The role of the gut microbiome in chronic liver disease: the clinical evidence revised

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Summary
Recent research has suggested a role for the intestinal microbiota in the pathogenesis and potential treatment of a wide range of liver diseases. The intestinal microbiota and bacterial products may contribute to the development of liver diseases through multiple mechanisms including increased intestinal permeability, chronic systemic inflammation, production of short-chain fatty acids and changes in metabolism. This suggests a potential role for pre-, pro- and synbiotic products in the prevention or treatment of some liver diseases. In addition, there is emerging evidence on the effects of faecal microbial transplant. Herein, we discuss the relationship between the intestinal microbiota and liver diseases, as well as reviewing intestinal microbiota-based treatment options that are currently being investigated.

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Introduction
The human intestinal microbiota (IM) is made up of bacteria, archaea and eukaryotic microorganisms and viruses.1–5 Currently, there are 1,000 known species of bacteria6 and approximately 1014 microorganisms.4 Two dominant phyla, Bacteroidetes and Firmicutes, comprise 90% of bacteria in the human digestive tract.3–7 The IM plays an essential role in the digestion of food, synthesis of vitamins, metabolism, immune system function, inflammation and cell proliferation.8–9 Recently, disturbances in the IM, or dysbiosis, have been associated with several diseases, including a wide range of hepatic disorders.4,8–12

Emerging evidence supports the bidirectional relationship between the IM and the liver, which results from the liver receiving 75% of its blood supply from the intestines via the portal vein13 and the liver releasing bile acids into the biliary tract.14 As a result, the IM may contribute to liver diseases through several mechanisms that can be influenced by bacterial composition, IM metabolism of bile acids, diet, environmental factors and genetics, with bacteria, bacterial products and metabolites translocating through the intestinal barrier into the portal system, and then the liver.1,12

The aim of this review is to outline how the IM and liver interact with each other. We will focus on the IM’s role in the pathogenesis and treatment of liver diseases, specifically non-alcoholic fatty liver disease (NAFLD), alcohol-related liver disease (ALD), primary sclerosing cholangitis, primary biliary cholangitis, hepatocellular carcinoma (HCC) and cirrhosis. This review will focus on clinical data and interventions for each of these pathologies.

Intestinal microbiota and liver disease: Overall mechanisms
Bile acid metabolism
Synthesised from cholesterol in the liver, bile acids (BAs) are essential in cholesterol metabolism and lipid digestion.15 BAs are stored in the gallbladder and are secreted during digestion into the small intestine.16 Over 95% of BAs are reabsorbed in the terminal ileum and transported back to the liver via the portal vein. BAs promote the absorption of dietary fats, cholesterol and fat-soluble vitamins.16 In addition, BAs also function as signalling molecules that influence physiological processes,16 which include the regulation of glucose and lipid metabolism through farnesoid X receptor (FXR) activation and binding of G-protein-coupled bile acid receptor 1.17,18 BAs can also influence the IM as it has been shown to be directly associated with intestinal mucosal integrity and synthesis of antibacterial peptides.20 When BAs bind to FXR, antimicrobial peptides, such as angiogenin 1, are produced. These peptides can inhibit IM overgrowth by increasing the intestinal epithelial cell potential to prevent bacterial uptake, improving gut-barrier function.20 In turn, the IM can influence the size and composition of the BA pool through the conversion of primary to secondary BAs.21,22 This may subsequently change the composition of the circulating BAs, which act as signalling molecules affecting, for example, lipid and glucose metabolism.

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Keywords: microbiome; intestinal microbiota; liver diseases; prebiotics; probiotics; fecal microbiota; liver diseases; gut-liver axis.
metabolism and predisposing individuals to non-alcoholic fatty liver disease (NAFLD). Therefore, both the dysbiosis of IM and/or imbalance of BAs can contribute to the pathogenesis and progression of liver diseases, which will be discussed.21,22

Intestinal permeability
The intestinal epithelium plays an essential role in restricting toxins, antigens and enteric flora from entering the circulation, while selectively permitting the absorption of nutrients across the tight junctions.23 The intestinal barrier is comprised of enterocytes that are bound to each other by transmembrane proteins including desmosomes, adherens junctions and tight junctions.23 The intestinal barrier is also strengthened by immunoglobulins, mucins and commensal bacteria.23 The IM can alter the intestinal barrier by altering tight junctions, degrading the mucus layer or inhibiting the production of mucus, which subsequently increases the permeability of the epithelium.23 One way in which the IM is associated with increased tight junction permeability is through the presence of luminal endotoxins.23 Endotoxins found on the outer membrane of gram-negative bacteria increase tight junction permeability by increasing toll-like receptor (TLR)4 expression.24 Widening of the tight junctions leads to increased intestinal permeability, resulting in increased translocation of bacterial fragments and endotoxins into the portal circulation and subsequently the liver.25 This in turn can cause systemic and hepatic inflammation and hepatic injury.25 Bacterial fragments and products can also recruit and activate hepatic immune cells, further contributing to liver disease progression.25

Chronic inflammation
The IM contributes to chronic inflammation not only through the production of endotoxins but also through cytokines and inflammasome dysfunction. Translocation of IM-derived endotoxins into the circulatory system increases TLR4 expression, which activates the proinflammatory cytokines tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6,26 thus triggering systemic inflammation. Inflammasomes, which consist of leucine-rich-repeat containing proteins and nucleotide-binding domains, govern the cleavage of proinflammatory cytokines. Dysbiosis has been shown to be associated with inflammasome deficiency, specifically NLRP 3 and 6, resulting in the increased expression of TNF-α.27 The increased activation and production of TLR4 and proinflammar-atory cytokines from dysbiosis can also lead to the recruitment and activation of hepatic immune cells, contributing to liver disease progression.27

Immune system activation
The recruitment and activation of hepatic immune cells can be caused either by local signals or signals from sources such as the IM.23 The immune system is divided into the innate and adaptive immune systems. The innate immune system defends against microorganisms and toxins, whereas the adaptive immune system is antigen specific and requires self-non-self-recognition.28 Kupffer cells (KCs) are critical components of the innate immune system, residing within the sinusoidal vascular space.29 KCs can be activated by various endogenous and exogenous stimuli including endotoxins.30 Activation of KCs triggers the production of inflammatory cytokines, such as TNF-α, as well as reactive oxygen species (ROS)29 which can produce tissue damage. These cytokines can also play a key role in regulating the phenotype and function of neighbouring parenchymal and non-parenchymal cells.30 For instance, cytokines have been shown to polarise and activate the proinflammatory M1 phenotype in KCs.30

Natural killer (NK) and natural killer T (NKT) cells may also play a role in the pathogenesis of liver diseases and can be affected by the IM. Recent murine studies have shown that IM-derived antigens could influence the composition and activation of hepatic NKT cells.31,32 NK cells in the liver play a role in linking the innate and adaptive immune response.33 Activated NK cells were found to have anti-fibrotic effects, by releasing interferon-γ (IFN-γ) which induces hepatic stellate cell (HSC) cycle arrest and apoptosis.34 However, IFN-γ also results in hepatocyte apoptosis and thus causes hepatic injury.34 NKT cells, which can be expressed by hepatocytes and antigen presenting cells, share properties of both T cells and NK cells.35 NKT cells can secrete cytokines and therefore play a critical role in directing the immune system.35 They are able to do this through their ability to produce T helper 1 cells, which are proinflammatory, and T helper 2 cells, which are anti-inflammatory.35 Overall, the activation of hepatic immune cells by the IM could contribute to the pathogenesis of several liver diseases.

Short-chain fatty acids
Another mechanism by which the IM can contribute to liver disease is through the production of short-chain fatty acids (SCFAs). The IM breaks down non-digestible carbohydrates releasing SCFAs in the human gut.36 The primary SCFAs are acetate, propionate and butyrate, which are metabolised by the muscle, liver and epithelium, respectively. Research has predominantly focussed on butyrate, a primary source of energy for colonocytes, which improves colonic barrier function36 and therefore
positively impacts on intestinal permeability. Butyrate has been shown to improve the gut barrier by induction of tight junction proteins and mucus, specifically Mucin 2, and enhanced expression of claudin-1. In the liver, butyrate can induce apoptosis and can inhibit cell proliferation in hepatic cells by suppressing sirtuin 1 expression while upregulating miR-22 expression. Therefore, butyrate can also inhibit hepatic cancer cells. Butyrate has also been shown to increase satiety, decrease food intake and delay gastric emptying through activation of free fatty acid receptor 3. Free fatty acid receptor 3 upregulates the production of gut hormones peptide YY and glucagon-like peptide-1. Therefore, the IM can affect the metabolism, including diet-induced obesity. Finally, butyrate can also impact on inflammation. In the intestinal tract, studies have found that butyrate binds and activates peroxisome proliferator-activated receptor gamma (PPAR-γ), which antagonises nuclear factor-kappa B (NF-κB) transduction, thus causing an anti-inflammatory effect. Therefore, the presence and/or abundance of butyrate produced by the IM could impact on the pathogenesis of liver diseases through several mechanisms.

**Choline**

Choline is an essential nutrient and a phospholipid component of the cell membrane, which can be metabolised by the IM. There are several mechanisms through which choline deficiency may impact the liver, including decreased very-low density lipoprotein (VLDL) formation, mitochondrial dysfunction and endoplasmic reticulum stress. Phosphatidylcholine, which is a phospholipid that contains choline in the headgroup, is a key component of the VLDL envelope. Choline deficiency, either due to diet or as a result of IM metabolism, leads to a decrease in VLDL formation and triglyceride export from the liver, resulting in the development of a fatty liver. Choline is also an essential component of the mitochondrial membrane. Choline deficiency decreases the mitochondrial membrane concentrations of phosphatidylethanolamine and phosphatidylcholine, resulting in decreased membrane potential, which, in turn, causes oxidative damage.

The IM may contribute to decreased choline bioavailability by metabolising dietary choline found in eggs, milk and red meat into trimethylamine (TMA). This increases the production of TMA, which is absorbed into the blood, and has been associated with an increased risk of cardiovascular disease. In addition, once TMA reaches the liver it is further metabolised by flavin-containing monooxygenases 1 and 3 to generate trimethylamine-N-oxide (TMAO). This may lead to increased hepatic triglyceride accumulation as TMAO effects BA pool size by decreasing BA synthesis through the inhibition of key enzymes and by limiting the enterohepatic circulation of BAs through repression of the organic anion transporter and multidrug resistance family protein expression. Therefore, it is possible that choline deficiency, either through the diet or the conversion of IM to TMA, may cause fat to accumulate in the liver.

**Ethanol**

Ethanol, which comes primarily from food and beverages, is absorbed through the mucosa of the gastrointestinal tract. However, ethanol can also be produced and metabolised by the IM in the absence of alcohol consumption. Ethanol is formed from *Escherichia coli* and under anaerobic conditions during the fermentation of carbohydrates. *E. coli* can metabolise pyruvic acid to generate acetaldehyde, which can be reduced to ethanol. Acetaldehyde has been shown to decrease the gut barrier function by weakening tight junctions and therefore facilitates the translocation of microbial products into the systemic circulation. Furthermore, studies have shown that acetaldehyde can stimulate an inflammatory and adaptive immune response by downregulating antimicrobial peptide expression in the intestine, thus leading to further hepatic injury.

Taken together, the IM and bacterial products can directly and indirectly affect the liver through various mechanisms (Fig. 1), leading to a wide variety of liver diseases (Fig. 2).

**Gut bacteria and specific liver diseases**

**Non-alcoholic fatty liver disease**

NAFLD is one of the most common causes of liver disease worldwide, affecting 15–30% of the general population. NAFLD ranges from simple fat deposition in the liver (steatosis) to inflammation (non-alcoholic steatohepatitis or NASH) to fibrosis and cirrhosis. Research studies have shown that altered IM composition, so-called “dysbiosis”, contributes to the pathogenesis of NAFLD, however causality has yet to be proven.

Despite a large number of preclinical data investigating and demonstrating a relationship between dysbiosis and NAFLD, only a limited number of human studies, mostly cross-sectional, have investigated the role of the IM in NAFLD, with variable results. In adults, patients with NASH were found to have lower amounts of Bacteroidetes, independent of body mass index and diet. Studies have shown that there are differences in the IM between patients with NAFLD and healthy controls. One study found that NAFLD severity is associated with IM dysbiosis and shifts in the metabolic function of the IM. Specifically, they found that the abundance of *Bacteroides* was independently associated with NASH and *Ruminococcus* with fibrosis. More recently, another cross-sectional study found that those with NAFLD had significantly decreased Bacteroidetes and Firmicutes, along with increased *Lactobacillus* compared with healthy controls, while those with NASH had decreased *Ruminococcus*,...
Faecalibacterium prausnitzii and Coprococcus compared to healthy controls, independently of body mass index and insulin resistance. In paediatrics, results showed an increased abundance of E. coli in patients with NASH compared to healthy controls, which was associated with higher blood alcohol levels. One intervention study, which included 15 adult women placed on a choline-deficient diet found that pre-diet microbiota composition, specifically a lower abundance of Gammaproteobacteria or a higher abundance of Erysipelotrichi increased vulnerability to the development of a fatty liver during choline depletion. Furthermore, they found that host genotypes (single nucleotide polymorphism in the PEMT gene) and specific IM can predict choline deficiency-induced fatty liver (assessed by magnetic resonance imaging). One study assessed faecal ester volatile organic compounds and found that specific patterns were associated with differences in the IM when patients with NAFLD, diagnosed on ultrasound, were compared to controls. Recently, a study investigating the relationship between the IM and immune function in NAFLD found that specific immune cells in the portal or lobular areas correlated with specific faecal IM. Specifically, Faecalibacterium prausnitzii was negatively correlated with CD45+ and CD163+ cells in the portal tract and Prevotella was negatively correlated with CD20+ cells in the liver lobule. Taken together, several studies showed associations between the IM or bacterial products and NAFLD.

**Alcohol-related liver disease**

ALD occurs in patients who chronically abuse alcohol. Like NAFLD, non-progressive ALD is characterised by fat accumulation in the liver, whereas progressive ALD (alcoholic steatohepatitis) exhibits...
hepatic inflammation. Recently, research has investigated the role of the IM in ALD, specifically focussing on how alcohol can cause microbiota-related dysbiosis which in turn, may contribute to the pathogenesis of ALD.

Many studies at the preclinical and experimental level have shed light on the relationship between ALD and dysbiosis. Through these many studies, multiple pathogens, toxic components and pathways have been shown to participate in the development of ALD. The amount of clinical data is unfortunately not as extensive. Studies including both mouse models and human participants found that alcohol consumption provokes a change in the IM leading to dysbiosis. Specifically, patients with ALD have lower levels of *Bifidobacterium*, *Enterobacterium* and *Lactobacillus* spp., while cirrhotic patients with ALD exhibit a significant reduction in Bacteroidetes and Firmicutes phyla. On the other hand, the Proteobacteria, Fusobacteria and Actinobacteria phyla were increased. Other studies using faecal samples from alcoholic patients showed a reduction in the *Lactobacillus* spp., whereas cirrhotic patients were shown to have lower faecal amounts of *Bifidobacterium* spp. When comparing IM of alcoholic patients with liver cirrhosis to those without, it was found that the IM of those with cirrhosis contained more *Enterobactericeae*. Based on some of these findings, the term cirrhosis dysbiosis ratio (CDR) was suggested, representing the ratio of autochthonous or beneficial bacteria to potentially pathogenic bacteria, with a low ratio correlating with a more advanced disease state.

Compared to other aetiologies of cirrhosis, ALD had the lowest ratio. Studies have also demonstrated an increase in the overall number of organisms in the small bowel of alcoholic patients. An evaluation of chronic alcoholics compared to patients without a history of alcohol abuse, using the hydrogen breath test, showed a significantly higher prevalence of small-intestinal bacteria overgrowth (SIBO) in alcoholics compared to controls. However, no differences were found between alcoholic patients with liver cirrhosis and those without liver cirrhosis. The presence of SIBO has been shown to significantly correlate with a higher prevalence of spontaneous bacterial peritonitis and with the severity of alcoholic cirrhosis. These changes in the IM of alcoholic patients seem to be accompanied by changes in colonic pH and liver steatosis. It also correlates with a higher level of serum endotoxin and increased intestinal TNF-α levels, as well as increased levels of nitric oxide, IL-6, and IL-8. Other studies also found higher levels of bacterial products in the blood of alcoholic patients compared to healthy controls. Additionally, endotoxemia after acute alcohol intoxication has been shown to correlate with haemodynamic derangement in cirrhotic portal hypertension. These findings suggest a potential link between dysbiosis and ALD, with alcohol promoting dysbiosis and leading to increased gut barrier permeability, consequently causing translocation of IM and endotoxins into the portal circulation, and eventually the liver. This, in turn, triggers hepatic inflammation and liver damage, particularly through the
interaction between lipopolysaccharides (LPS) and TLRs.  

**Primary sclerosing cholangitis**

Primary sclerosing cholangitis (PSC) is characterised by inflammation and scarring of the bile ducts.  

The few studies investigating the IM in PSC have shown an overall reduction in IM diversity, however, there are inconsistencies in these findings at the genus and species level.

The evaluation of the IM in patients with PSC and PSC-inflammatory bowel disease (IBD) demonstrated low bacterial diversity, and an overrepresentation of *Rothia*, *Enterococcus*, *Streptococcus*, *Clostridium*, *Veillonella*, *Haemophilus*, *Fusobacterium* and *Lactobacillus* genera regardless of concomitant IBD.  

Another study confirmed that *Veillonella* abundance was markedly increased in PSC compared to healthy controls.  

Studies looking at intestinal biopsies found that the overall microbiota profile of those with PSC was characterised by enrichment of *Barnesiellaceae* and a reduction in *Clostridiales*.  

According to the aforementioned studies, these changes lead to IM dysbiosis and are associated with the pathogenesis of PSC by inducing bacterobilia, which in turn activates a proinflammatory pathway in the cholangiocytes leading to fibrosis and inflammation. Bacterobilia may also play a role in molecular mimicry, through endotoxemia, and inflammation. Bacterobilia may also play a pathway in the cholangiocytes leading to fibrosis and a reduction in IM diversity, which in turn activates a proinflammatory pathway in the cholangiocytes leading to fibrosis and inflammation. Bacterobilia may also play a role in molecular mimicry, through endotoxemia, and inflammation. 

**Cirrhosis and hepatic encephalopathy**

Cirrhosis is considered end-stage liver disease that is characterised by severe fibrosis and a loss of liver cells. Each of the aforementioned liver diseases can result in a cirrhotic liver. Research has found that patients with cirrhosis have lower levels of *Bacteroidetes* and higher levels of *Proteobacteria*, *Enterococcus*, *Veillonella*, *Megasphaera*, *Burkholderia*, *Prevotella* and *Fusobacteria*.  

In addition, cirrhotic patients also have lower levels of autochthonous taxa such as *Blautia*, *Roseburia*, *Faecalibacterium*, *Dorea*, *Lachnospiraceae* and *Ruminococcaceae*.  

When analysing the duodenal mucosal microbiota of 30 cirrhotic patients, Chen *et al.* found that cirrhotic patients’ colonisation was significantly different than that of 28 healthy controls. There seemed to be an overrepresentation of *Veillonella*, *Megasphaera*, *Dialister*, *Atoptobium*, and *Prevotella* in cirrhotic patients compared to controls. *Veillonella*, *Prevotella*, *Neisseria*, and *Haemophilus*, were the taxa best able to discriminate between those with cirrhosis and healthy controls. Other studies have demonstrated higher levels of *buccal*-derived microbiota in the stool samples of patients with cirrhosis, as well as a significantly altered salivary microbiome in cirrhotic patients. This could suggest that the oral microbiota has a great impact upon duodenal and possibly intestinal microbiota in this population. Another author even mentioned the possibility that the duodenal microbiota might directly contribute to hepatic encephalopathy in cirrhosis.

Hepatic encephalopathy, which is defined as cognitive impairment, occurs as a result of severe liver disease. Studies have found that there is a link between hepatic encephalopathy and by-products of the IM, specifically endotoxemia. One study compared the IM in patients with hepatic encephalopathy to other cirrhotic patients and healthy controls and found that those with hepatic encephalopathy had higher levels of *Alcaligenaceae*, *Enterobacteriaceae* and *Fusobacteriaceae* along with lower *Ruminococcaceae* and *Lachnospiraceae*. Of those, *Alcaligenaceae* and *Porphyromonadaceae* were positively correlated with cognitive impairment whereas *Prevotella* was linked to improvement of cognition and decreased inflammation. Their study also showed a higher concentration of *Veillonellaceae*, endotoxemia and inflammation in patients with hepatic encephalopathy.

Another study demonstrated that the composition of the IM could predict decompensation and hospitalisation of cirrhotic patients.
Higher serum endotoxin levels, lower CDR and increased pathogenic taxa were significantly linked to death secondary to multi-organ failure when compared to patients who survived. In the same study the salivary microbiome was shown to independently correlate with liver-related 90-day hospitalisation regardless of the model for end-stage liver disease (MELD) score or the status of hepatic encephalopathy.128

Several mechanisms have been suggested for the association between IM and cirrhosis that include increased small bowel permeability and decreased small bowel motility, leading to small bowel overgrowth. This in turn leads to translocation of the IM and endotoxins into the portal circulation, activation of inflammatory pathways in HSCs through TLR4 and subsequently the development of fibrosis.113 For HE, ammonia plays a central role in the development of the disease. Some studies have shown that in patients with cirrhosis, in addition to bacterial translocation and activation of proinflammatory cytokines, there is an increased quantity of urease active bacteria, which would lead to increased production of ammonia in the small bowel and increased levels in the portal blood.115,116

**Hepatocellular carcinoma**

HCC can be a complication of many liver diseases. Dysbiosis may contribute to HCC pathogenesis by increasing steatosis, oxidative stress and inflammation.14

Changes in the microbiota have been suspected of playing a role in carcinogenesis. One study by Grat et al. investigated the IM of 15 patients with HCC undergoing liver transplantation and compared them to 15 patients who did not have HCC but had a similar aetiology of cirrhosis and a similar MELD stage. The study showed that the presence of HCC was significantly associated with increased faecal Escherichia coli.117 Another study evaluated liver tissue samples in patients with HCC and found the presence of Helicobacter spp., suggesting intestinal translocation as a potential mechanism for carcinogenesis.118 However, Helicobacter could not be found in patients with viral-induced HCC.119

Mechanistically, murine studies suggested that the IM can contribute to HCC pathogenesis through its interaction with the TLRs, particularly TLR4. However, more clinical research is needed to further characterise the causal role of the IM in the pathogenesis of HCC.119

**Limitations to IM and liver disease studies**

Research in the area of IM and liver diseases is rapidly evolving, but there are several limitations to consider when interpreting this association. First of all, differences in genetics120 and environmental factors such as diet,121 alcohol122 and medication/antibiotic use122 have been shown to contribute to variations in IM. Additionally, the use of different liver diagnostic tools that are used for primary endpoints in clinical trials is another limitation. Some studies will use a liver biopsy,107 the gold standard for diagnostics, while others use non-invasive and less reliable tools such as imaging or blood tests,108 which could explain the differences seen in clinical research. Another limitation in human IM studies is how the stool sample is collected. Although similar IM phyla predominate across the stomach, small intestine and colon, there are variations in IM composition and abundance.123 The majority of human studies analyse stool samples, however 1 study found differences in the IM when comparing stool samples to caecal luminal contents,124 therefore limiting the generalisability of the results. Furthermore, there are variations in the sequencing methods used, which all produce different results. These include quantitative PCR,125 16S rRNA sequencing126 or shotgun sequencing.112 Additionally, differences in bioinformatic analysis platforms, such as QIIME,125 Mothur126 and PICRUST,127 can contribute to variations in results. Overall, it is important to consider these limitations when analysing IM and liver disease research and they should be considered when designing future studies.

**Future directions**

Based on the above studies, there is likely an association between the IM and liver disease, but a causal relationship has yet to be confirmed. Several studies looking at the effect of IM manipulation by pre-, pro- and symbiotics, as well as faecal microbiota transplant (FMT), suggest that the IM has a role in liver diseases.

**Pre- and probiotic treatment**

Several studies have investigated the role of pre- and probiotic treatment in patients with liver disease. A summary of these studies can be found in Table 1. However, no studies have been carried out in patients with PSC or PBC.

Over all, pre-, pro- and symbiotics seem to improve various liver parameters in patients with NAFLD, ALD, cirrhosis or HE, supporting a role for the IM in liver disease pathology. However, interventions vary in terms of the product type and amount used and most of the studies have small sample sizes. Therefore, more research is needed with larger randomised controlled trials before any recommendations can be made. Answers may come from the clinical trials currently being conducted; for studies currently recruiting see Table 2.

**Faecal microbiota transplantation**

FMT has recently become a standard of care for treating antibiotic-resistant Clostridium difficile.144,145 As a result, FMT is becoming frequently investigated as a potential therapeutic option for a variety of diseases, including those that are liver related. As previously stated, the liver-gut axis...
plays an essential role in the pathogenesis of liver disease, with recent research suggesting that FMT could be beneficial. A pilot study, investigating the effects of FMT in 8 male patients with severe alcoholic hepatitis compared to historical controls found that those receiving FMT had reduced hospitalisation rates and improved cognition and dysbiosis. Furthermore, in the 5 months

| Disease                  | Treatment                                      | Study design                     | Outcome                                                                 | Reference |
|--------------------------|------------------------------------------------|----------------------------------|--------------------------------------------------------------------------|-----------|
| NAFLD                    | VSL#3 (combination of 8 probiotic strains) or placebo for 4-months | RCT, n = 48 children             | VSL#3 improved NAFLD                                                    | 128       |
| NAFLD                    | Multi-probiotic product or placebo              | RCT, n = 58 adults               | Probiotic resulted in reductions of AST, GGT, TNF-a and IL-6             | 129       |
| NAFLD                    | Symbiotic or placebo                            | RCT, n = 50                      | Symbiotic group had significant decrease in AST, total cholesterol, triacylglycerol and steatosis (based on Fibroscan) | 130       |
| ALD                      | Bifidobacterium bifidum and P Bacillus plantarum daily for 5 days | Open-label randomised, n = 66    | Reductions in AST and ALT                                               | 85        |
| ALD                      | Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium spp., Lactobacillus acidophilus, Lactobacillus rhamnosus GG, Streptococcus thermophilus 5x10^8 1 capsule twice daily for 28 days or placebo | Double-blind RCT, n = 50        | Decrease in SIBO, however no difference in intestinal permeability       | 131       |
| ALD/HE                   | VSL#3 or placebo                                | RCTI, n = 130                    | No difference in incidence of encephalopathy or mortality. However, reductions in Child-Pugh, MELD, plasma TNF-a, IL-1B and IL-6 seen | 132       |
| ALD/Cirrhosis            | Lactobacillus subtilis and Streptococcus faecium (daily for 7 days) or placebo | Double-blind RCT, n = 117        | Decrease in TNF-a and an increase in albumin levels. Stabilisation of LPS levels in cirrhotic patients | 133       |
| ALD/Cirrhosis            | Lactobacillus casei Shirato (6.5x10^9) three times a day for 4 weeks | Open-label randomised, n = 12    | Normalised neutrophil phagocytic capacity. No improvement in disease control and no change on TNF-a and IL10 | 134       |
| ALD/Cirrhosis            | VSL#3 1 sachet daily for 60 days or placebo     | Double-blind RCT, n = 63         | Reduction of hepatovenous pressure gradient and TNF-a                   | 135       |
| ALD/Cirrhosis            | VSL#3 2 sachets twice a day for 60 days         | Open pilot study, n = 8          | Trending reduction of endotoxin levels and TNF-a                        | 136       |
| ALD/Cirrhosis            | VSL#3 2 sachets twice daily for 60 days or placebo | Double-blind RCT, n = 11 ALD and n = 15 cirrhosis | No impact on IM, endotoxins, liver function, hepatovenous pressure. Reduction in plasma aldosterone. | 137       |
| Cirrhosis/HE             | Lactobacillus rhamnosus GG twice daily for 8 weeks or placebo | RCT, n = 30                     | No change in cognition. However, decrease in endotoxemia and TNF-a      | 138       |
| Cirrhosis/HE             | VSL#3 1 capsule three times a day for 3 months or placebo | RCT, n = 86                     | Reduction of ammonia, SIBO and OCTT. Increased psychometric HE scores and CFF threshold. Significantly less patients developed overt HE. | 139       |
| Cirrhosis                | Escherichia coli Nissle for 42 days             | Double-blind RCT, n = 39         | Improvement in intestinal colonisation. Lowering of endotoxemia and Improvement of liver function/Child-Pugh score. | 140       |
| Cirrhosis/HE             | Lactulose and lactitol                          | Cochrane review of randomised control trials, n = 1828 | Beneficial effect of non-absorbable disaccharides on mortality, HE, reduction of serious adverse events associated with liver disease (liver failure, hepatorenal syndrome, variceal bleed) | 141       |
| Cirrhosis/HE             | Bifidobacterium longum plus fructo-oligosaccharides for 90 days or placebo | Double-blind RCT, n = 60         | Decrease in ammonium (NH4) and performance on Trial Making Test A and B. Significant improvement of symbol digit modalities test; block design and MMSE | 142       |
| Cirrhosis/HE             | Symbiotic treatment daily for 30 days or placebo | RCTI, n = 55                    | Increase in faecal Lactobacillus. Reduction in endotoxemia, blood ammonia and reversal of minimal HE in 50%. Improvement of Child-Pugh class in 50%. | 143       |

ALD, alcohol-related liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CFF, critical flicker frequency; GGT, gamma-glutamyl transferase; HE, hepatic encephalopathy; IL-, interleukin-; IM, intestinal microbiota; LPS, lipopolysaccharide; MELD, model for end-stage liver disease; MMSE, mini-mental state examination; NAFLD, non-alcoholic fatty liver disease; OCTT, orooccale transit time; RCT, randomised controlled trial; SIBO, small intestine bacteria overgrowth; TNF-a, tumour necrosis factor-alpha.
following the FMT procedure, no FMT recipients’ developed hepatic encephalopathy, whereas 5 of those receiving standard of care did.\(^{127}\) Relevant to NAFLD, 1 pilot study reported that FMT significantly reduced insulin resistance associated with changes in the IM.\(^{148}\) Again, more clinical trials are needed.

### Table 2. Ongoing pre-, pro- and synbiotic trials.

| Type of liver disease | Type of pre-, pro or synbiotic | Study design | Primary outcome | Location | ClinicalTrial.gov ID |
|-----------------------|--------------------------------|--------------|----------------|----------|----------------------|
| NAFLD                 | 2x probiotic/day: *Lactobacillus acidophilus* 1x10⁹ CFU + *Bifidobacterium lactis* 1x10⁹ CFU + *Lactobacillus rhamnosus* 1x10⁹ CFU + *Lactobacillus paracasei* 1x10⁹ CFU | RCT; n = 46 adults | Change in fibrosis by hepatic elastography | Brazil | NCT03467282 |
| NAFLD                 | 1x probiotic/day: *Lactobacillus acidophilus* 10⁹, B. lactis 10⁹ | RCT; n = 58 adults | Hepatic changes based on FIBROMAX test | Brazil | NCT02764047 |
| NAFLD                 | Symbiotic: Fructo-oligosaccharide with a degree of polymerisation <10 at 4 g/twice a day plus *Bifidobacterium animals* subsp. lactis BB-12 as minimum of 10 billion CFU/day (1 capsule a day). | RCT; n = 100 adults | Change in liver fat by MRS | United Kingdom | NCT01680640 |
| NAFLD                 | 2x prebiotic/day: oligofructose-enriched inulin (Synergy1) | RCT; n = 60 adults | Change in liver fat by MRI, change in liver fibrosis by FibroScan, change in liver injury by Fibrotest Score | Canada | NCT02568605 |
| NAFLD                 | Prebiotic 16 g/day: inulin and oligofructose | RCT; n = 60 adults | Change in liver fat by MRS, biochemistry | Israel | NCT02642172 |
| Acute alcoholic hepatitis | 1x probiotic/day: *Lactobacillus rhamnosus* GG | RCT; n = 130 adults | MELD Score | United States of America | NCT01922895 |
| NASH                  | Frozen faecal material from lean healthy donors infused into the duodenum by endoscopy | Open label; n = 5 adults | Change in liver fat by MRI | United States of America | |
| NASH-related cirrhosis | Recipient will receive healthy donor faecal samples through a naso-gastric tube, 100 ml once a month for 5 months. | RCT; n = 60 adults | Reduction in hepatic venous pressure gradient | India | |
| Alcoholic hepatitis   | Healthy donor FMT administered by naso-gastric tube for 7 days | RCT; n = 130 adults | Proportion of participants with Overall Survival at 3 months | India | |
| Cirrhosis             | FMT by endoscope and/or enema | RCT; n = 60 adults | Number of adverse events complication rate | China | |
| Cirrhosis             | FMT (200 ml) from donated healthy samples will be administered into the duodenum via a gastroscope | RCT; n = 32 adults | Assessment of the feasibility of FMT and assessment of the incidence of treatment-emergent adverse events | United Kingdom | |
| Cirrhosis             | One-dose of 90 ml of FMT enema from healthy donor stool sample | RCT; n = 20 adults | Proportion of participants with a related serious adverse event, with newly acquired transmissible infectious diseases and related adverse event | United States of America | |
| Hepatic encephalopathy | Single-arm open-label healthy donor FMT administered at Week 0 by colonoscopy and at Weeks 1–4 by enema | Open label; n = 10 adults | Time to hepatic encephalopathy breakthrough | Canada | |
| Hepatic encephalopathy | Single-centre open-label trial of RBX2660 (microbiota suspension). Healthy donor FMT administered at Week 0 by colonoscopy and at Weeks 1–4 by enema | Open label; n = 30 adults | Time to hepatic encephalopathy breakthrough | Canada | |
| Hepatic encephalopathy | Subjects will receive 15 oral capsules of FMT on days 1, 2, 7, 14, and 21. FMT prepared from healthy donors. | RCT; n = 30 adults | Psychometric Hepatic Encephalopathy Score | United States of America | |
| Acute liver failure    | FMT administered by enema | RCT; n = 40 adults | Survival | India | |

CFU, colony forming units; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RCT, randomised controlled trial.

### Table 3. Current faecal microbiota transplantation trials.

| Type of liver disease | Type of faecal microbiota transplantation | Study design | Primary outcome | Location |
|-----------------------|------------------------------------------|--------------|----------------|----------|
| NASH                  | Frozen faecal material from lean healthy donors infused into the duodenum by endoscopy | Open label; n = 5 adults | Change in liver fat by MRI | United States of America |
| NASH-related cirrhosis | Recipient will receive healthy donor faecal samples through a naso-gastric tube, 100 ml once a month for 5 months. | RCT; n = 60 adults | Reduction in hepatic venous pressure gradient | India |
| Alcoholic hepatitis   | Healthy donor FMT administered by naso-gastric tube for 7 days | RCT; n = 130 adults | Proportion of participants with Overall Survival at 3 months | India |
| Cirrhosis             | FMT by endoscope and/or enema | RCT; n = 60 adults | Number of adverse events complication rate | China |
| Cirrhosis             | FMT (200 ml) from donated healthy samples will be administered into the duodenum via a gastroscope | RCT; n = 32 adults | Assessment of the feasibility of FMT and assessment of the incidence of treatment-emergent adverse events | United Kingdom |
| Cirrhosis             | One-dose of 90 ml of FMT enema from healthy donor stool sample | RCT; n = 20 adults | Proportion of participants with a related serious adverse event, with newly acquired transmissible infectious diseases and related adverse event | United States of America |
| Hepatic encephalopathy | Single-arm open-label healthy donor FMT administered at Week 0 by colonoscopy and at Weeks 1–4 by enema | Open label; n = 10 adults | Time to hepatic encephalopathy breakthrough | Canada |
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| Hepatic encephalopathy | Subjects will receive 15 oral capsules of FMT on days 1, 2, 7, 14, and 21. FMT prepared from healthy donors. | RCT; n = 30 adults | Psychometric Hepatic Encephalopathy Score | United States of America |
| Acute liver failure    | FMT administered by enema | RCT; n = 40 adults | Survival | India |

FMT, faecal microbiota transplantation; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; RCT, randomised controlled trial.
to fully investigate the beneficial effects of FMT on specific liver diseases, several of which are underway (Table 3).

Conclusion
There is evidence of associations between dysbiosis and liver disease, particularly as it relates to NAFLD, ALD, cirrhosis and hepatic encephalopathy. Specifically, molecules produced by the IM such as endotoxin and proinflammatory cytokines play a role in the pathogenesis of liver diseases. Furthermore, the IM can be influenced by several environmental factors, particularly diet and alcohol in the case of NAFLD and ALD Other than dietary changes or alcohol abstinence, manipulations of the IM by various interventions show promise. The majority of studies investigate the use of pre-, pro- and symbiotics in NAFLD, ALD and cirrhosis/HE and have found that these products improved clinical and biochemical markers of liver disease, however studies in patients with PSC and PBC are lacking. In conclusion, even though these studies show promise, more clinical research is required, particularly larger randomised controlled trials to bridge the gap between experimental/preclinical data and the small amount of human data on the subject.

Supplementary data
Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2019.04.004.

References
[1] Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. Microbiome 2015;3:31.
[2] Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. J Physiol 2009;587:4153–4158.
[3] Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science 2006;312:1355–1359.
[4] Dore J, Simren M, Bottle L, Guarner F. Hot topics in gut microbiota. United European Gastroenterol J 2013;1:311–318.
[5] Eckburg PB, Relman DA. The role of microbes in Crohn’s disease. Clin Infect Dis 2007;44:254–262.
[6] Gosalbes MJ, Abellan JJ, Durban A, Perez-Cobas AE, Latorre A, Moya A. Metagenomics of human microbiome: beyond 16s rDNA. Clin Microbiol Infect 2012;18:47–49.
[7] Arrese M, Cabrera D, Kalergis AM, Feldstein AE, Arendt BM, Zhang L, et al. Innate Immunity and the Intestinal Microbiota. In: Marchesi JR, Ravel J, editors. The Vocabulary of Microbiome Research: A Proposal. Microbiome; 2015. p. 31–31.
[8] Blaut M, Clavel T. Metabolic diversity of the intestinal microbiota: implications for health and disease. J Nutr 2007;137:7515–7555.
[9] Simren M, Barbaro G, Flint H, Spiegel BM, Spiller RC, Vanner S, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut 2013;62:159–176.
[10] Ahmed I, Roy BC, Khan SA, Septer S, Umar S. Microbiome, Metabolome and Inflammatory Bowel Disease. Microorganisms; 2016:4.
[11] Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 2009;15:1546–1559.
[12] Duseja A, Chawla YK. Obesity and NAFLD: The role of bacteria and microbiota. Clin Liver Dis 2014;18:59–71.
[13] Henoa-Mejia J, Elinav E, Thaiss CA, Flavell RA. The intestinal microbiota in chronic liver disease. Adv Immunol 2013;117:73–97.
[14] Tripathi A, Debels J, Brenner DA, Karin M, Loomba R, Snabl B, et al. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018;15:397–411.
[15] Stakey C, Weinagarden AR, Khoruts A, Sadowsky MJ. Interaction of gut microbiota with bile acid metabolism and its influence on disease states. Appl Microbiol Biotechnol 2017;101:47–64.
[16] Long SL, Gahan CGM, Joyce SA. Interactions between gut bacteria and bile in health and disease. Mol Aspects Med 2017;56:54–65.
[17] Sinal CJ, Nishimura N, Miyata M, Ward JM, Lambert C, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 2002;102:731–744.
[18] Hylemon PB, Zhou H, Pandak WM, Ren S, Gill G, Dent P. Bile acids as regulatory molecules. J Lipid Res 2009;50:1509–1520.
[19] Cupple BL, Li T. Pharmacology of bile acid receptors: Evolution of bile acid receptors from simple detergent to complex signalling molecules. Pharmacol Res 2016;104:9–21.
[20] Parsaei A, Sommer N, Sommer F, Caesar R, Molinaro A, Stahlman M, et al. Microbiota-induced obesity requires farnesoid X receptor. Gut 2017;66:429–437.
[21] Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. Curr Opin Gastroenterol 2014;30:332–338.
[22] Mouzaki M, Wang AY, Bansma R, Comelli EM, Arendt BM, Zhang L, et al. Bile Acids and Dysbiosis in Non-Alcoholic Fatty Liver Disease. PLoS One 2016;11:e0151829.
[23] Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol 2009;124:3–20 quiz 21–22.
[24] Gao S, Al-Sadi R, Said HM, Ma TY. Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. Am J Pathol 2013;182:375–387.
[25] Arrese M, Cabrera D, Kalergis AM, Feldstein AE. Innate Immunity and Inflammation in NAFLD/NASH. Dig Dis Sci 2016;61:1294–1303.
[26] Aron-Wisnewsky J, Gaborit B, Doutou A, Clement K. Gut microbiota and non-alcoholic fatty liver disease: new insights. Clin Microbiol Infect 2013;19:338–348.
[27] Henao-Mejia J, Elinav E, Jin C, Hao L, Melah WZ, Strowig T, et al. Inflammamma-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 2012;482:179–185.
[28] Vonghia L, Michielsen P, Francque S. Immunological mechanisms in the pathophysiology of non-alcoholic steatohepatitis. Int J Mol Sci 2013;14:19867–19890.
[29] Balfy G. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. J Hepatol 2009;51:212–223.
[30] Tosello-Trampont AC, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS. Kupffer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-alpha production. J Biol Chem 2012;287:40161–40172.
[31] Chen J, Wei Y, He J, Cui G, Zhu Y, Lu C, et al. Natural killer T cells play a necessary role in modulating of immune-mediated liver injury by gut microbiota. Sci Rep 2014;4:7259.
[32] Liang S, Webb T, Li Z. Probiotic antigens stimulate hepatic natural killer T cells. Immunology 2014;141:203–210.
[33] Tian Z, Chen Y, Gao B. Natural killer cells in liver disease. Hepatology 2013;57:1654–1662.
[34] Jeong WJ, Gao B. Innate immunity and alcoholic liver fibrosis. J Gastroenterol Hepatol 2008;23:S112–S118.
[35] Godfrey DL, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what’s in a name? Nat Rev Immunol 2004;4:231–237.
[36] Hamer HM, Jonkers D, Venema K, Vannoutven S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther 2008;27:104–119.
[37] Gaudier E, Rival M, Buisine MP, Robineau I, Hoebler C. Butyrate enemas upregulate Muc genes expression but decrease adherent mucin thickness in mice colon. Physiol Res 2009;58:111–119.
[38] Willemse LF, Koetsier MA, van Deventer SJ, van Tol EA. Short chain fatty acids stimulate epithelial mucus 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. Gut 2003;52:1442–1447.
[39] Augenlicht L, Shi L, Mariadason J, Laboisse C, Velich A. Repression of MUC2 gene expression by butyrate, a physiological regulator of intestinal cell maturation. Oncogene 2003;22:4983–4992.
[40] Wang HB, Wang PY, Wang X, Wan YL, Liu YC. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Dig Dis Sci 2012;57:3126–3135.
[41] Pint K, Yadav AK, Gupta P, Islam R, Saraya A, Venugopal SK. Butyrate induces ROS-mediated apoptosis by modulating miR-22/SIRT-1 pathway in hepatic cancer cells. Redox Biol 2017;12:340–349.
[42] Lin HV, Frassetto A, Kowalik EW, Navrocki AR, Lu MM, Kosinski JR, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via non-alcoholic fatty acid receptor 3-independent mechanisms. PLoS One 2012;7:e33520.

[43]Kinoshita M, Suzuki Y, Saito Y. Butyrate reduces colonic paracellular permeability by enhancing PPARgamma activation. Biochem Biophys Res Commun 2002;293:827–831.

[44]Corbin KD, Zeisel SH. Choline metabolism provides novel insights into non-alcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 2012;28:159–165.

[45]Zeisel SH, da Costa K-A. Choline: an essential nutrient for public health.

[46]Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, et al. Flavin coenzyme metabolism is altered in human non-alcoholic fatty liver disease. J Hepatol 2011;54:62–70.

[47]Corbin KD, Zeisel SH. Choline metabolism provides novel insights into non-alcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 2012;28:159–165.

[48]Corbin KD, Zeisel SH. Choline metabolism provides novel insights into non-alcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 2012;28:159–165.

[49]Howitt MR, Garrett WS. A complex microworld in the gut: gut microbiota and cardiovascualr disease connectivity. Nat Med 2012;18:1188–1199.

[50]Chaudhry KK, Shukla PK, Mir H, Manda B, Gangwar R, Yadav N, et al. Acetaldehyde-induced barrier disruption and paracellular permeability in Caco-2 cell monolayer. Methods Mol Biol 2008;447:171–183.

[51]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Dysbiosis in non-alcoholic steatohepatitis (NASH): a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–609.

[52]Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, et al. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015;5:8096.

[53]Wong VW, Tse CH, Lam TT, Wong GL, Chin AM, Chu WC, et al. Molecular characterization of the faecal microbiota in patients with nonalcoholic steatohepatitis—a longitudinal study. PLoS One 2013;8:e62085.

[54]Bourrier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology 2016;63:764–775.

[55]Da Silva HE, Teterina A, Comelli EM, Taiba A, Arendt BM, Bonengel J, Fung SE, et al. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. Sci Rep 2018;8:14466.

[56]Schwenger KP, Chen L, Chelliah A, Da Silva HE, Teterina A, Comelli EM, et al. Markers of activated inflammatory cells are associated with disease severity and intestinal microbiota in adults with nonalcoholic fatty liver disease. Int J Mol Med 2018;42:2229–2237.

[57]Kalogeris K, Seth A, Sheth P. Recent Advances in Alcoholic Liver Disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. Am J Physiol Gastrointest Liver Physiol 2004;286:G881–G884.

[58]Tuomisto S, Pessi T, Collin P, Vuento R, Altimiemi J, Karhunen P, et al. Evaluation of intestinal leakage to xenobiotics and the effect of Lactobacillus rhamnosus GG treatment. PLoS One 2013;8:e53028.

[59]Chen Y, Yang F, Lu H, Wang B, Chen Y, Ye D, et al. Characterization of faecal microbial communities in patients with liver cirrhosis. Hepatology 2016;63:1340–1347.

[60]Tuomisto S, Pess I, Collin P, Vuento R, Altimiemi J, Karhunen P, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. PLoS One 2013;8:e53028.

[61]Hauge T, Persson J, Danielsson D. Mucosal bacterial growth in the upper gut of alcoholics. Scand J Gastroenterol 1990;25:955–962.

[62]Bluemel S, Williams B, Knight R, Schnabl B. Precision medicine in alcoholic liver disease: an integrative approach. JHEP Reports 2019;vol. 1| 214–226

[63]Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SE, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology 2013;58:120–127.

[64]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen J, et al. Human gut barrier dysfunction and fatty liver in mice. J Nutr Biochem 2002;13:385–392.

[65]Vernon G, Baranova A, Yousouf ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;34:274–285.

[66]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:52–59.

[67]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.

[68]Vernon G, Baranova A, Yousouf ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;34:274–285.

[69]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:52–59.

[70]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.

[71]Vernon G, Baranova A, Yousouf ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;34:274–285.

[72]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:52–59.

[73]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.

[74]Vernon G, Baranova A, Yousouf ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;34:274–285.

[75]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:52–59.

[76]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.

[77]Vernon G, Baranova A, Yousouf ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2011;34:274–285.

[78]Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:52–59.

[79]Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.
Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, Puri P, Sterling RK, et al. Randomised clinical trial: Lactobacillus GG modulates gut microbiome, metabolome and endotoxemia in patients with cirrhosis. Aliment Pharmacol Ther 2014;39:1113–1125.

Lunia MK, Sharma BC, Sharma P, Sachdeva S, Srivastava S. Probiotics prevent hepatic encephalopathy in patients with cirrhosis: a randomized controlled trial. Clin Gastroenterol Hepatol 2014;12:1003–1008 e1001.

Lata J, Novotny I, Pribramska V, Jurankova J, Fric P, Kroupa R, et al. The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. Eur J Gastroenterol Hepatol 2007;19:1111–1113.

Gluud LL, Vilstrup H, Morgan MY. Non-absorbable disaccharides versus placebo/no intervention and lactulose versus lactitol for the prevention and treatment of hepatic encephalopathy in people with cirrhosis. Cochrane Database Syst Rev 2016;4CD003044.

Malaguarnera M, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. Dig Dis Sci 2007;52:3259–3265.

Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Symbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology 2004;39:1441–1440.

Khanna S. Microbiota Replacement Therapies: Innovation in Gastrointestinal Care. Clin Pharmacol Ther 2018;103:102–111.

Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human faecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. J Clin Gastroenterol 2010;44:354–360.

Philips CA, Pande A, Shasthry SM, Jamwal KD, Khillan V, Chandel SS, et al. Healthy Donor Faecal Microbiota Transplantation in Steroid-Ineligible Severe Alcoholic Hepatitis: A Pilot Study. Clin Gastroenterol Hepatol 2017;15:600–602.

Bajaj JS, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, et al. Faecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. Hepatology 2017;66:1727–1738.

Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913–916 e917.