 3α; REG3B, regenerating islet-derived protein 3β.

These data demonstrate that there are major changes in gut microbial composition and function in patients with alcoholic hepatitis, which have the potential to change the natural history of ALD.

Metabolomic changes in ALD

Untargeted metabolomics approaches using techniques such as NMR spectroscopy, gas chromatography and liquid chromatography mass spectroscopy can increase the pathophysiological insight into the disease process by identifying other disease-associated molecules[74]. However, it is somewhat difficult to pinpoint the source of these metabolites, and only a few of them, such as secondary bile acids, endotoxin and SCFAs, are distinctly microbial in origin. Changes in metabolites in the serum and/or plasm and urine can be used to gauge the extent of alcohol intake in community settings or determine the presence of preclinical ALD. Preclinical ALD and

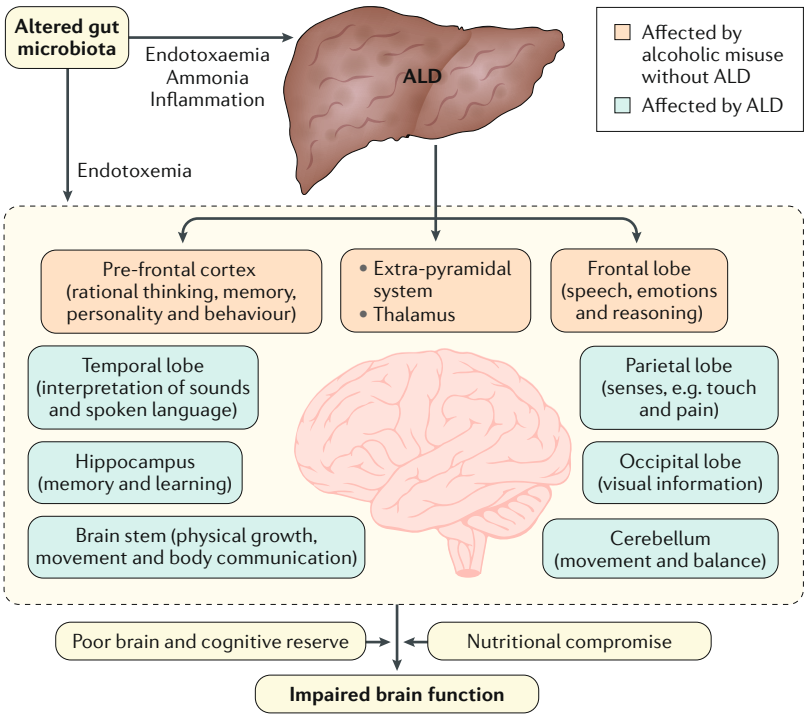

Fig. 4 | **Altered gut–brain axis in alcohol misuse.** Alcohol misuse can directly affect many areas of the brain, including the cortical lobes, cerebellum and brain stem, depending on the extent and age of onset of alcohol intake. With the development of added alcoholic liver disease (ALD), there is additional impairment of the extra-pyramidal system and synergistic effects on the frontal and pre-frontal cortex. The gut microbiota changes can potentially be associated with these brain alterations and endotoxaemia, systemic inflammation and hyperammonaemia. These effects result in astrocytic swelling and microglial activation, which can impair brain function. Concomitant with nutritional deficiencies and resultant poor cognitive and brain reserve, these microbiota-associated changes lead to impaired brain function with alcohol use.

high levels of alcohol consumption are associated with inflammatory, amino acid and bioenergetic perturbations (including higher plasma levels of threonine, glutamine and guanidinosuccinate, higher urinary levels of 3-hydroxytetradecanedioic acid and isocitric acid and lower urinary concentrations of sebacic acid) that result from alcohol metabolism rather than microbial metabolism[75,76]. However, gut microbiota-associated metabolites from faecal samples were different between patients with alcoholism and healthy control individuals and between patients with cirrhosis who were drinking versus patients with cirrhosis who were not drinking and healthy controls[51,57]. The study by Couch et al. found that levels of SCFAs, such as propionate and isobutyrate, and products of microbial decomposition, dimethyl disulfide and dimethyl trisulfide were altered in the stool volatile organic compounds from patients with alcoholism[57]. In the second study, actively drinking patients with cirrhosis had lower stool metabolite concentrations in metabolites largely related to bioenergetics (citrate, malate and phosphate), amino acids (threonine, ornithine and serine) and pyrimidine intermediates (ribosine, orotic acid and hexanoate) than non-drinking or abstinent patients with cirrhosis[51]. These findings were accompanied by profound perturbations of serum, faecal and duodenal fluid bile acid profiles in actively drinking patients with cirrhosis related to higher total and secondary bile acid concentrations in the faeces that were reflected in the duodenal fluid profiles that are freshly released from the biliary system.

Patients with alcoholic hepatitis show major alterations in levels of serum metabolites related to lipolysis and oxidative stress (higher levels of eicosapentaenoate and docosapentaenoate and lower levels of monoacylglycerols) and bioenergetics (malate, fumarate and citrate) compared with ambulatory patients with cirrhosis[77]. Changes in serum levels of microbiota-associated bile acids (lower concentrations of deoxycholate and glycodeoxycholate and higher concentrations of conjugated primary bile acids) and benzoic acid metabolites were also seen. Levels of 15 individual metabolites were associated with alcoholic hepatitis survival, independent of therapy effects. Therefore, gut microbiota-associated metabolites, in addition to SCFAs, bile acids and endotoxin, might be relevant in ALD progression in addition to microbial composition.

Non-bacterial microbiota changes in ALD

The gut microbiota consists of microorganisms other than bacteria, such as fungi, archaea and viruses, which can modulate the interactions between and directly influence the host and bacteria. Patients with ALD have a higher risk of bacterial infections[13] than those without ALD, and patients with advanced cirrhosis have a high risk of fungal infections, which could have a strong effect on the post-antibiotic gut microbiota[78]. Fungal infections in patients with cirrhosis and alcoholic hepatitis are independently associated with increased mortality compared with that seen in those without fungal infections[78,79]. In a mouse model of ALD, alcohol intake caused fungal overgrowth, especially of *Candida* spp., that was associated with increased liver injury[20]. The investigators also found that fungal cell wall β-glucan induced liver inflammation by binding the C-type lectin-like receptor CLEC7A on Kupffer cells through upregulation of IL-1β. Extending these findings to humans, patients with ALD had increased systemic exposure and increased immune responses to intestinal fungi compared with healthy individuals or those with cirrhosis not related to alcohol. In another study of patients with more advanced cirrhosis, stool fungal diversity was correlated with bacterial diversity and both fungal and bacterial diversities reduced after antibiotic use[19]. The ratio of Bacteroidetes to Ascomycota could also independently predict hospital readmission[19]. The yield of fungi per sample is typically lower than bacteria, and therefore, greater finesse is needed in performing analysis and interpreting mycobiota changes.

Ultimately, it is important to realize that non-bacterial components of the microbiota in the gut, such as fungi, can influence the progression, pathogenesis and clinical outcomes in patients with ALD. In patients with alcoholic cirrhosis and alcoholic hepatitis who have infections, there should be a high suspicion of fungal aetiologies.

Gut–brain axis changes in ALD

The gut–brain axis (the connection between the brain and the gut mediated by metabolites, neural connections and hormonal influences) is an important concept

in several diseases, including AUDs. Given the addictive nature of alcohol, alternative strategies to prevent relapse after withdrawal beyond the often-ineffective current management approaches are being investigated[80]. The brain is affected throughout the spectrum of AUDs, ranging from acute intoxication to changes in personality and behaviour to dementia. In patients with cirrhosis and advanced liver disease, the added effect of nutritional deficiencies and hepatic encephalopathy can further worsen the brain reserve and functioning in patients with AUDs[14,81,82] (FIG. 4). Thus, the interaction of the brain with the gut and liver, the gut–brain axis, is important in fully understanding the pathogenesis of AUDs and ALD. Evaluating the gut as a potential pathway by which brain function is altered in patients with AUDs has attracted considerable interest, as efforts to approach addictive behaviour and liver disease need to be combined with reduction in the inflammatory potential of gut-related changes to affect the entire gut–brain axis[44]. Leclercq et al.[58] showed that depression, anxiety and alcohol craving correlated with increased intestinal permeability in patients with early AUDs. They also found that patients with high intestinal permeability continued to have depression, anxiety and cravings for alcohol even after alcohol withdrawal. Brain connectivity and activation alterations in primary psychiatric disorders such as depression and schizophrenia can alter the gut–brain axis in the absence of AUDs, but alcohol use has the potential to worsen these effects[83]. With the development of dysbiosis in AUDs, systemic inflammatory mediators, ammonia and endotoxaemia can worsen neuroinflammation[84]. The gut–brain axis is altered in patients with pre-cirrhosis with AUDs and can influence concomitant eating disorders, cocaine use and anxiety disorders[84–86]. In addition, there are effects of alcohol on the brain directly or through concomitant nutritional deficiencies that worsen the brain function[82,87]. In patients with ALD and cirrhosis, alterations in the gut–brain axis usually manifest as worsened hepatic encephalopathy. Hepatic encephalopathy is associated with systemic inflammation, hyperammonaemia, endotoxaemia and microglial activation, which are enhanced by altered gut microbial composition and function independent of alcohol use[88,89]. In patients with cirrhosis related to ALD, there is a greater likelihood of persistent cognitive impairment than in patients without alcoholism with cirrhosis, which can affect the daily function of these patients[14,52]. Given the role of the microbiota and intestinal permeability in patients with AUDs, it might be prudent to investigate the gut microbiota as a potential target to reduce alcohol cravings. To this effect, a randomized trial of faecal microbiota transplantation (FMT) is underway in patients with cirrhosis who continue to drink alcohol (NCT03416751).

Microbiota-related therapies in ALD

As many patients with ALD are not candidates for life-saving liver transplantation and are either not willing or unable to abstain from alcohol, other options to improve their prognosis are needed[15,90,91]. On the basis of growing evidence of an important role in ALD, the gut microbiota could be a relevant therapeutic target.

In mouse models, administration of *A. muciniphila* and *L. rhamnosus GG* improves alcohol-induced injury through complex changes to the intestinal barrier and systemic inflammation[45,70]. However, human intervention data are scarce. The few published studies that have investigated the effect of gut microbiota modification in patients with ALD are encouraging but are usually small (TABLE 2). In patients hospitalized for alcohol treatment, Kirpich et al. demonstrated that administration of probiotics for 5 days (*Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3) increased levels of potentially beneficial bacteria such as bifidobacteria

Table 2 | **Clinical trials of therapies to alter the gut microbiota in ALD**

Study	Intervention	Trial design and participants	Summary of results
Kirpich et al. 2008 (REF.[92])	*Bifidobacterium bifidum* and *Lactobacillus plantarum* 8PA3 versus standard therapy	• Randomized open-label trial • Hospitalized male patients with alcoholic psychosis ($n = 66$; $n = 26$ had alcoholic hepatitis)	Reduced serum AST and ALT levels and increased relative abundance of *Lactobacillus* spp. and bifidobacteria in patients receiving probiotic
Stadlbauer et al. 2008 (REF.[93])	*Lactobacillus casei* Shirota 3 times per day for 4 weeks	• Open-label study • Patients with compensated alcoholic cirrhosis ($n = 10$)	Neutrophil phagocytic capacity was improved compared with baseline
Han et al. 2015 (REF.[66])	1,500 mg *Bacillus subtilis* and *Enterococcus faecium* (formerly known as *Streptococcus faecium*) per day versus placebo for 7 days	• Placebo-controlled trial • Hospitalized patients with alcoholic hepatitis ($n = 117$)	Liver function, systemic inflammation and endotoxaemia improved, with lower CFU of *Escherichia coli* in patients receiving probiotic
Phillips et al. 2017 (REF.[100])	Daily FMT from several donors via a nasojejunal tube for 7 days	• Open-label study with 1 year of follow-up • Male patients with steroid-resistant alcoholic hepatitis ($n = 8$)	FMT improved survival compared with historical controls, improved liver function and reduced potentially pathogenic species
Phillips et al. 2018 (REF.[101])	FMT daily via a nasojejunal tube for 7 days compared with corticosteroids, nutritional therapy and pentoxifylline	Open-label study with 3 months of follow-up. Male patients with alcoholic hepatitis treated with: • FMT ($n = 16$) • Pentoxifylline ($n = 10$) • Corticosteroids ($n = 8$) • Nutritional therapy ($n = 17$)	• Survival at 3 months of follow-up was highest in the FMT group • Changes in microbial function and composition were found after FMT

ALD, alcoholic liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFU, colony-forming units; FMT, faecal microbiota transplantation.

Box 1 | **Future directions and unanswered questions**

- The role of the gut microbiota in determining why some patients develop certain phenotypes of alcohol-associated injury and others are not affected
- Determining the specific point in the liver disease natural history at which microbial changes persist despite alcohol abstinence
- Determining the role of an altered gut–brain axis in the promotion of relapse, misuse and other affective disorders in alcoholic liver disease
- Functional changes in the microbiota, including bile acid modifications, could be a promising source of future gut microbiota-directed therapies
- A focus on the role of the small intestine and non-bacterial microbial changes in human alcoholic liver disease is required
- Larger and long-term clinical trials are needed focusing on gut microbial modification to translate these into practice

and lactobacilli as determined by serial plating[92]. These results were accompanied by an improvement in levels of liver-associated enzymes (alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transpeptidase) in patients with mild alcoholic hepatitis. Although encouraging, this study was open-label and used conventional culture methods rather than modern 16S rRNA or metagenomic sequencing. Stadlbauer et al. investigated the effects of a different probiotic strain, *Lactobacillus casei* Shirota, in an open-label study in outpatients with compensated cirrhosis. This probiotic was well tolerated, improved neutrophil phagocytic function and reduced endotoxin and TLR4 responses[93] compared with non-treated patients with cirrhosis and healthy control individuals. However, the gut microbiota were not analysed, and long-term outcomes were not studied. In the largest study to date, Han et al. randomly assigned 117 patients with alcoholic hepatitis from 4 centres to receive a probiotic containing *Bacillus subtilis* and *Enterococcus faecium (formerly known as Streptococcus faecium)* or placebo for 7 days[66]. Using serial plating, the team found that probiotic administration reduced stool *Escherichia coli* abundance. Endotoxaemia and liver-associated enzyme levels were also improved by the probiotic, but levels of inflammatory cytokines were unchanged. Smaller, nonrandomized trials have been conducted investigating the minimally absorbed antibiotic rifaximin for patients with alcoholic cirrhosis who had quit alcohol. Kalambokis et al.[94] investigated the effects of rifaximin given for 4 weeks on 13 patients, and Vlachogiannakos et al.[95] assessed the effects of rifaximin administered for 5 years to 23 patients with decompensated cirrhosis and ascites matched with 46 patients not given rifaximin. Kalambokis et al. showed that haemodynamics, renal function, systemic inflammation and endotoxaemia improved over 4 weeks compared with baseline values. Vlachogiannakos et al. demonstrated a long-term reduction in development of portal hypertensive complications such as hepatic encephalopathy, spontaneous bacterial peritonitis and variceal bleeding, but microbial data were not collected[94,95].

FMT has been investigated for an increasing number of applications over the past few years[96]. Within liver disease, the approach has been explored for fatty liver disease and hepatic encephalopathy[97–99]. Phillips et al. performed an open-label study of FMT in eight patients with steroid-resistant alcoholic hepatitis using stool from several donors and followed up recipient patients for 1 year[100]. Compared with a historical cohort of patients who did not receive FMT, patients receiving FMT had improved overall survival (87.5% versus 33.3%). FMT was provided using a nasojejunal tube daily for 7 days and resulted in reduced levels of Proteobacteria and increased levels of Firmicutes at 1 year after transplantation. These results are encouraging because there is always a concern for potential infection after FMT in those with advanced liver disease, although studies of FMT in cirrhosis have not borne this out[98]. Another open-label study compared outcomes in male patients with alcoholic hepatitis treated with FMT, nutritional therapy, corticosteroids or pentoxifylline. FMT was associated with improved survival at 90 days compared with other treatments (75% for FMT versus 38% in the steroid group, 29% in the nutrition group and 30% in the pentoxifylline group), with favourable changes in gut microbiota composition and predicted functionality[101]. Another open-label trial is currently comparing FMT with steroids in alcoholic hepatitis (NCT03091010). However, further targeted, blinded trials are needed in this population.

Future strategies

The gut microbiota has an important role in the occurrence and progression of ALD, but other factors that make this synergism possible need to be investigated (BOX 1). For instance, transfer of stool from actively drinking human donors into germ-free mice can cause bacterial translocation and hepatic inflammation, but histological liver injury or cirrhosis does not occur unless the mice are fed alcohol[102]. In addition, when germ-free mice that receive stool transfers from active drinkers with alcoholic hepatitis are fed alcohol, their liver injury is far greater than that of those who received stool from patients with less severe ALD without alcoholic hepatitis[69]. Thus, the relative importance of the gut microbiota with respect to alcohol cessation needs to be placed in context, and efforts to improve both aspects are needed concurrently. Focusing on the small intestine, which shows dysbiosis and increased secondary bile acid production in human studies, would be of future interest given that this is where bile acids and alcohol first interact with the gut microbiota[51,103]. Modulation of the gut microbiota composition and function using precise techniques is needed; this tailoring will enable specific therapies to be delivered depending on the stage of ALD and take into account the dietary, psychosocial and ethnic backgrounds of the patient. Bile acid physiology modification to bolster the intestinal barrier function and favourably modulate the gut–liver axis is one promising area of therapy development focus. This modification could be achieved using directed therapies such as bile acid binders, FXR agonists, upregulation of specific strains such as *A. muciniphila* and exploring specific microbial consortia and the role and safety of targeted FMT in this population. Using gut microbial modification to alleviate the psychological factors that often coexist or propagate the alcohol

use is another promising area of research that requires dedicated investigation.

Conclusions

The gut microbiota is intricately linked with the development and propagation of liver injury in patients who misuse alcohol. Evidence is compelling that specific changes in the human gut microbiota can accelerate this injury through changes in microbial function, especially bile acid metabolism. Large and long-term clinical trials are needed to adequately translate these microbial findings into practice.

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1. Rehm, J., Samokhvalov, A. V. & Shield, K. D. Global burden of alcoholic liver diseases. *J. Hepatol.* **59**, 160–168 (2013).
2. World Health Organization. Global status on alcohol and health 2014. *WHO* http://www.who.int/substance_abuse/publications/global_alcohol_report/msb_gsr_2014_1.pdf (2014).
3. Mathurin, P. & Bataller, R. Trends in the management and burden of alcoholic liver disease. *J. Hepatol.* **62**, S38–S46 (2015).
4. Yoon, Y. & Chen, C. M. Surveillance report #105. *NIAAA* https://pubs.niaaa.nih.gov/publications/surveillance105/Cirr13.pdf (2016).
5. Mills, S. J. & Harrison, S. A. Comparison of the natural history of alcoholic and nonalcoholic fatty liver disease. *Curr. Gastroenterol. Rep.* **7**, 32–36 (2005).
6. Singal, A. K., Bataller, R., Ahn, J., Kamath, P. S. & Shah, V. H. ACG clinical guideline: alcoholic liver disease. *Am. J. Gastroenterol.* **113**, 175–194 (2018).
7. National Institute on Alcohol Abuse and Alcoholism. Alcohol facts and statistics. *NIAAA* https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/alcohol-facts-and-statistics (2018).
8. Zakhari, S. & Li, T. K. Determinants of alcohol use and abuse: impact of quantity and frequency patterns on liver disease. *Hepatology* **46**, 2032–2039 (2007).
9. Mutlu, E. A. et al. Colonic microbiome is altered in alcoholism. *Am. J. Physiol. Gastrointest. Liver Physiol.* **302**, G966–G978 (2012).
10. Szabo, G. Gut-liver axis in alcoholic liver disease. *Gastroenterology* **148**, 30–36 (2015).
11. Soyka, M. et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of substance use and related disorders, part 1: alcoholism. *World J. Biol. Psychiatry* **9**, 6–23 (2008).
12. Moos, R. H. & Moos, B. S. Rates and predictors of relapse after natural and treated remission from alcohol use disorders. *Addiction* **101**, 212–222 (2006).
13. Rosa, H., Silverio, A. O., Perini, R. F. & Arruda, C. B. Bacterial infection in cirrhotic patients and its relationship with alcohol. *Am. J. Gastroenterol.* **95**, 1290–1293 (2000).
14. Ahluwalia, V. et al. The etiology of cirrhosis is a strong determinant of brain reserve: a multimodal magnetic resonance imaging study. *Liver Transpl.* **21**, 1123–1132 (2015).
15. Martin, P., DiMartini, A., Feng, S., Brown, R. Jr & Fallon, M. Evaluation for liver transplantation in adults: 2013 practice guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* **59**, 1144–1165 (2014).
16. Altamirano, J. et al. Alcohol abstinence in patients surviving an episode of alcoholic hepatitis: prediction and impact on long-term survival. *Hepatology* **66**, 1842–1853 (2017).
17. Human Microbiome Project, C. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012).
18. Sokol, H. et al. Fungal microbiota dysbiosis in IBD. *Gut* **66**, 1039–1048 (2016).
19. Bajaj, J. S. et al. Fungal dysbiosis in cirrhosis. *Gut* **67**, 1146–1154 (2018).
20. Yang, A. M. et al. Intestinal fungi contribute to development of alcoholic liver disease. *J. Clin. Invest.* **127**, 2829–2841 (2017).
21. Hooks, K. B. & O'Malley, M. A. Dysbiosis and its discontents. *mBio* **8**, e01492–17 (2017).
22. Rodriguez, J. M. et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb. Ecol. Health Dis.* **26**, 26050 (2015).
23. Vandeputte, D. et al. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut* **65**, 57–62 (2016).
24. Rothschild, D. et al. Environment dominates over host genetics in shaping human gut microbiota. *Nature* **555**, 210–215 (2018).
25. Wu, G. D. et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* **334**, 105–108 (2011).
26. Bajaj, J. S. et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* **68**, 234–247 (2018).
27. Iebba, V. et al. Eubiosis and dysbiosis: the two sides of the microbiota. *New Microbiol.* **39**, 1–12 (2016).
28. Sampson, T. R. et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* **167**, 1469–1480 (2016).
29. Hughes, H. K., Rose, D. & Ashwood, P. The gut microbiota and dysbiosis in autism spectrum disorders. *Curr. Neurol. Neurosci. Rep.* **18**, 81 (2018).
30. Ni, J., Wu, G. D., Albenberg, L. & Tomov, V. T. Gut microbiota and IBD: causation or correlation? *Nat. Rev. Gastroenterol. Hepatol.* **14**, 573–584 (2017).
31. Bajaj, J. S., Betrapally, N. S. & Gillevet, P. M. Decompensated cirrhosis and microbiome interpretation. *Nature* **525**, E1–E2 (2015).
32. Young, V. B. The role of the microbiome in human health and disease: an introduction for clinicians. *BMJ* **356**, j831 (2017).
33. Ridlon, J. M., Harris, S. C., Bhowmik, S., Kang, D. J. & Hylemon, P. B. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* **7**, 22–39 (2016).
34. Gillevet, P., Sikaroodi, M., Keshavarzian, A. & Mutlu, E. A. Quantitative assessment of the human gut microbiome using multitag pyrosequencing. *Chem. Biodivers.* **7**, 1065–1075 (2010).
35. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **489**, 220–230 (2012).
36. Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
37. Segal, Z. V. et al. Antidepressant monotherapy versus sequential pharmacotherapy and mindfulness-based cognitive therapy, or placebo, for relapse prophylaxis in recurrent depression. *Arch. Gen. Psychiatry* **67**, 1256–1264 (2010).
38. Vancamelbeke, M. & Vermeire, S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev. Gastroenterol. Hepatol.* **11**, 821–834 (2017).
39. Spadoni, I. et al. A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* **350**, 830–834 (2015).
40. Bajaj, J. S. & Hylemon, P. B. Gut-liver axis alterations in alcoholic liver disease: are bile acids the answer? *Hepatology* **67**, 2074–2075 (2018).
41. Wiest, R., Albillos, A., Trauner, M., Bajaj, J. S. & Jalan, R. Targeting the gut-liver axis in liver disease. *J. Hepatol.* **67**, 1084–1103 (2017).
42. Bala, S., Marcos, M., Gattu, A., Catalano, D. & Szabo, G. Acute binge drinking increases serum endotoxin and bacterial DNA levels in healthy individuals. *PLOS ONE* **9**, e96864 (2014).
43. Voigt, R. M. et al. Diurnal variations in intestinal barrier integrity and liver pathology in mice: implications for alcohol binge. *Am. J. Physiol. Gastrointest. Liver Physiol.* **314**, G131–G141 (2018).
44. de Timary, P., Leclercq, S., Starkel, P. & Delzenne, N. A dysbiotic subpopulation of alcohol-dependent subjects. *Gut Microbes* **6**, 388–391 (2015).
45. Bull-Otterson, L. et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLOS ONE* **8**, e53028 (2013).
46. Wang, Y. et al. *Lactobacillus rhamnosus* GG treatment potentiates intestinal hypoxia-inducible factor, promotes intestinal integrity and ameliorates alcohol-induced liver injury. *Am. J. Pathol.* **179**, 2866–2875 (2011).
47. Chen, P. et al. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver injury in mice. *Gastroenterology* **148**, 203–214 (2015).
48. Shao, T. et al. Intestinal HIF-1alpha deletion exacerbates alcoholic liver disease through inducing intestinal dysbiosis and barrier dysfunction. *J. Hepatol.* **69**, 886–895 (2018).
49. Forsyth, C. B. et al. *Lactobacillus* GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol* **43**, 163–172 (2009).
50. Nath, B. et al. Hepatocyte-specific hypoxia-inducible factor-1alpha is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice. *Hepatology* **53**, 1526–1537 (2011).
51. Bajaj, J. S. et al. Continued alcohol misuse in human cirrhosis is associated with an impaired gut-liver axis. *Alcohol. Clin. Exp. Res.* **41**, 1857–1865 (2017).
52. Bajaj, J. S. et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J. Hepatol.* **60**, 940–947 (2014).
53. Kakiyama, G. et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **306**, G929–G937 (2014).
54. Wang, L. et al. Methods to determine intestinal permeability and bacterial translocation during liver disease. *J. Immunol. Methods* **421**, 44–53 (2015).
55. Grant, B. F., Dufour, M. C. & Harford, T. C. Epidemiology of alcoholic liver disease. *Semin. Liver Dis.* **8**, 12–25 (1988).
56. Forsyth, C. B., Voigt, R. M., Burgess, H. J., Swanson, G. R. & Keshavarzian, A. Circadian rhythms, alcohol and gut interactions. *Alcohol* **49**, 389–398 (2015).
57. Couch, R. D. et al. Alcohol induced alterations to the human fecal VOC metabolome. *PLOS ONE* **10**, e0119362 (2015).
58. Leclercq, S. et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc. Natl Acad. Sci. USA* **111**, E4485–E4493 (2014).
59. Lucey, M. R., Mathurin, P. & Morgan, T. R. Alcoholic hepatitis. *N. Engl. J. Med.* **360**, 2758–2769 (2009).
60. Hartmann, P. et al. Modulation of the intestinal bile acid-FXR-FGF15 axis improves alcoholic liver disease in mice. *Hepatology* **67**, 2150–2166 (2017).
61. Dubinkina, V. B. et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome* **5**, 141 (2017).
62. Qin, N. et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* **513**, 59–64 (2014).
63. Bajaj, J. S. et al. Salivary microbiota reflects changes in gut microbiota in cirrhosis with hepatic encephalopathy. *Hepatology* **62**, 1260–1271 (2015).
64. Raghava, K. V., Shivananda, H., Mundinamane, D., Boloor, V. & Thomas, B. Evaluation of periodontal status in alcoholic liver cirrhosis patients: a comparative study. *J. Contemp. Dent. Pract.* **14**, 179–182 (2013).
65. Bajaj, J. S. et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am. J. Physiol. Gastrointest. Liver Physiol.* **302**, G168–G175 (2012).
66. Han, S. H. et al. Effects of probiotics (cultured *Lactobacillus* subtilis/*Streptococcus* faecium) in the treatment of alcoholic hepatitis: randomized-controlled multicenter study. *Eur. J. Gastroenterol. Hepatol.* **27**, 1300–1306 (2015).
67. Dhiman, R. K. et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* **147**, 1327–1337 (2014).
68. Tuomisto, S. et al. Changes in gut bacterial populations and their translocation into liver and

ascites in alcoholic liver cirrhotics. *BMC Gastroenterol.* **14**, 40 (2014).
69. Llopis, M. et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* **65**, 830–839 (2016).
70. Grander, C. et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. *Gut* **67**, 891–901 (2018).
71. Brandl, K. et al. Dysregulation of serum bile acids and FGF19 in alcoholic hepatitis. *J. Hepatol.* **69**, 396–405 (2018).
72. Ciocan, D. et al. Bile acid homeostasis and intestinal dysbiosis in alcoholic hepatitis. *Aliment. Pharmacol. Ther.* **48**, 961–974 (2018).
73. Puri, P. et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. *Hepatology* **67**, 1284–1302 (2018).
74. Zhong, W. & Zhou, Z. Alterations of the gut microbiome and metabolome in alcoholic liver disease. *World J. Gastrointest. Pathophysiol.* **5**, 514–522 (2014).
75. Harada, S. et al. Metabolomic profiling reveals novel biomarkers of alcohol intake and alcohol-induced liver injury in community-dwelling men. *Environ. Health Prev. Med.* **21**, 18–26 (2016).
76. Liang, Q., Wang, C., Li, B. & Zhang, A. Metabolomics of alcoholic liver disease: a clinical discovery study. *RSC Adv.* **5**, 80381–80387 (2015).
77. Rachakonda, V. et al. Serum metabolomic profiling in acute alcoholic hepatitis identifies multiple dysregulated pathways. *PLOS ONE* **9**, e113860 (2014).
78. Bajaj, J. S. et al. Prediction of fungal infection development and their impact on survival using the NACSELD cohort. *Am. J. Gastroenterol.* **113**, 556–563 (2018).
79. Gustot, T. et al. Invasive aspergillosis in patients with severe alcoholic hepatitis. *J. Hepatol.* **60**, 267–274 (2014).
80. Addolorato, G., Mirijello, A., Barrio, P. & Gual, A. Treatment of alcohol use disorders in patients with alcoholic liver disease. *J. Hepatol.* **65**, 618–630 (2016).
81. Davis, B. C. & Bajaj, J. S. Effects of alcohol on the brain in cirrhosis: beyond hepatic encephalopathy. *Alcohol. Clin. Exp. Res.* **42**, 660–667 (2018).
82. Butterworth, R. F. Thiamine deficiency-related brain dysfunction in chronic liver failure. *Metab. Brain Dis.* **24**, 189–196 (2009).
83. Cenit, M. C., Sanz, Y. & Codoner-Franch, P. Influence of gut microbiota on neuropsychiatric disorders. *World J. Gastroenterol.* **23**, 5486–5498 (2017).
84. Temko, J. E. et al. The microbiota, the gut and the brain in eating and alcohol use disorders: a 'menage a trois'? *Alcohol Alcohol.* **52**, 403–413 (2017).
85. Xiao, H. W. et al. Gut microbiota modulates alcohol withdrawal-induced anxiety in mice. *Toxicol. Lett.* **287**, 23–30 (2018).
86. Volpe, G. E. et al. Associations of cocaine use and HIV infection with the intestinal microbiota, microbial translocation, and inflammation. *J. Stud. Alcohol Drugs* **75**, 347–357 (2014).
87. Butterworth, R. F. Pathogenesis of hepatic encephalopathy in cirrhosis: the concept of synergism revisited. *Metab. Brain Dis.* **31**, 1211–1215 (2015).
88. Bajaj, J. S. The role of microbiota in hepatic encephalopathy. *Gut Microbes* **5**, 397–403 (2014).
89. Kang, D. J. et al. Gut microbiota drive the development of neuroinflammatory response in cirrhosis in mice. *Hepatology* **64**, 1232–1248 (2016).
90. Mathurin, P. et al. Early liver transplantation for severe alcoholic hepatitis. *N. Engl. J. Med.* **365**, 1790–1800 (2011).
91. Lee, B. P. et al. Outcomes of early liver transplantation for patients with severe alcoholic hepatitis. *Gastroenterology* **155**, 422–430 (2018).
92. Kirpich, I. A. et al. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. *Alcohol* **42**, 675–682 (2008).
93. Stadlbauer, V. et al. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. *J. Hepatol.* **48**, 945–951 (2008).
94. Kalambokis, G. N. et al. Rifaximin improves systemic hemodynamics and renal function in patients with alcohol-related cirrhosis and ascites. *Clin. Gastroenterol. Hepatol.* **10**, 815–818 (2012).
95. Vlachogiannakos, J. et al. Long-term administration of rifaximin improves the prognosis of patients with decompensated alcoholic cirrhosis. *J. Gastroenterol. Hepatol.* **28**, 450–455 (2013).
96. Kelly, C. R. et al. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. *Gastroenterology* **149**, 223–237 (2015).
97. Kao, D. et al. Fecal microbiota transplantation in the management of hepatic encephalopathy. *Hepatology* **63**, 339–340 (2016).
98. Bajaj, J. S. et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology* **66**, 1727–1738 (2017).
99. Vrieze, A. et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913–916 (2012).
100. Philips, C. A. et al. Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: a pilot study. *Clin. Gastroenterol. Hepatol.* **15**, 600–602 (2017).
101. Philips, C. A., Phadke, N., Ganesan, K., Ranade, S. & Augustine, P. Corticosteroids, nutrition, pentoxifylline, or fecal microbiota transplantation for severe alcoholic hepatitis. *Indian J. Gastroenterol.* **37**, 215–225 (2018).
102. Kang, D. J. et al. Gut microbial composition can differentially regulate bile acid synthesis in humanized mice. *Hepatol. Commun.* **1**, 61–70 (2017).
103. Wang, L. et al. Intestinal REG3 lectins protect against alcoholic steatohepatitis by reducing mucosa-associated microbiota and preventing bacterial translocation. *Cell Host Microbe* **19**, 227–239 (2016).
104. Chen, Y. et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* **54**, 562–572 (2011).
105. Kakiyama, G. et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. *J. Hepatol.* **58**, 949–955 (2013).

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Competing interests
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