Review

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Gut dysbiosis as a driver in alcohol-induced liver injury

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Summary

Alcohol-related liver disease characterises a broad spectrum of hepatic diseases that result from heavy alcohol use, and include alcohol-related steatosis, steatohepatitis, fibrosis, cirrhosis, and alcoholic hepatitis. Amongst heavy drinkers, progression to more severe forms of alcohol-related liver disease is not universal, with only 20% developing cirrhosis and up to one-third developing alcoholic hepatitis. Non-alcohol-related triggers for severe disease are not well understood, but the intestinal microbiome is thought to be a contributing factor. This review examines the role of the microbiome in mild alcohol-related liver disease, cirrhosis, and alcoholic hepatitis. While most of the literature discusses bacterial dysbiosis, we also discuss the available evidence on fungal (mycobiome) and virome alterations in patients with alcohol-related liver disease. Additionally, we explore the mechanisms by which the microbiome contributes to the pathogenesis of alcohol-related liver disease, including effects on intestinal permeability, bile acid dysregulation, and production of hepatotoxic virulence factors.

Keywords: Microbiome; Mycobiome; Virome; Alcohol; Cirrhosis; Hepatitis; Bile acids

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Introduction

The microbiome in health and disease

More than 100 trillion microbes reside within the human digestive system and are collectively referred to as the *gut microbiome*.[1] The composition of the microbiome can vary widely amongst individuals and is affected by numerous host factors. Beginning *in utero*, the human gut microbiome undergoes a variable evolution throughout the human lifecycle.[2] Its composition is affected by a multitude of elements (both modifiable and unmodifiable), including the birthing process, aging, geography, stress, exercise, and diet.[2] The variation of dietary and other environmental inputs, such as pharmaceuticals and alcohol consumption, can have a significant impact on the makeup of the microbiome.

The physiologic connection between the microbiome and the human host is expansive, and includes important roles in digestion, metabolism, and immunity. The microbiome produces essential nutrients and vitamins, notably vitamin K and B group vitamins.[3] It is also integral to fatty acid and glucose metabolism via independent production of short-chain fatty acids (SCFAs), such as butyrate and propionate, and induction of glucagon-like peptide 1 secretion.[1] The human immune system is also tightly connected with the intestinal microbial environment. The balance of commensal and pathologic bacteria is essential for homeostasis within the gut, but also for protection against various systemic disease states. Gut microbes are involved in mucosal immunity by directly contributing to the production of the mucus layer, but also indirectly by regulating the presence of immune cells within the lamina propria and maintaining the integrity of the intestinal-blood barrier.[1] The SCFAs produced by bacterial fermentation within the microbiome provide the necessary energy source for the adjacent enterocytes to uphold durable tight junctions.[4] Strong reinforcement of the intestinal-blood interface prevents translocation of luminal contents (including microbial products), which once in systemic circulation can trigger inflammatory changes in the liver and elsewhere in the body.[1] Dysbiosis of the gut microbiome has been implicated in the pathogenesis of many extraintestinal diseases, such as obesity, diabetes, autoimmune disease, neurodegenerative conditions, and certain malignancies.[1,5,6] However, the degree of evidence to determine causality between changes in the microbiome and these conditions is highly variable. This review will focus specifically on the enteric microbial changes associated with alcohol-related liver disease (ALD).

Spectrum of alcohol-related liver disease

ALD is a broad term encompassing a spectrum of liver pathologies that result from excessive alcohol intake.[7] Nearly all heavy alcohol drinkers will develop some degree of steatosis, which can develop within as little as 2 weeks of heavy alcohol use.[8] Though largely subclinical, approximately one-third of these patients will develop the histologic inflammation known as alcohol-related steatohepatitis.[8] This underlying hepatic inflammation is the driver of disease progression to fibrosis, and

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ultimately cirrhosis in up to 20% of cases.[8] With continued heavy drinking, this pathway to alcohol-related cirrhosis progresses in a relatively predictable linear fashion. Alcoholic hepatitis (AH), however, is a separate entity, and can coexist with any degree of underlying liver disease but occurs predominantly in patients with underlying cirrhosis. AH is characterised by hepatic inflammation, severe cholestasis, and a systemic inflammatory response. Disease severity varies widely, but mortality can be as high as 50% within 1 month.[8] A minority of patients with heavy alcohol use will develop AH (at any point in the cirrhosis pathway), but the triggers and non-alcohol related risk factors remain unclear.[8]

Key point

Only a minority of heavy drinkers will develop severe forms of alcohol-related liver disease, and there is evidence to suggest that the microbiome is a contributing factor to this disease progression.

Microbiome demonstrates pathologic and therapeutic potential in alcohol-related liver disease

Only a minority of heavy drinkers develop more severe forms of liver disease, and there are a variety of factors associated with progression such as sex, age, genetic factors, drinking pattern, obesity, smoking, and concomitant viral hepatitis infections.[9] There is increasing evidence to suggest that the gut microbiome might be an additional factor. For instance, when the microbiome of a patient with severe AH and a heavy drinking control are transplanted into germ-free mice and fed an ethanol-containing diet, a significantly higher degree of liver inflammation and intestinal permeability is induced in mice harbouring the microbiome of the patient with AH.[10] The liver injury is then ameliorated when the microbiome of a healthy control is subsequently transplanted into the mouse with hepatitis, despite ongoing alcohol intake.[10] While this study investigates the role of human dysbiosis in a mouse model, it is demonstrable of the microbiome's pathologic potential in ALD.

Historically, most studies investigate the effects of the bacterial microbiome on ALD. More recently there have been efforts to describe the other microbial inhabitants of the gut, such as commensal fungi and viruses, and their associations with ALD. For the present review, we searched the current literature on bacterial, viral, and/or fungal changes seen in all forms of ALD (including non-cirrhotic ALD, cirrhotic ALD, and AH). A literature search for changes in bile acids, SCFA production, and endotoxemia for all stages of ALD was subsequently performed. Search results were narrowed to faecal analysis in human patients with ALD.

Changes in the bacterial microbiome in alcohol-related liver disease

Bacterial dysbiosis in mild alcohol-related liver disease

Several studies have sought to evaluate changes in the intestinal microbiome in patients with ALD. When comparing the results of these studies, it is necessary to consider the significant variation in design and data collection methodology. The analysis often includes patients at different stages of ALD. We will first examine the changes in microbiota in patients with ALD without cirrhosis.

In the 1980s, Bode *et al.* were among the earliest to describe changes in intestinal bacteria in patients following heavy alcohol use.[11] Via analysis of jejunal aspirate cultures, they noted an increased quantitative bacterial burden within the small bowel of patients following high alcohol consumption.[11] There were, however, no significant differences between the jejunal microbiomes of patients with different stages of ALD.[11] With advances in faecal analysis by way of PCR fingerprinting, more recent literature has identified specific microbial changes in patients with ALD. Mutlu *et al.* described changes in the microbiome of heavy drinkers without cirrhosis. They utilised PCR fingerprinting from colonic biopsies to compare the microbiome in patients with alcohol use disorder (AUD) with or without mild liver disease (severe disease or cirrhosis excluded) to healthy controls. There was a stepwise reduction in *Bacteriodaceae* in healthy controls, heavy drinkers without liver disease, and patients with ALD.[12] Not all alcohol-consuming patients were dysbiotic, but among the subset who were, the microbiota were characterised by reductions in *Bacteroidetes* and increases in *Proteobacteria*.[12] There was no significant difference in alpha diversity between patients with AUD and healthy controls.[12]

Dubinkina *et al.* compared the enteric microbiome by shotgun metagenomic sequencing of patients with alcohol-related cirrhosis to patients with AUD and an external healthy control group. The AUD group included some patients with mild liver disease but excluded patients with evidence of cirrhosis or hepatic synthetic dysfunction. This group had distinct alterations, with notable increases in *Klebsiella pneumoniae*, *Lactobacillus salivarius*, *Citrobacter koseri*, and *Lactococcus lactis* subsp. *cremoris* compared with healthy controls.[13] Alternatively, genera *Akkermansia*, *Coprococcus*, and unclassified *Clostridiales* were significantly reduced in patients with AUD.[13] This study also did not observe significant changes in diversity between any of the groups.[13]

Bacterial dysbiosis in alcohol-related cirrhosis

Several studies have evaluated the microbiome in patients with more severe ALD. Of note, some studies included analysis of patients with any aetiology of cirrhosis. Furthermore, variables such as ongoing alcohol use or compensation of cirrhosis are not always controlled for. Comparator groups can be healthy controls, heavy alcohol users without liver disease, or patients with non-alcohol-related cirrhosis.

Chen *et al.* were among the first to evaluate the microbiome using 16s rRNA analysis in patients with cirrhosis. This smaller study compared the microbiome of patients with cirrhosis (1/3 of whom had AC) to healthy controls. The intestinal microbiome of the cirrhosis group was characterised by a reduction in the phylum *Bacteroidetes*, as well as enrichment of *Enterobacteriaceae*, *Veillonellaceae*, and *Streptococcaceae* at the family level.[14] The dysbiotic changes were largely irrespective of cirrhosis aetiology, with the exception of family *Prevotellaceae*, which was significantly more abundant in patients with alcohol-related cirrhosis than those with HBV-related cirrhosis.[14] It was thus postulated that this change might be more uniquely related to alcohol metabolism in the gut. Cirrhotic patients had reduced bacterial diversity, though this did not reach statistical significance.[14]

In a similar study by Kakiyama *et al.*, *Enterobacteriaceae* and *Veillonellaceae* were increased in the microbiota of patients with cirrhosis compared to healthy controls.[15] Notably, *Lachonospiraceae* and *Ruminococcaceae* families were reduced in the cirrhotic gut.[15] There was also a significant reduction in the genus *Blautia*, an enteric anaerobe of the *Lachanospiraceae* family.[15] While alcohol-related cirrhosis was included, it only

comprised about 15% of the cirrhosis group, and results were not stratified by aetiology.

The degree of dysbiosis also appears to correlate with the severity of cirrhosis. The cirrhosis dysbiosis ratio (CDR) was developed by Bajaj *et al.*, and can be used to quantify the extent of dysbiosis in patients with cirrhosis.[16] More specifically, the CDR is the proportion of classically commensal enteric microbes (such as *Lachnospiraceae*, *Ruminococcaceae*, *Veillonellaceae,* and *Clostridialies Incertae Sedis XIV*) to potentially pathogenic taxa associated with cirrhosis (such as *Enterobacteriaceae* and *Bacteroidaceae*), with lower values equating to more dysbiosis.[16] While *Veillonellaceae* is included in the autochthonous category, its degree of pathogenicity in ALD appears to be more complex and its abundance is sometimes seen to increase in patients with cirrhosis.[13–15,17] Model for end-stage liver disease (MELD) scores correlate negatively with the CDR, suggesting that there is more dysbiosis in the microbiome as liver disease worsens. While this analysis by Bajaj *et al.* includes all aetiologies of cirrhosis, a *post hoc* analysis comparing dysbiosis in alcohol-related cirrhosis to non-alcoholic cirrhosis showed a comparative enrichment of *Enterobacteriaceae* and *Halomonodaceae* and a further reduction of *Lachnospiraceae*, *Ruminococcaceae*, and *Clostridialies XIV* in the alcohol-related cirrhosis group.[16] Thus, despite similar MELD scores, alcohol-related cirrhosis was associated with a lower CDR.[16]

Dubinkina *et al.* performed one of the few studies investigating microbial alterations solely in alcohol-related cirrhosis. Microbiome analysis by shotgun metagenomic sequencing has the advantage of distinguishing alterations at the species level. They noted an increase in *Bifidobacterium* (*B. longum, dentium,* and *breve*), *Streptococcus* (*S. thermophilus* and *mutans*), and *Lactobacillus* (*L. salivarius*, *antri*, and *crispatus*) within the microbiomes of the alcohol-related cirrhosis group compared to healthy controls,[13] as well as a significant decline in *Paraprevotella*, *Alistipes,* and *Prevotella* .[13] Of note, this is seemingly discordant with the findings of Chen *et al.*, who proposed that increased *Prevotellaceae* (of which *Paraprevotella* and *Prevotella* are descendants) might be specific to alcohol-related changes.[14] Both *Lactobacillus* and *Bifidobacterium* are largely considered to be beneficial inhabitants of the microbiome and are commonly incorporated into probiotic supplements.[13] A direct comparison between 2 alcohol-consuming groups found that patients with alcohol-related cirrhosis had higher quantities of *Streptococcus constellatus*, *Streptococcus salivarius*, *Veillonella atypica*, *Veillonella dispar*, and *Veillonella parvula* compared to patients with AUD without cirrhosis.[13] These microbes are common inhabitants of the oral cavity, which suggests that the intrusion of oral microbes into the enteric environment might be triggered after the onset of liver injury. The postulated mechanism for the distal migration of oral microbiota in liver disease is related to the dysregulation of enterohepatic bile acid circulation (discussed in detail later in this review). It is important to note that proton pump inhibitors (PPIs) also promote oralization of the intestinal microbiome by reducing gastric acidity, however, Dubinkina *et al.* excluded patients taking PPIs to eliminate this confounder. Microbiome heterogeneity was similar between all groups, with no difference in alpha diversity between patients with AUD, alcohol-related cirrhosis, or controls.[13]

Active drinking was not consistently controlled for in the aforementioned studies. In 2017, Bajaj *et al.* sought to explore the effects of continued drinking on the microbiome in patients with alcohol-related cirrhosis. They compared 16s rRNA from faecal samples and from mucosal biopsies taken at various points in the alimentary tract between patients who were or were not actively drinking. There was a significant reduction in *Lachnospiraceae, Ruminococcaeae,* and *Clostridiales cluster XIV* in faecal samples and all mucosal samples of the actively drinking cirrhotic group compared to the abstinent cirrhotic group and the control group.[18]

Most recently, Addolorato *et al.* published their analysis of the microbiome of patients with ALD. They utilised 16s rRNA sequencing and compared 36 active drinkers with at least stage F2 hepatic fibrosis (including 14 with cirrhosis) to an equal number of non-drinking healthy controls. Overall, their microbiomes were significantly different by principal coordinate analysis and there was a significant reduction in alpha diversity in the ALD group.[17] The changes which characterised the dysbiosis are vast and at multiple taxonomic levels. At the genus level, some of the notable expansions in patients with ALD include: *Staphylococcus, Paraprevotella, Streptococcus, Veillonella, Enterococcus, Lactobacillus, Bilophila, Citrobacter, Turicibacter, Desulfovibrio, Parabacteroides, Bacteroides,* and *Prevotella* genera. Some significant genera reductions include*: Akkermansia, Blautia, Bifidobacterium, Anaerostipes,* and *Ruminococcus*.[17] Patients with alcohol-related cirrhosis had a significant expansion in *Methanobrevibacter* (genus of anaerobic archaea) and a significant reduction in *Catenibacterium* (a known SCFA producer) compared to the patients with advanced fibrosis.[17] It is important to note that 25% of patients in the ALD group were diagnosed with AH, which is associated with distinct alterations to the microbiome.

Bacterial dysbiosis in alcoholic hepatitis

Investigation of the microbiome in patients with AH may improve our understanding of the variability of disease presentation. In the few studies which have examined the microbiome in patients with AH, there is significant variation in size and design, and most do not control for underlying cirrhosis (since these entities very often coexist). All of the studies evaluating faecal samples utilised 16s rRNA pyrosequencing.

Llopis *et al.* performed one of the first studies analysing the microbiome in patients with AH. They studied the microbiome in hospitalised patients with AUD, non-severe AH, and severe AH (by histologic scoring system). The prevalence of cirrhosis in each group was not specified. They noted a reduction in *Atopobium*, but an enrichment in *Streptococci*, *Bifidobacteria*, and *Enterobacteria* in patients with severe AH.[10] These changes were not seen in the comparison between non-severe AH and heavy drinking controls, suggesting that these changes are unique to severe disease.[10] Furthermore, they noted that *Streptococci* and *Enterobacteria* abundance correlated positively with disease severity determined by the histologic AH score.[10] Conversely, *Atopobium* and *Clostridium leptum* negatively correlated with serum bilirubin and degree of fibrosis.[10]

In 2018, Ciocan *et al.* conducted a study comparing the microbiome of patients with severe AH (by histologic scoring system) to patients with chronic alcoholic pancreatitis and alcoholic controls. All patients in the AH group also had cirrhosis. When comparing the AH group to alcoholic controls, there were numerous expansions at the genus level including: *Lactobacillus*, *Bifidobacterium*, *Haemophilus*, *Enterococcus*, *Streptococcus, Rothia,* and *Aggregatibacter*.[19] In contrast to the prior study, Ciocan *et al.* also noted an expansion of *Atopobium* in patients with AH.[10,19] The genera *Ruminococcus*, *Parabacteroides*, *Bilophila,*

Odoribacter, Desulfovibrio, and *Oscillospira* were diminished in patients with AH.[19] There was no difference in alpha-diversity between patients with AH and alcoholic controls.[19]

Key point

Patients with alcohol-related cirrhosis and alcoholic hepatitis have an enrichment of more commonly pathogenic taxa, such as *Enterobacteriaceae, Streptococcaceae, and Enterococcus*.

Most recently, Smirnova *et al.* compared the microbiome of patients with moderate or severe AH to heavy drinking and non-drinking controls. The presence of cirrhosis was not specified. When comparing all patients with AH to the heavy drinking controls, they observed an increase in *Fusobacterium, Megasphaera,* and *Veillonella*.[20] Like Ciocan *et al.*, they also noted expansion of *Atopobium* in patients with AH, though other genera of the *Coriobacteriaceae* family were comparatively reduced.[19,20] Consistent with prior literature, patients with AH had a reduction in multiple genera of the SCFA producers *Lachnospiraceae* and *Ruminococcaceae*.[19,20] The overall composition of the microbiome could distinguish patients with AH from heavy drinking controls, though the most discriminating taxa were observed at minute levels.[20] Among the top 20 taxa which comprised the predictive model, *Veillonella* and *Bacteroides* were the most abundant genera enriched in patients with AH, whereas *Lachnospiraceae Lachnospiracea incertae sedis* was more abundant in the heavy drinkers without hepatitis.[20] There was an overall reduction in alpha diversity in patients with AH compared to both healthy and heavy drinking controls.[20] Results were then stratified by AH severity, with MELD score greater than 20 characterising severe disease. A direct comparison between the AH groups demonstrated that patients with severe disease had higher quantities of *Actinomyces* and *Fusobacterium*, while those with moderate disease had comparatively more *Blautia, Dorea, Sporacetigenium*, and *Hydrogenoanaerobacterium*.[20]

Key point

There is a reduction in short-chain fatty acid-producing bacteria, such as *Lachonospiraceae* and *Ruminococcaceae*, within the microbiome of patients with alcohol-related liver disease.

There is evidence to suggest that particular microbiome alterations are associated with disease severity in patients with AH. We recently completed an analysis of faecal samples of 74 patients with AH from 9 centres internationally, with particular attention paid to the association between dysbiosis and disease severity. As a surrogate for disease severity, severe hyperbilirubinemia was associated with a significant reduction in unclassified *Enterobacteriaceae* and *Akkermansia.*[21] The most significant expansions were among taxa of very low abundance, however, there was a prominent expansion of *Veillonella* in the high bilirubin group (the relative abundance of which also positively correlated with degree of hyperbilirubinemia on a continuum).[21] A MELD score exceeding 21 was associated with increases in unclassified *Neisseriaceae* and reductions in unclassified *Clostridiales*, unclassified *Prevotellaceae*, *Anaerostipes*, and *Morganella*.[21] There was a significant reduction in alpha diversity in the high MELD group, and increasing MELD scores correlated with reduced diversity (as a continuous variable).[21] Patients with coexisting cirrhosis on liver biopsy had a significant increase in *Clostridium sensu stricto* and unclassified *Gammaproteobacteria* compared to non-cirrhotic patients with AH.[21] There was an increase in *Enterococcus, Bifidobacterium, Lactococcus, Oribacterium, Desulfovibrio*, and *Veillonella* in patients with high grade steatosis.[21] *Veillonella* was also more abundant in patients with a more severe hepatic inflammation on liver biopsy.[21]

Akkermansia is largely considered to be a beneficial inhabitant of the microbiome, and its role in fatty acid metabolism contributes to the barrier function of the human intestine.[22] Depletion of *Akkermansia* is implicated in cirrhosis secondary to non-alcoholic steatohepatitis, however, there is increasing evidence of its involvement in the dysbiosis of ALD.[17,21,22] In 2018, Grander *et al.* specifically evaluated changes in *Akkermansia* abundance and found a significant reduction in *Akkermansia muciniphila* in patients with AH compared to healthy controls.[22] *Akkermansia muciniphila* abundance was significantly negatively correlated with histologic disease severity and degree of fibrosis.[22] They further demonstrated that supplementation of *Akkermansia* was protective against the development of ethanol-mediated liver disease and could reduce already established ethanol-induced liver disease in mouse models.[22]

Common themes in bacterial dysbiosis in patients with alcohol-related liver disease

Overall, there are significant enteric microbial alterations in patients with various stages of ALD. Drawing conclusions about the microbial makeup of these patients is limited by the wide variation in study design and methodology. Most of the aforementioned studies are from a single centre and sample sizes are often small. The definition of liver disease severity varies, and many studies do not utilise liver biopsy to evaluate fibrosis stage or histologic inflammation. Despite these limitations, there are several common themes in microbial composition worth mentioning. For instance, while there appears to be no difference in alpha diversity in patients with AUD or mild ALD compared to healthy controls,[12,13] there is a trend towards reduced diversity in patients with alcohol-related cirrhosis.[14,17] Enteric microbial diversity is similarly reduced in patients with AH, and there is evidence to support a correlation between reduced diversity and disease severity.[20,21]

Key point

There is an overall reduction in bacterial diversity in the microbiome of patients with all forms of alcohol-related liver disease.

In terms of specific bacterial taxa, there are some common microbial associations in patients with ALD. Alcohol use alone, without the presence of significant liver disease, is associated with reductions in *Bacteroidaceae* and increases in *Proteobacteria* more broadly.[12,13] A higher prevalence of studies investigating alcohol-related cirrhosis and AH has enabled characterisation of microbial changes at more precise taxonomic levels. Alcohol-related cirrhosis and AH are both associated with reductions in *Lachonospiraceae* and *Ruminococaceae*, which are SCFA producers and widely considered beneficial inhabitants of the microbiome.[15,16,18,20] Reductions in *Clostridiales XIV* and *Blautia* are more consistently seen in alcohol-related cirrhosis ,[15–18] while diminishment of *Akkermansia* is described in patients with AH.[21,22] The commonly pathogenic families *Enterobacteriaceae* and *Streptococcaceae* are increased in both diseases,[10,13–17,19,20] as well as the genus *Enterococcus*.[16,17,19,21] Interestingly, they also share common enrichments in the genera *Bifidobacterium* and *Lactobacillus*, which are usually viewed as beneficial

Table 1. Summary of compositional microbiota changes in patients with alcohol-related liver diseases.

	AUD/mild ALD	Alcohol-related cirrhosis	AH
Bacterial changes	↓*Bacteriodaceae* (f) ↓*Akkermansia* (g) ↓*Coprococcus* (g) ↑*Proteobacteria* (p) ↑*Klebsiella pneumoniae* (s) ↑*Lactobacillus salivarius* (s) ↑*Citrobacter koseri* (s) ↑*Lactococcus lactis* subsp. *cremoris* (s)	↓*Lachonospiraceae* (f) ↓*Ruminococcaceae* (f) ↓*Clostridiales XIV* (f) ↓*Blautia* (g) ↑*Enterobacteriaceae* (f) ↑*Streptococcaceae* (f) ↑*Bifidobacterium* (g) ↑*Streptococcus* (g) ↑*Lactobacillus* (g) ↑*Enterococcus* (g)	↓*Lachnospiraceae* (f) ↓*Ruminococcaceae* (f) ↓*Ruminococcus* (g) ↓*Akkermansia* (g) ↑*Fusobacterium* (g) ↑*Veillonella* (g) ↑*Streptococcus* (g) ↑*Enterobacteria* (g) ↑*Lactobacillus* (g) ↑*Bifidobacterium* (g) ↑*Enterococcus* (g)
Fungal changes	↓*Epicoccum* (g) ↓*Galactomyces* (g) ↓*Debaryomyces* (g) ↑*Candida* (g)	↑*Candida* (g)	↓*Penicillum* (g) ↑*Candida* (g)
Viral changes			↑*Parvoviridae* ↑*Herpesviridae* ↑*Epstein-Barr virus* ↑*Staphylococcus phages* ↑*Escherichia phages* ↑*Enterobacteria phages* ↑*Enterococcus phages*

AH, alcoholic hepatitis; ALD, alcohol-related liver disease; AUD, alcohol use disorder.

commensals.[13,17,19] Literature describing the microbiome of patients with AH has recurrently demonstrated expansion in *Veillonella*,[20,21] however, this is less consistent in alcohol-related cirrhosis studies.[13,14,16,17]

Fungal dysbiosis in patients with alcohol-related liver disease

Until recently, investigation of microbial alterations and associations with ALD has been limited to the bacterial domain. Fungi are commensal in the human intestinal tract and fungal dysbiosis is similarly associated with progression of ALD.[23] There are only a few studies which analysed the mycobiome in patients with ALD.[23–25] In 2017, Yang *et al.* compared faecal samples from 4 patients with alcohol-related cirrhosis, 6 with AH, 10 with mild ALD, and 8 healthy controls. Alcohol-consuming groups exhibited a significant reduction in fungal diversity, coupled with a significant expansion in *Candida* and diminishment in *Epicoccum*, unclassified fungi, *Galactomyces*, and *Debaryomyces*.[23] Lang *et al.* compared the mycobiota of 59 patients with AH to 15 patients with AUD (varying degree of liver disease) and 11 non-drinking controls. Their results supported the prior study, demonstrating less fungal diversity and enrichment of *Candida* in alcohol-consuming patients.[24] *Penicillium* was the most abundant genus in the control group and was significantly reduced in alcohol-consuming groups.[24] Despite the marked differences in degree of liver disease, there were no significant mycobiome differences between patients with AUD and AH.[24] Bajaj *et al.* also noted expansion of *Candida* in patients with cirrhosis, one-third of whom had ALD.[25]

Key point

Dysbiosis of the mycobiome in patients with alcohol-related liver disease is characterised by an increased abundance of *Candida* and reduction in fungal diversity.

Intestinal virome changes in patients with alcohol-related liver disease

The human digestive tract is also inhabited by numerous commensal viruses, which collectively comprise the enteric virome. It is predominantly made up of bacteriophages (lytic phages which can infect and lyse bacterial hosts), but also includes eukaryotic viruses, many of which are known to cause human disease.[26] Alterations in the enteric virome have been seen in certain gastrointestinal conditions such as inflammatory bowel disease and colorectal malignancy, however, there is a paucity of data in patients with liver disease.[27,28] Jiang *et al.* utilised a multicentre and international design to specifically extract and analyse virus-like particles from faecal samples of patients with AH, AUD, and healthy controls.

Patients with AH had a marked increase in mammalian viruses, particularly *Parvoviridae* and *Herpesviridae*.[29] The *Herpesviridae* family was exclusively found in patients with AH, and was predominantly comprised of Epstein-Barr virus, which is a well described hepatic pathogen.[29] *Herpesviridae*, as well as *Staphylococcus* phages, were associated with higher MELD scores and increased mortality.[29] Other bacteriophages were more abundant in the AH group, including *Escherichia*-, *Enterobacteria*-, and *Enterococcus* phages.[29] In contrast to bacterial and fungal diversity, viral diversity was increased in patients in alcohol-consuming groups and was most pronounced in patients with AH.[29] This may be attributed to phage-bacteria dynamics and incorporation of phage genetic material into their bacterial hosts. The presence of phages often (though not always) correlates positively with their respective hosts.[29] In contrast, the virome in patients with non-alcoholic fatty liver disease does not show an enrichment in eukaryotic viruses, therefore this seems to be specific for AH.[30]

Table 1 summarises the most common changes of the bacterial microbiota, mycobiome and virome in patients with ALD.

Key point

Patients with alcohol-related liver disease demonstrate an expansion of eukaryotic viruses and increased viral diversity in the enteric environment known as the virome.

Key point

Alcohol-related dysbiosis is associated with deleterious shifts in bile acid quantity and composition, which are implicated in the pathogenesis of alcohol-related liver disease.

Mechanisms of dysbiosis-driven alcohol-related liver disease

Dysregulation of bile acid metabolism

Due to the close physiologic relationship between the gut and the liver, enteric dysbiosis is thought to be a central component to ALD and possibly one of the drivers of disease progression from simple steatosis to more advanced disease. The gut-liver axis is a well described bidirectional relationship, whereby the luminal components (delivered by portal circulation) affect liver physiology and disease, and hepatic-derived components (delivered by way of the biliary system) affect the makeup of the luminal environment. The enterohepatic circulation of bile acids, for example, is critically important to gut eubiosis. Nearly all of the primary bile acids secreted into the intestines are reabsorbed back into the portal circulation and reused by the liver, while the remaining 5% are converted into secondary bile acids by the colonic microbiota.[4] Thus, disruption of the normal intestinal microbiota can change bile acid metabolism, augment the degree of secondary bile acid conversion, and therefore reduce the rate of primary bile acid reabsorption .[4] The signalling pathway involved in primary bile acid reabsorption involves activation of the farnesoid x receptor (FXR), which results in the production of antimicrobial peptides in the lumen .[4] Thus, reduced utilisation of this pathway makes the intestinal environment more susceptible to bacterial overgrowth. Furthermore, FXR has also been shown to modulate liver inflammation.[31]

As a compensatory response to an increase in total bile acid burden, the ileal enterocytes generate fibroblast growth factor 19 (FGF19), which travels to the liver by way of the portal vein and is responsible for negative feedback on *de novo* bile acid synthesis.[32] There is evidence to suggest that alcohol-related dysbiosis is associated with the deleterious shifts in bile acid quantity and composition described above. Several studies have shown a significant increase in secondary bile acids among actively drinking patients with liver disease.[10,18,33] The cholestatic nature of severe forms of ALD contributes to an increase in total bile acids, triggering high concentrations of circulating FGF19 and a reduction in *de novo* bile acid synthesis.[32] Patients with AH have marked elevations in both circulating and hepatic expression of FGF19, a finding which is not observed in patients with non-alcoholic steatohepatitis.[32] Additionally, the concentration of FGF19 correlates positively with MELD score in patients with AH, suggesting an association with disease severity.[32]

Patients with AUD have higher amounts of faecal secondary bile acids and ileal bile acid transporters (namely, the apical sodium-dependent bile acid transporter) [18] and expression of inflammatory cytokines in colonic mucosa,[33] which would allow translocation of luminal products to the portal circulation. Mice transplanted with the microbiome of humans with AH have been found to have reductions in the primary bile acid chenodeoxycholic acid and its secondary bile acid derivative ursodeoxycholic acid, which has been used as a therapeutic tool in liver disease (such as primary biliary cholangitis) .[10,34] These findings demonstrate that the physiology of the gut microbiome and bile acid metabolism are intimately connected, with the composition of each being highly dependent on the function of the other (dysfunction of either can lead to hepatic inflammation).

Microbial products contribute to liver inflammation and disease

The liver, being the first organ to see the unadulterated intestinal products, is highly susceptible to toxins absorbed into the portal circulation. Microbial products including bacterial endotoxins (such as lipopolysaccharide [LPS] secreted by gram-negative bacteria), bacterial exotoxins (such as cytolysin secreted by *Enterococcus*), fungal exotoxins (such as candidalysin), and microbial pathogen-associated molecular patterns (PAMPs) from all types of microbiota can promote hepatocellular injury. Endotoxins bind hepatic toll-like receptors and PAMPs directly bind to pattern-recognition receptors on Kupffer and hepatic stellate cells. All of the above microbial products can result in an inflammatory cascade of cytokine activation, oxidative stress, and fibrotic changes .[4]

Particular exotoxins have demonstrated pathogenicity in patients with ALD. The abundance of cytolysin-producing *Enterococcus faecalis* is increased in patients with AH compared with heavy drinking controls, with the amount of cytolysin correlating with both the severity of disease and mortality.[35] The fungal exotoxin candidalysin is similarly seen in higher concentrations in patients with AH and is associated with disease severity and mortality.[36] Mice colonised with candidalysin-producing *Candida* develop worse liver injury after an ethanol-containing diet.[36]

There appears to be a link between the dysbiosis in ALD and the degree of circulating endotoxin. Several of the aforementioned studies demonstrating dysbiosis associated with ALD also note a concomitant rise in circulating LPS, and the correlation holds true for alcohol-related cirrhosis and AH.[12,16,17,37] In a comparison of alcohol *vs.* non-alcohol induced cirrhosis, there appears to be a higher degree of endotoxemia in alcohol-related cirrhosis, despite similar MELD scores.[16] Gut permeability is likely a key facilitator of endotoxemia. Notably, only about half of patients with AUD demonstrate increased intestinal permeability, which is associated with alterations to the microbiome.[38,39] Thus, dysbiosis appears to be an important prerequisite for gut permeability and progression to ALD.[38,39]

Short-chain fatty acids

Regulation of intestinal permeability involves numerous processes, many of which are affected by alcohol use. Chronic alcohol consumption weakens enterocyte tight junctions both directly and through dysbiosis characterised by shifts away from the SCFA-producing commensals involved in maintaining barrier integrity.[4] In addition, SCFAs can attenuate hepatic adiposity and inflammation.[40] Genera of the *Lachnospiraceae* and *Ruminococcaceae* families are well-described SCFA producers, and their reduction in the microbiomes of patients with all forms of ALD has been demonstrated with relative consistency.[13,15–17,19,20] Conversely, *Veillonella* is also known to produce SCFAs and is often expanded in patients with ALD.[13–15,17,20,21] Regardless of particular microbial changes, patients with AH have been shown to have a quantitative reduction of stool SCFAs compared to heavy drinking controls.[20] Overall, a waning production of SCFAs is postulated to create a more permeable gut membrane and contribute to hepatic inflammation.

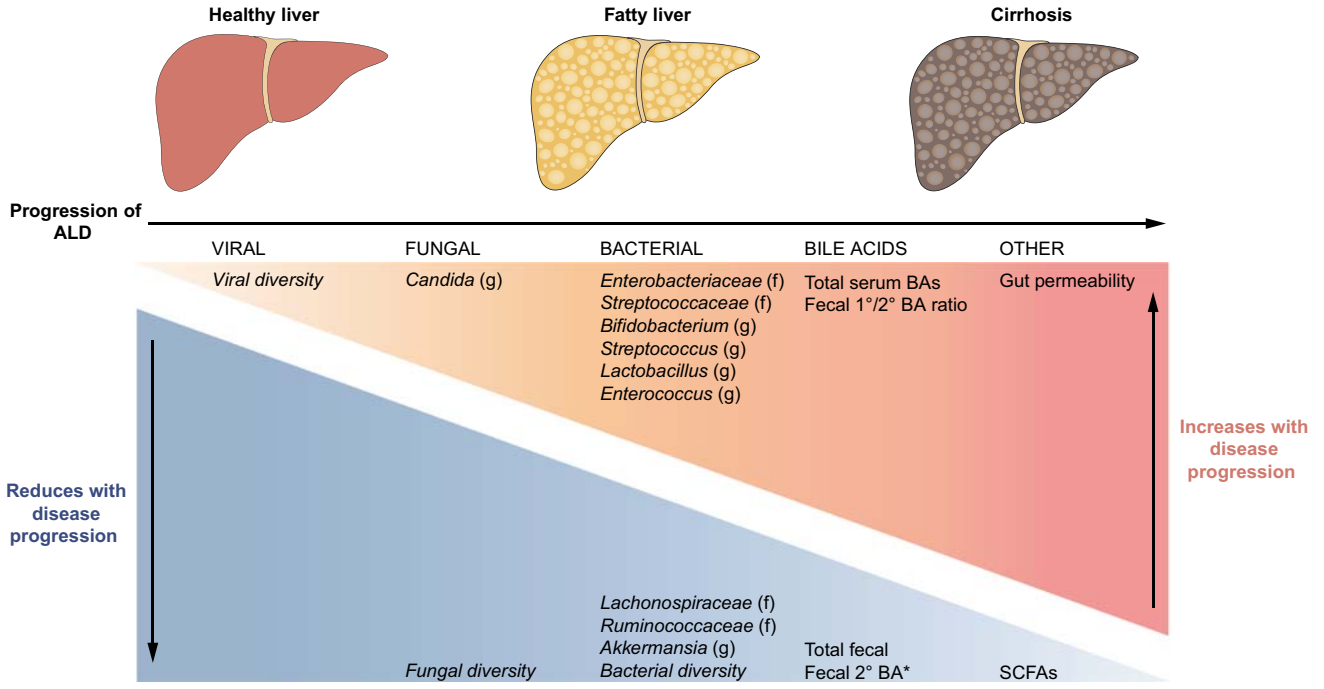

Fig. 1. Summary of major microbial changes associated with progression of alcohol-related liver disease. Alcohol-associated dysbiosis is characterised by changes in bacteria, fungi and viruses during the onset and progression of ALD. Dysbiosis contributes to liver disease via different mechanisms, which include increased intestinal permeability, changes in bile acids and in bacterial metabolites such as short-chain fatty acids. *Active drinking is independently associated with an increase in faecal secondary bile acids regardless of disease stage. 1°, primary; 2°, secondary; ALD, alcohol-related liver disease; BA, bile acid; g, genus; f, family; SCFA, short-chain fatty acid.

Conclusion

There are well described modifications in microbiome composition in patients with all stages of ALD. Most of the available literature focuses on bacterial alterations in alcohol-related cirrhosis and AH. Microbiomes of both groups are largely characterised by reductions in beneficial commensals, such as *Lachnospiraceae* and *Ruminococcaceae,* and enrichment in more pathogenic taxa, such as *Enterobacteriacea, Streptococcaceae*, and *Enteroccocus*. There are also changes in the enteric mycobiome in ALD, characterised by increased abundance of *Candida*. Patients with ALD often exhibit a reduction in both bacterial and fungal diversity compared to healthy controls. The virome increases in diversity, incorporates more eukaryotic viruses (such as *Herpesviridae*), and demonstrates an increase in bacteriophages which correspond with more pathologic bacterial hosts. Mechanisms by which the microbiome might exert its pathologic potential in ALD are multifactorial. The underlying premise postulates that a dysbiotic shift away from taxa which reinforce barrier integrity (by way of SCFA production) and towards taxa which dysregulate bile acid metabolism and produce exo- and endotoxins drives the liver injury observed in ALD. Studies vary significantly in methodology and quality of design, limiting definitive conclusions regarding the role of dysbiosis in ALD. A summary of the major changes in microbial composition, bile acid makeup, enteric SCFAs, and overall gut permeability in ALD is illustrated in Fig. 1. The data suggest that the microbiome or its byproducts might make an attractive therapeutic target for ALD. Only a handful of studies have examined the use of probiotics or faecal microbiota transplant in patients with ALD.[22,41–43] There is some evidence to suggest that faecal microbiota transplant improves survival compared to standard medical therapies in patients with AH, however, these trials are underpowered.[44,45] Further mechanistic studies are needed to clarify the role of the microbiome in driving the hepatoxicity observed in ALD and to elucidate potential therapeutic strategies.

Key point

The microbiome could be an attractive therapeutic target in alcohol-related liver disease, though most current studies on probiotics or faecal microbiota transplant are small and lack long-term follow-up data.

Abbreviations

AH, alcoholic hepatitis; ALD, Alcohol-related liver disease; AUD, alcohol use disorder; CDR, cirrhosis dysbiosis ratio; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; LPS, lipopolysaccharide; MELD, model for end-stage liver disease; PAMPs, pathogen-associated molecular patterns; PPI, proton pump inhibitor; SCFA, short-chain fatty acid.

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Conflicts of interest

B.S. has been consulting for Ferring Research Institute, Intercept Pharmaceuticals, HOST Therabiomics, Mabwell Therapeutics, Patara Pharmaceuticals and Takeda. B.S.'s institution UC San Diego has received grant support from BiomX, NGM Biopharmaceuticals, CymaBay Therapeutics, Synlogic Operating Company and Axial Biotherapeutics.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

B.F. wrote the manuscript and B.S. edited the manuscript.

Supplementary data

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References

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[1] Cani PD. Human gut microbiome: hopes, threats and promises. Gut 2018;67(9):1716–1725.
[2] Cresci GA, Bawden E. Gut microbiome: what we do and don't know. Nutr Clin Pract 2015;30(6):734–746.
[3] Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr 2018;57(1):1–24.
[4] Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, et al. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018;15(7):397–411.
[5] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007;56(7):1761–1772.
[6] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444(7122):1027–1031.
[7] Masarone M, Rosato V, Dallio M, Abenavoli L, Federico A, Loguercio C, et al. Epidemiology and natural history of alcoholic liver disease. Rev Recent Clin Trials 2016;11(3):167–174.
[8] Singal AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG clinical guideline: alcoholic liver disease. Am J Gastroenterol 2018;113(2):175–194.
[9] Osna NA, Donohue Jr TM, Kharbanda KK. Alcoholic liver disease: pathogenesis and current management. Alcohol Res 2017;38(2):147–161.
[10] Llopis M, Cassard AM, Wrzosek L, Boschat L, Bruneau A, Ferrere G, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Gut 2016;65(5):830–839.
[11] Bode JC, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. Hepatogastroenterology 1984;31(1):30–34.
[12] Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol 2012;302(9):G966–G978.
[13] Dubinkina VB, Tyakht AV, Odintsova VY, Yarygin KS, Kovarsky BA, Pavlenko AV, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. Microbiome 2017;5(1):141.
[14] Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 2011;54(2):562–572.
[15] Kakiyama G, Pandak WM, Gillevet PM, Hylemon PB, Heuman DM, Daita K, et al. Modulation of the fecal bile acid profile by gut microbiota in cirrhosis. J Hepatol 2013;58(5):949–955.
[16] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol 2014;60(5):940–947.
[17] Addolorato G, Ponziani FR, Dionisi T, Mosoni C, Vassallo GA, Sestito L, et al. Gut microbiota compositional and functional fingerprint in patients with alcohol use disorder and alcohol-associated liver disease. Liver Int 2020.
[18] Bajaj JS, Kakiyama G, Zhao D, Takei H, Fagan A, Hylemon P, et al. Continued alcohol misuse in human cirrhosis is associated with an impaired gut-liver Axis. Alcohol Clin Exp Res 2017;41(11):1857–1865.
[19] Ciocan D, Rebours V, Voican CS, Wrzosek L, Puchois V, Cassard AM, et al. Characterization of intestinal microbiota in alcoholic patients with and without alcoholic hepatitis or chronic alcoholic pancreatitis. Sci Rep 2018;8(1):4822.
[20] Smirnova E, Puri P, Muthiah MD, Daitya K, Brown R, Chalasani N, et al. Fecal microbiome distinguishes alcohol consumption from alcoholic hepatitis but does not discriminate disease severity. Hepatology 2020.
[21] **Lang S**, **Fairfied B**, Gao B, Duan Y, Zhang X, Fouts DE, et al. Changes in the fecal bacterial microbiota associated with disease severity in alcoholic hepatitis patients. Gut Microbes 2020:1–12.
[22] Grander C, Adolph TE, Wieser V, Lowe P, Wrzosek L, Gyongyosi B, et al. Recovery of ethanol-induced Akkermansia muciniphila depletion ameliorates alcoholic liver disease. Gut 2018;67(5):891–901.
[23] Yang AM, Inamine T, Hochrath K, Chen P, Wang L, Llorente C, et al. Intestinal fungi contribute to development of alcoholic liver disease. J Clin Invest 2017;127(7):2829–2841.
[24] Lang S, Duan Y, Liu J, Torralba MG, Kuelbs C, Ventura-Cots M, et al. Intestinal Fungal Dysbiosis and Systemic Immune Response to Fungi in Patients With Alcoholic Hepatitis. Hepatology 2020;71(2):522–538.
[25] Bajaj JS, Liu EJ, Kheradman R, Fagan A, Heuman DM, White M, et al. Fungal dysbiosis in cirrhosis. Gut 2018;67(6):1146–1154.
[26] Shkoporov AN, Clooney AG, Sutton TDS, Ryan FJ, Daly KM, Nolan JA, et al. The human gut virome is highly diverse, stable, and individual specific. Cell Host Microbe 2019;26(4):527–541. e5.
[27] Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 2015;160(3):447–460.
[28] Nakatsu G, Zhou H, Wu WKK, Wong SH, Coker OO, Dai Z, et al. Alterations in enteric virome are associated with colorectal cancer and survival outcomes. Gastroenterology 2018;155(2):529–541. e5.
[29] Jiang L, Lang S, Duan Y, Zhang X, Gao B, Chopyk J, et al. Intestinal virome in patients with alcoholic hepatitis. Hepatology 2020.
[30] Lang S, Demir M, Martin A, Jiang L, Zhang X, Duan Y, et al. Intestinal Virome Signature Associated With Severity of Nonalcoholic Fatty Liver Disease. Gastroenterology 2020.
[31] Wu WB, Chen YY, Zhu B, Peng XM, Zhang SW, Zhou ML. Excessive bile acid activated NF-kappa B and promoted the development of alcoholic steatohepatitis in farnesoid X receptor deficient mice. Biochimie 2015;115:86–92.
[32] Brandl K, Hartmann P, Jih LJ, Pizzo DP, Argemi J, Ventura-Cots M, et al. Dysregulation of serum bile acids and FGF19 in alcoholic hepatitis. J Hepatol 2018;69(2):396–405.
[33] Kakiyama G, Hylemon PB, Zhou H, Pandak WM, Heuman DM, Kang DJ, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. Am J Physiol Gastrointest Liver Physiol 2014;306(11):G929–G937.
[34] Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med 1994;330(19):1342–1347.
[35] Duan Y, Llorente C, Lang S, Brandl K, Chu H, Jiang L, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. Nature 2019;575(7783):505–511.
[36] Chu H, Duan Y, Lang S, Jiang L, Wang Y, Llorente C, et al. The Candida albicans exotoxin candidalysin promotes alcohol-associated liver disease. J Hepatol 2020;72(3):391–400.
[37] Puri P, Liangpunsakul S, Christensen JE, Shah VH, Kamath PS, Gores GJ, et al. The circulating microbiome signature and inferred functional metagenomics in alcoholic hepatitis. Hepatology 2018;67(4):1284–1302.
[38] Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A 2014;111(42):E4485–E4493.
[39] Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, et al. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. Gut Microbes 2020;12(1):1782157.
[40] Sahuri-Arisoylu M, Brody LP, Parkinson JR, Parkes H, Navaratnam N, Miller AD, et al. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. Int J Obes (Lond) 2016;40(6):955–963.
[41] Dhiman RK, Rana B, Agrawal S, Garg A, Chopra M, Thumburu KK, et al. Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. Gastroenterology 2014;147(6):1327–1337. e3.
[42] Han SH, Suk KT, Kim DJ, Kim MY, Baik SK, Kim YD, et al. Effects of probiotics (cultured Lactobacillus subtilis/Streptococcus faecium) in the

treatment of alcoholic hepatitis: randomized-controlled multicenter study. Eur J Gastroenterol Hepatol 2015;27(11):1300–1306.
[43] Kirpich IA, Solovieva NV, Leikhter SN, Shidakova NA, Lebedeva OV, Sidorov PI, et al. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol 2008;42(8):675–682.
[44] Philips CA, Pande A, Shasthry SM, Jamwal KD, Khillan V, Chandel SS, et al. Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: a pilot study. Clin Gastroenterol Hepatol 2017;15(4):600–602.
[45] Philips CA, Phadke N, Ganesan K, Ranade S, Augustine P. Corticosteroids, nutrition, pentoxifylline, or fecal microbiota transplantation for severe alcoholic hepatitis. Indian J Gastroenterol 2018;37(3):215–225.