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Changes in the Intestinal Microbiome and Alcoholic- and Non-alcoholic Liver Diseases—Causes or Effects?

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Abstract

The prevalence of fatty liver diseases is increasing rapidly worldwide; after treatment of hepatitis C virus infection becomes more widespread, fatty liver diseases are likely to become most prevalent liver disorders. Although fatty liver diseases are associated with alcohol, obesity, and the metabolic syndrome, their mechanisms of pathogenesis are not clear. Development and progression of fatty liver, alcoholic, and non-alcoholic liver disease (ALD) all appear to be influenced by the composition of the microbiota. The intestinal microbiota have been shown to affect pre-cirrhotic and cirrhotic stages of liver diseases, which could lead to new strategies for their diagnosis, treatment, and study. We review differences and similarities in the cirrhotic and pre-cirrhotic stages of non-alcoholic fatty liver disease (NAFLD) and ALD. Differences have been observed in these stages of alcohol-associated disease in patients who continue to drink compared with those who stop, with respect to the composition and function of the intestinal microbiota and intestinal integrity. NAFLD and the intestinal microbiota also differ between patients with and without diabetes. We also discuss the potential of microbial therapy for patients with NAFLD and ALD.

Effects of the Gut Microbiota on the Liver

The microbiota maintains a symbiotic relationship within the intestine and contributes to various functions such as digestion, synthesis of vitamins, and resistance to colonization of intestine by pathogens[1]. The microbiota is hugely diverse. An estimated 10–100 trillion microorganisms are present in each gram of stool, with approximately 500–1000 highly prevalent species; [2] these strongly linked to an individual's gut metabolome. The microbiota provide its host with an extensive set of otherwise inaccessible metabolic capabilities and approximately 150-fold more genes than human cells [3]. There are several methods to define and interpret the composition of the gut microbiota (Table 1). Ultimately bacteria are

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presented as phylum, order, family, genus or species, in relative abundance values. Before comparing different studies, the uniformity of the depth of coverage of each subject in the study (i.e. number of reads per sample) should be taken into consideration.

The gut microbiota elicits innate and adaptive immune mechanisms that cooperate to protect the host and maintain intestinal homeostasis. Activation of innate host defense depends on specific pattern recognition receptors, including the family of toll-like receptors (TLRs) and NOD-like receptors (NLRs). Of the 11 TLRs that have been identified in humans, TLRs 2, 4, and 9 are involved in interactions between the gut microbiota host immune response, recognizing and becoming activated by Gram-positive and Gram-negative bacteria [4].

The liver regulates systemic metabolism and the distribution of substances through the human gut, and also regulates numerous hormone and immune responses [5]. Communication between the liver and the intestine is facilitated by bile acids, which mediate absorption of dietary fats and vitamins and act as ligands for receptors that include nuclear receptor farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (GPBAR1 or TGR5), which regulate the entero–hepatic circulation [1]. A decrease in total fecal bile acids directly affects overgrowth of intestinal bacteria. FXR-deficient mice are protected from genetic- and diet-induced obesity but not hepatic steatosis [6]. The intestinal microbiota might therefore contribute to liver disease by modifying intestinal bile acids and regulating FXR signaling. Studies of expression patterns of bacterial genes and profiles of bile acids might help determine how modulation of FXR could contribute to liver disease.

Role of microbiota in digestion and effect of bile acids

Humans do not have enzymes that digest cellulose, xylans, resistant starch, or inulin. Intestinal microbes ferment these carbohydrates to produce short-chain fatty acids [7]. Cholic acid and chenodeoxycholic acid are the primary bile acids synthesized from cholesterol in the human liver. However, these primary acids can be converted into secondary bile acids by the intestinal microbiota [8]. Intestinal microorganisms therefore have an important role in metabolizing bile acid. For example, *Clostridium* spp help catalyze the breakdown of the most abundant bile acid, cholic acid, to deoxycholic acid, via a 7α-dehydroxylation reaction [9].

Bile acids suppress overgrowth of bacteria in the gut and have a strong anti-microbial role in maintaining a healthy gut [10]. Bile acids have been proposed to be entero-protective, probably via their detergent properties and a sophisticated mechanism of activation of FXR, which protects the distal small intestine from bacterial proliferation and its detrimental effects. This mechanism involves activation of genes regulated by FXR in the ileum, including angiopoietin 1 (*ANG1),* nitric oxide synthase 2 *(NOS2),* and interleukin-18 *(IL18)* [11]. In of 8–10 month old mice with bile duct ligation, as well as FXR-knockout mice, expression levels of *Ang1, Fgf15, Shp, Car12,* and *Ibabp* correlated with FXR-mediated entero-protection, indicating that the protective effects of FXR involve expression of these genes. [11] These pathways are part of inflammatory signaling pathways that are activated in mice with bile duct ligation, demonstrating that FXR is important for protecting the distal small intestine against bacterial overgrowth and the resulting disruption of the epithelial barrier. Microbes that can tolerate physiologic concentrations of bile acids survive in the gut;

feeding cholic acid to rats significantly increased the ratio of *Firmicutes* to *Bacteriodetes* [12]. Therefore the deconjugation and 7α-dehydroxylation of bile acids in stool are important markers of gut health.

Gut hormones

Gut hormones promote intestinal epithelial proliferation and reduce gut permeability. Glucagon like peptide-1 (GLP1) is an incretin secreted by intestinal L cells that maintains glucose-dependent insulin secretion and augmentation of β-cell mass; GLP1 inhibits glucagon release, gastric emptying, and food intake [13]. A healthy gut microbiota produces short-chain fatty acids that activate the G protein-coupled receptors GPR41 and GPR43, promoting secretion of GLP1 [9]. GLP2 is secreted along with GLP1 and helps maintain the gut barrier integrity, slows gastric emptying, improves nutrient absorption, and increases immune function [1415].

Effects of type 2 diabetes and obesity

Microbial dysbiosis is associated with type 2 diabetes as well as obesity[1617]. Studies have also shown an increase in the relative abundance of *Bacteriodetes* and *Betaproteobacteria* and reductions in *Firmicutes* and *Clostridia*. These findings associate obesity and diabetes with reductions in butyrate-producing bacteria and increases in pathogens [181920].

The gut microbiota is partly responsible for body fat deposition in mice—colonized animals have higher fat content than germ-free animals. Inoculation of germ-free mice with microbiota from colonized adult mice resulted in a 57% increase in total body fat [212223]. The proportions of *Firmicutes* and *Bacteriodetes* vary between obese and lean mice—obese have a higher ratio of *Firmicutes* to *Bacteriodetes*, which has also been observed in humans [2425]. A different balance of *Bifidobacterium* species and *Staphylococcus aureus* has been observed in children of normal weight compared to those that become overweight or obese, indicating that the microbiome might be used to predict obesity [26]. A high-fat diet can cause reduce proportions of *Eubacterium rectale, Clostridium coccoides,* and *Bifidobacterium* species [27]. Ultimately, studies of changes in the gut microbiota must be performed in the context of their function and composition, as well as their effect on the host.

Role for the Intestinal Microbiota

Nonalcoholic fatty liver disease (NAFLD)

NAFLD, one of the most common cause of chronic liver diseases, is characterized by fat accumulation, mainly as triglycerides, in the hepatocytes. The disease is associated with factors such as obesity, metabolic syndrome, insulin resistance, and dyslipidemia [2829]. An energy-rich diet of fat and carbohydrates leads to dysregulation of adipocytes to adapt in terms of proliferation and differentiation [30]. NAFLD encompasses a spectrum of hepatic pathologies, and can progress to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma.

Patients with NAFLD have lower proportions of *Bacteroidetes* and higher proportions of *Prevotella* and *Porphyromonas* spp compared to healthy controls [31]. Predisposition to

NAFLD is associated with increased expression of TLR4, TLR9, or the tumor necrosis factor (TNF) receptor. The gut microbiota might control the severity of NAFLD by increasing production of ethanol, activating TLR signaling and TNF production in the liver, or altering the bile acid profile. In a study of C129S6 mice, a high-fat diet shifted the metabalome of the intestinal microbiota toward a choline-degradation profile, resulting in low circulation levels of plasma phosphatidylcholine and high urinary excretion of methylamines [32].

Alterations to the intestinal microbiota are also thought to affect development of NASH, by affecting digestion, development of obesity, the immune response, and production of gut hormones [213334]. Patients with NASH have an increased abundance of ethanol-producing bacteria in their gut microbiome and increased blood concentrations of ethanol, indicating a role for alcohol-producing microbiota in the pathogenesis of NASH [31]. Fecal samples from patients with NASH have decreased proportions of *Bacteriodetes* and increased proportions of *Clostridium coccoides* [31]. In a study that included 16 healthy children (controls), 25 obese children, and 22 children with biopsy-proven NASH, microbial diversity was reduced in fecal samples from the obese children and from children with NASH, compared with controls [31]. Children with NASH and obese children had similar increases in *Bacteriodetes* and decreases in *Firmicutes*. Proportions of *Proteobacteria* were significantly greater children with obesity or NASH than controls. However, proportions of *Lachnospiraceae* and *Ruminococcaceae* decreased, along with the proportion of *Firmicutes*, and there was an even greater reduction in *Blautia* and *Faecalibacterium* genera in obese children and those with NASH, compared with controls. The increase in *Proteobacteria* correlated with an increased proportion of *Enterobacteriaceae*—especially *Escherichia*.

Escherichia produce ethanol, and serum concentrations of ethanol are significantly higher in patients with NASH compared to obese or control groups. In a study of patients with NASH and F0–F3 fibrosis, proportions of *Bacteroides* and *Ruminococcus* were greater in patients with higher-stage fibrosis [31]. These findings support observations from previous studies, which found patients with NASH and cirrhosis to have significantly greater proportions of *Bacteroidaceae* than patients with NASH without cirrhosis [31]. Patients with type 2 diabetes also have higher proportions of Bacteroides and Ruminococcus than patients without [35].

When mice with disruption of *Nlrp3* or *Nlrp6* are placed on a methionine-choline deficient diet, to induce steatosis, their intestinal microbiota is altered and they develop colonic inflammation and NASH [36]. In other knockout mice that develop severe diet-induced NASH, steatohepatitis was found to arise via influx of intestinal TLR4 ligands and TLR9 activation, leading to production of TNF in the liver [36]. Liver tissues from patients with NASH also have higher levels of TNF than those from patients with simple hepatic steatosis [37].

Alcoholic liver disease (ALD)

Alcohol abuse is one of the leading causes of chronic liver disease. The prognosis for patients with ALD worsens as the disease progresses from steatohepatitis to liver fibrosis, cirrhosis, and end-stage liver disease. ALD has a unique clinical presentation in the form of alcoholic hepatitis, which is associated with a significant inflammation[38]. During progression of ALD, the composition of the microbiota changes through pre-cirrhotic,

cirrhotic, and alcoholic hepatitis forms. These can vary with patterns of alcohol intake, such as with binge drinking vs social drinking or chronic dependence. Studies of the relationship between ALD and the intestinal microbiota should be performed in patients with different patterns of alcohol consumption and different stages of liver disease. The pathogenesis of ALD is poorly understood, because the effects of alcohol on the intestine and the microbiome begin before there is evidence of liver disease.

Patients without cirrhosis

In healthy subjects, binge drinking causes significant increases in the blood level of endotoxin (produced by Gram-negative) and systemic inflammation, which might be caused by increased gut permeability after the binge [39]. Rodents have also been shown to have increased endotoxemia after binge consumption of ethanol [40]. Interestingly, germ-free mice given an alcohol gavage developed more severe acute alcohol-associated injury than mice with a control microbiome [41].

Studies of chronic alcohol drinkers without cirrhosis or alcoholic hepatitis found that bacterial overgrowth and translocation are required for disease progression. Higher numbers of aerobic and anaerobic bacteria were detected in jejunal aspirates from alcoholic patients than from non-alcoholics [42]. Gut leakiness, caused by intestinal barrier dysfunction, has been reported in patients with alcohol-induced endotoxemia and liver damage [434445]. The permeability of the gut increases via of the breakdown of alcohol into acetaldehyde and allows endotoxin and bacterial DNA into the liver [4046], which activate Kupffer cells via TLR4 or TLR9. Kupffer cells then begin to produce inflammatory cytokines [47]. Chronic alcohol abuse can induce changes in the colonic mucosal microbiota that can be detected in fecal samples. Fecal samples from patients with alcoholic cirrhosis have a lower proportion of *Bacteriodetes* and higher proportions of *Proteobacteria* in the colon as compared to alcoholic patients without cirrhosis [48]. Once patients abstain from alcohol abuse, intestinal permeability is reduced and proportions of some autochthonous taxa, such as *Ruminococcus*, normalize [49].

In rodent, bacterial translocation can be detected as early as 2–3 weeks after chronic alcohol consumption begins, before changes are observed in the microbiome [5051]. Rats that consumed alcohol for 10 weeks developed alterations in the colonic mucosa associated with the composition of the microbiome [52]. Mice fed alcohol for 3 weeks had increased proportions of *Bacteriodetes* and *Verrucomicrobia* in the cecum, whereas control mice had higher proportions of *Firmicutes* [51]. Feces from mice given chronic alcohol for 8 weeks had reduced proportions of *Bacteroidetes* and *Firmicutes* and proportional increases in Gram-negative *Proteobacteria* and Gram-positive *Actinobacteria* [53]. This dysbiosis was associated with significant reductions in *Lactobacillus, Bacteriodaceae, Pediococcus, Leuconostoc, and Lactococcus* [51].

Although it is tempting to speculate that alcohol simply has direct effects on intestinal integrity and the microbiota, leading to development of liver injury, it is important to remember that alcohol also affects the composition of bile acids. The gastrointestinal tract of rats fed alcohol for 8 weeks contained many bile acid alterations, increased levels of fatty acids and steroids, and decreased levels of carnitines, amino acids, branched amino acids,

and short-chain fatty acids [54]. Fatty acids that increased included 17-HDoHE and 19,20-DiHDPA, which are metabolites of docosahexaenoic acid (DHA). Increased levels of DHA and its metabolites in the large intestine indicate disrupted absorption of DHA. All 21 bile acids were perturbed along the length of the gastrointestinal tract, but the largest changes were observed in the ileum. Levels of taurine-conjugated bile acids were reduced in the small intestine and liver, compared to control rats. The bile salt taurine to glycine ratio was 30:1 in control rats, vs 1:1 in the alcohol-fed rats. The overgrowth of microbiota in alcohol-fed rats contributed to the degradation of taurine to inorganic sulfate, thereby reducing their availability [54]. Chronic consumers of alcohol were found to have significantly higher synthesis of bile acids, regardless of cirrhosis, which contributed to gut injury; FXR signaling was not found to be involved in this process[55]. Further studies are needed to determine how alcohol consumption alters the intestinal microbiota.

Alcoholic hepatitis and cirrhosis

Patients with alcoholic hepatitis and cirrhosis have an altered immune response and frequently develop infections, associated with poor outcomes. Alcoholic hepatitis, in particular, has high mortality, partly due to systemic inflammatory response syndrome [56]. Many factors are likely to contribute to inflammation in these patients. There have been few studies of their microbiomes, due to the presence of multiple confounders, including alcohol abstinence or level of intake and concurrent use of proton pump inhibitors and/or antibiotics. Transfer of gut microbiota from a patient with alcoholic hepatitis to germ-free mice led to increased liver inflammation, compared to microbiota from alcoholic patients without any liver injury, indicating that alcoholic hepatitis-associated microbes contribute to liver injury. The microbiota from the patient with alcoholic hepatitis had increased dysbiosis, with reduced proportions of *Fecalibacterium* spp, compared to the microbiota from patients without liver injury[57]. More studies are needed in these populations of patients.

Studies of patients with alcoholic cirrhosis are usually performed as sub-group analyses of larger studies of cirrhosis. Further complicating the analyses are patients with alcoholic cirrhosis who continue to drink but do not have alcoholic hepatitis. Patients with alcoholic cirrhosis have been consistently found to have higher levels of microbial dysbiosis than patients with non-alcoholic cirrhosis, despite similar level of cirrhosis severity [58]. Animal studies are also a challenge; mouse models of ALD do not develop cirrhosis. Patients with alcoholic cirrhosis who continue to drink have evidence of colonic inflammation with significantly increases in total fecal and secondary bile acid proportions [55].

Manipulating the Microbiome

NAFLD

GLP1 is secreted into bloodstream in response to nutrient ingestion and induces secretion of insulin in response to glucose, inhibits secretion of postprandial glucagon, delays gastric emptying, and promotes weight loss [59]. Liraglutide, a GLP1 agonist, induces weight loss in obese patients and improves eating behavior. Mice given GLP1 agonists have reduced hepatic triglyceride content compared to mice given vehicle (controls) [60].

GLP1 is involved in lipid metabolism, reducing serum levels of triglycerides, total cholesterol levels, low density lipoprotein–cholesterol, and serum high-density lipoprotein–cholesterol. GLP1 agonists can improve the lipid profile and increase metabolism via activation of peroxisome proliferator-activated receptor-α on the surface of hepatocytes, reducing the synthesis of apolipoprotein C, degrading fat in plasma, and removing triglycerides [61626364]. Administration of the probiotic VSL#3 for 4 months significantly reduced NASH in children, increasing levels of GLP1 [65]. In mice with steatosis, VSL#3 reduced fat deposits and damage to the liver parenchyma and decreased serum levels of alanine aminotransferase (ALT). The probiotic also reduced oxidative and inflammatory liver damage [666768].

The butyrate-producing probiotic MIYAIRI 588 reduced hepatic oxidative stress in a rat model of NASH [69]. Interestingly, simply adding butyrate to the diets of mice with steatosis reduced liver injury [70]. A meta-analysis found that this probiotic use can reduce serum levels of ALT and aspartate aminotransferase (AST), inflammation, and insulin resistance in NAFLD patients [71]. However, the microbes and amounts given varied among groups.

Obeticholic acid is a potent activator of the FXR that reduces liver fat content and fibrosis in animal models of NAFLD. Adult patients with NASH given obeticholic acid for 72 weeks had reduced histologic features of NASH. The long-term benefits of obeticholic acid require further study [72].

ALD

Abstinence is the best treatment for ALD, because it is associated with improvements in the microbiota and intestinal permeability [49], but there is often a residual dysbiosis. The intestinal microbiome has been manipulated in patients and in animal models of ALD using antibiotics, prebiotics, and probiotics. The effects of antibiotics that decrease endotoxin signaling (alcohol-induced endotoxemia), have been explored [7374]. Affecting the intestinal microbiota with ampicillin increased intestinal expression of the solute carrier family 10 (sodium and bile acid cotransporter) member 2 (SLC10A2 or ASBT), increasing the bile acid transport from the intestine into portal blood [67]. SLC10A2 is the primary mechanism for uptake of intestinal bile acids by apical cells in the distal ileum.

Short-term administration of *Bifidobacterium bifidum* and *Lactobacillus plantarum 8PA3* to alcoholic patients lowered plasma levels of ALT and AST, restored the intestinal microbiota, and reduced alcoholic liver injury[75]. Neutrophils from patients with alcoholic cirrhosis given *Lactobacillus casei Shirota* (live, heat inactivated, or culture supernatant) for 4 weeks had increased phagocytic capacity [76]. Administration of microencapsulated *L plantarum* to mice after chronic alcohol feeding reduced endotoxemia, serum levels of aminotransferase, activation of nuclear factor-κB, and expression of TNF and IL12B. Intestine and liver tissues from these mice had reduced histologic features of alcohol-induced injury. Alcoholic patients given *Bifidobacteria* and *lactobacillus* over a 5 day period had increased numbers of these bacteria in their intestine and lower serum levels of AST and ALT, indicating that these probiotics can quickly alter the gut microbiota and aide in recovery from liver injury induced by chronic alcohol consumption [75]. Probiotics are likely to reduce oxidative stress and inflammation in the intestine and preserve its barrier function.

Administration of prebiotics to alcohol-fed mice reduced bacterial overgrowth and steatohepatitis by partially restoring intestinal expression of the anti-microbial protein regenerating family member 3 gamma (REG3G) [51]. REG3G is a secreted, C-type lectin with activity against Gram-positive bacteria. Supplementation of the diet with milk osteopontin also reduces alcohol-induced liver injury, blocking translocation of enteric Gram-negative bacteria and reduces the effects of endotoxin on the liver [75]. Supplementing the diets of mice with long-chain saturated fatty acids increased intestinal barrier function by promoting expansion of lactobacilli, which attenuated alcohol-associated liver injury [77].

Changes in the Microbiome During Disease Development

In adult mice fed methionine-choline deficient diets[36], inflammasome-dependent processing of IL1B and IL18 were found to promote progression of fatty liver disease. Complex and cooperative effects of NLRs and TLR also regulate metabolic events that lead to abnormal accumulation of bacterial products in the portal circulation. Alterations in the intestinal microbiota, along with inflammasome deficiencies, could contribute to development of NAFLD.

A study of 244 patients with different cirrhosis etiologies and stages of cirrhosis (compensated, decompensated) [58] was used to define the cirrhosis dysbiosis ratio, which is a ratio of autochthonous or beneficial bacteria to potentially pathogenic ones. A lower CDR indicated a smaller ratio of autochthonous to non-autochthonous taxa. CDR was highest among individuals without cirrhosis (controls), lower among patients with compensated cirrhosis, and lowest in patients with decompensated cirrhosis. Over time, a reduced CDR was associated with disease progression and endotoxemia. Patients with cirrhosis had higher proportions of *Staphylococcaeae, Enterobacteriaceae,* and *Enterococcaceae* than controls; a higher CDR was associated with worse outcome.

The presence or relative abundance of certain bacterial taxa could be used as markers of intestinal disorders. The microbiome profile associated with endotoxemia reflects the microbiota effects as a whole. A study of patients with NASH [78] (30 with F0/1 fibrosis and 27 with F≥2 fibrosis) found increased proportions of Bacteroides relative abundance in NASH patients compared to compared to patients without NASH. The proportion of Prevotella was significantly decreased in patients with NASH and F≥2, compared to patients with F0/1 fibrosis. Meta-genomic profile analysis with KEGG associated NASH with fibrosis stage F≥2 with microbial changes in carbohydrate, lipid, and amino acid metabolism. The severity of NAFLD is therefore associated with dysbiosis of the intestinal microbiota and changes in its metabolic functions compared to patients without NAFLD or NASH.

More studies are needed to thoroughly evaluate the contribution of the microbiota to the etiology of liver disease. In-depth analyses will require a large, multi-center collaboration that collect many samples over time from patients with NASH and ALD. Understanding and reversing the severe dysbiosis that develops in patients with NAFLD, NASH, or ALD will require further insights into the microbial metagenome, transcriptome, and metabolome, as well as more studies of the interactions among the intestine, liver, and microbiome.

Future Directions

Although substantial progress has been made in increasing our understanding of the gut microbiota in patients with alcoholic and non-alcoholic steatohepatitis, many important questions remain. With the increasing epidemic of obesity and NAFLD, and the effects of alcohol misuse and diabetes in these patients are important to determine. Phenotypes of NASH vary among different populations, so multi-ethnic studies are needed to compare differences in microbiomes and other factors that might contribute to these differences [79]. Large, multi-center studies of many patients, over long time periods, are needed to determine how the microbiota might cause liver disease and how liver disease alters the microbiota. Antibiotics, synbiotics, probiotics, prebiotics, and putative microbial products might be developed to treat patients with ALD or NAFLD. However in studying the impaired interactions between the gut and liver in these patients, we should remember that NAFLD and ALD are multi-organ diseases that also involve metabolic syndrome and the widespread effects of alcohol. The composition of the intestinal microbiome varies widely among individuals, and its effects on development of liver disease involve additional environmental, dietary, genetic, social, and behavioral factors.

Microbiota Analysis Strategies

Human microbiome analysis takes raw sequence reads from the 16S rRNA gene or metagenomic reads (random genomic fragments) and uses these to identify the taxa composition or gene content of a biological sample. Two major challenges of analyzing the human microbiome comes from the fact that the distributions of taxa within a sample are non-parametric and the data matrices are sparse. The former issue is a result of the fact that the communities are dynamic and oscillating (REF), and thus, depends on what phase the sample is in when it is interrogated. The latter issues is due to the fact that many taxa perform the same function in the gut ecosystem and thus one individual may have taxa A whereas a second person may have taxa B performing the same function. These issues present challenges to microbiome analysis that the field is still trying to address.

Two popular approaches, Qiime and Mothur, take 16S rRNA reads and cluster them into Operational Taxonomic Units (OTUs) using greedy algorithms based on word tables and then perform phylogenetic analysis on these OTUs. These phylogenetic tree approaches compare the trees from the various samples to derive Alpha diversity (within sample variation), Beta diversity (variation between samples) and derive community statistics such as UNIFRAC that compare classes of samples (i.e. disease versus controls).

One of the major issues with the phylogenetic approaches is that the OTU construction can be problematic as the clusters may vary depending on the input order of the raw reads. An alternative approach that we routinely use is to just build the taxa tables directly from the raw reads using the RDP10 Bayesian algorithm. This algorithm is quite fast and practical for analyzing millions of 16S raw reads. However, the tool only classifies taxa down to the genera level but this is usually adequate for all practical clinical comparisons.

Once one has generated taxa abundance tables, on can do binary statistical comparison between experimental classes using non-parametric techniques such as Metastats and LEfSE. LEfSE has the added advantage that it does a linear discriminant analysis that identifies specific taxa that differentiate the clinical classes.

Metagenomic approaches (Metaphlan, MetAMOS) can also be used to define the species in a sample. This entails random shotgun sequencing of fragmented of all the DNA in a samples and identifying assembling clusters by comparing these clusters to sequenced genomes to identifying the genomic species in a sample. However, it has been reported that ligation of next-generation sequencing adapters to the genomic fragments is not very reproducible leading to analysis inconsistencies.

There are several techniques (Picrust, HUMAnN) that build metabolic pathway tables instead of taxa tables. Picrust takes the output 16S abundance tables from QIIME and builds a KEGG pathway table by comparing the identified tax to their closest phyogenetic relative whose genome has been completely sequenced and annotated. HUMAnN takes an alternative approach and builds the KEGG pathway tables directly from the metagenomic data.

One approach we have taken to interpret the dynamic nature of the microbial communities is to perform correlation network analysis and correlation difference network analysis. The methodology calculates the significant spearman correlations between feature tables from individual clinical classes. The identified correlated features are graphically represented using Cytoscape and interpreted by visual inspection to generate working hypotheses. The utility of the approach is that one can correlate different feature sets such as bacterial taxa, metabolic functions, immune cytokines, and clinical features. We have built an extension of the approach in which we calculate the correlation difference between the clinical classes that is we identify those correlations that are statistically different between the classes. This latter approach has proven very effective in the development of hypothesis for disease processes.

References

1. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014; 146:1513–1524. [PubMed: 24440671]
2. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. J Physiol. 2009; 587:4153–4158. [PubMed: 19491241]
3. Qin J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010; 464:59–65. [PubMed: 20203603]
4. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009; 136:65–80. [PubMed: 19026645]
5. Henao-Mejia J, Elinav E, Thaiss CA, Flavell RA. The intestinal microbiota in chronic liver disease. Adv Immunol. 2013; 117:73–97. [PubMed: 23611286]
6. Prawitt J, et al. Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. Diabetes. 2011; 60:1861–1871. [PubMed: 21593203]
7. Salyers AA, Gherardini F, O'Brien M. Utilization of xylan by two species of human colonic Bacteroides. Appl Environ Microbiol. 1981; 41:1065–1068. [PubMed: 7235704]
8. Prabha V, Ohri M. Review: Bacterial transformations of bile acids. World J Microbiol Biotechnol. 2005; 22:191–196.

9. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem. 2013; 288:25088–25097. [PubMed: 23836895]
10. Kurdi P, Kawanishi K, Mizutani K, Yokota A. Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. J Bacteriol. 2006; 188:1979–1986. [PubMed: 16484210]
11. Inagaki T, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A. 2006; 103:3920–3925. [PubMed: 16473946]
12. Islam KBMS, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology. 2011; 141:1773–1781. [PubMed: 21839040]
13. Lim GE, Brubaker PL. Glucagon-Like Peptide 1 Secretion by the L-Cell The View From Within. Diabetes. 2006; 55:S70–S77.
14. Dubé PE, Brubaker PL. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. Am J Physiol Endocrinol Metab. 2007; 293:E460–465. [PubMed: 17652153]
15. Shi X, et al. Central GLP-2 enhances hepatic insulin sensitivity via activating PI3K signaling in POMC neurons. Cell Metab. 2013; 18:86–98. [PubMed: 23823479]
16. Ley RE, et al. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005; 102:11070–11075. [PubMed: 16033867]
17. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444:1022–1023. [PubMed: 17183309]
18. Zhang X, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PloS One. 2013; 8:e71108. [PubMed: 24013136]
19. Qin J, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012; 490:55–60. [PubMed: 23023125]
20. Karlsson FH, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013; 498:99–103. [PubMed: 23719380]
21. Flint HJ. Obesity and the gut microbiota. J Clin Gastroenterol. 2011; 45:S128–S132. [PubMed: 21992951]
22. Finelli C, Tarantino G. Is there any consensus as to what diet or lifestyle approach is the right one for NAFLD patients? J Gastrointest Liver Dis JGLD. 2012; 21:293–302.
23. Wolf G. Gut microbiota: a factor in energy regulation. Nutr Rev. 2006; 64:47–50. [PubMed: 16491670]
24. DiBaise JK, et al. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc. 2008; 83:460–469. [PubMed: 18380992]
25. Kallus SJ, Brandt LJ. The intestinal microbiota and obesity. J Clin Gastroenterol. 2012; 46:16–24. [PubMed: 22064556]
26. Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr. 2008; 87:534–538. [PubMed: 18326589]
27. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des. 2009; 15:1546–1558. [PubMed: 19442172]
28. Vanni E, et al. From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis Off J Ital Soc Gastroenterol Ital Assoc Study Liver. 2010; 42:320–330.
29. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of Non-Alcoholic Fatty Liver Disease. Dig Dis. 2010; 28:155–161. [PubMed: 20460905]
30. Gregor MF, Hotamisligil GS. Thematic review series: Adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. J Lipid Res. 2007; 48:1905–1914. [PubMed: 17699733]
31. Zhu L, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. Hepatology. 2013; 57:601–609. [PubMed: 23055155]
32. Maher JJ. New insights from rodent models of fatty liver disease. Antioxid Redox Signal. 2011; 15:535–550. [PubMed: 21126212]

33. Vinolo MAR, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients. 2011; 3:858–876. [PubMed: 22254083]
34. Tilg H. Obesity, metabolic syndrome, and microbiota: multiple interactions. J Clin Gastroenterol. 2010; 44(Suppl 1):S16–18. [PubMed: 20535027]
35. Bajaj JS, et al. Gut Microbiota Alterations can predict Hospitalizations in Cirrhosis Independent of Diabetes Mellitus. Sci Rep. 2015; 5:18559. [PubMed: 26692421]
36. Henao-Mejia J, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012; 482:179–185. [PubMed: 22297845]
37. Farhadi A, et al. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis. Liver Int Off J Int Assoc Study Liver. 2008; 28:1026–1033.
38. Szabo G. Gut-liver axis in alcoholic liver disease. Gastroenterology. 2015; 148:30–36. [PubMed: 25447847]
39. Bala S, Marcos M, Gattu A, Catalano D, Szabo G. Acute Binge Drinking Increases Serum Endotoxin and Bacterial DNA Levels in Healthy Individuals. PLoS ONE. 2014; 9:e96864. [PubMed: 24828436]
40. Rivera CA, Bradford BU, Seabra V, Thurman RG. Role of endotoxin in the hypermetabolic state after acute ethanol exposure. Am J Physiol. 1998; 275:G1252–1258. [PubMed: 9843760]
41. Chen P, et al. Microbiota Protects Mice Against Acute Alcohol-Induced Liver Injury. Alcohol Clin Exp Res. 2015; doi: 10.1111/acer.12900
42. Bode JC, Bode C, Heidelbach R, Dürr HK, Martini GA. Jejunal microflora in patients with chronic alcohol abuse. Hepatogastroenterology. 1984; 31:30–34. [PubMed: 6698486]
43. Bjarnason I, Peters TJ, Wise RJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. Lancet Lond Engl. 1984; 1:179–182.
44. Keshavarzian A, et al. Leaky gut in alcoholic cirrhosis: a possible mechanism for alcohol-induced liver damage. Am J Gastroenterol. 1999; 94:200–207. [PubMed: 9934756]
45. Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol. 2000; 32:742–747. [PubMed: 10845660]
46. Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol. 1991; 12:162–169. [PubMed: 2050995]
47. Roh YS, Seki E. Toll-like receptors in alcoholic liver disease, non-alcoholic steatohepatitis and carcinogenesis. J Gastroenterol Hepatol. 2013; 28(Suppl 1):38–42. [PubMed: 23855294]
48. Mutlu EA, et al. Colonic microbiome is altered in alcoholism. Am J Physiol Gastrointest Liver Physiol. 2012; 302:G966–978. [PubMed: 22241860]
49. Leclercq S, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci U S A. 2014; 111:E4485–E4493. [PubMed: 25288760]
50. Napolitano LM, et al. Chronic ethanol intake and burn injury: evidence for synergistic alteration in gut and immune integrity. J Trauma. 1995; 38:198–207. [PubMed: 7869435]
51. Yan AW, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatol Baltim Md. 2011; 53:96–105.
52. Mutlu E, et al. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin Exp Res. 2009; 33:1836–1846. [PubMed: 19645728]
53. Bull-Otterson L, et al. Metagenomic Analyses of Alcohol Induced Pathogenic Alterations in the Intestinal Microbiome and the Effect of Lactobacillus rhamnosus GG Treatment. PLoS ONE. 2013; 8:e53028. [PubMed: 23326376]
54. Xie G, et al. Alteration of bile acid metabolism in the rat induced by chronic ethanol consumption. FASEB J Off Publ Fed Am Soc Exp Biol. 2013; 27:3583–3593.
55. Kakiyama G, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. Am J Physiol Gastrointest Liver Physiol. 2014; 306:G929–937. [PubMed: 24699327]

56. Jaruvongvanich V, Upala S, Sanguankeo A. Association between systemic inflammatory response syndrome and mortality in alcoholic hepatitis: A meta-analysis. Hepatol Baltim Md. 2015; doi: 10.1002/hep.28366
57. Llopis M, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Gut. 2015; doi: 10.1136/gutjnl-2015-310585
58. Bajaj JS, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol. 2014; 60:940–947. [PubMed: 24374295]
59. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet Lond Engl. 2006; 368:1696–1705.
60. Samson SL, et al. Exenatide decreases hepatic fibroblast growth factor 21 resistance in non-alcoholic fatty liver disease in a mouse model of obesity and in a randomised controlled trial. Diabetologia. 2011; 54:3093–3100. [PubMed: 21956711]
61. Wilkins JJ, Dubar M, Sébastien B, Laveille C. A drug and disease model for lixisenatide, a GLP-1 receptor agonist in type 2 diabetes. J Clin Pharmacol. 2014; 54:267–278. [PubMed: 24122776]
62. Sebokova E, et al. Taspoglutide, a novel human once-weekly analogue of glucagon-like peptide-1, improves glucose homeostasis and body weight in the Zucker diabetic fatty rat. Diabetes Obes Metab. 2010; 12:674–682. [PubMed: 20590744]
63. Aguilar RB. Evaluating treatment algorithms for the management of patients with type 2 diabetes mellitus: a perspective on the definition of treatment success. Clin Ther. 2011; 33:408–424. [PubMed: 21635988]
64. Wang XC, Gusdon AM, Liu H, Qu S. Effects of glucagon-like peptide-1 receptor agonists on non-alcoholic fatty liver disease and inflammation. World J Gastroenterol. 2014; 20:14821–14830. [PubMed: 25356042]
65. Alisi A, et al. Randomised clinical trial: The beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2014; 39:1276–1285. [PubMed: 24738701]
66. Li Z, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatol Baltim Md. 2003; 37:343–350.
67. Swann JR, et al. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci U S A. 2011; 108(Suppl 1):4523–4530. [PubMed: 20837534]
68. Wong VWS, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. Ann Hepatol. 2013; 12:256–262. [PubMed: 23396737]
69. Endo H, Niioka M, Kobayashi N, Tanaka M, Watanabe T. Butyrate-Producing Probiotics Reduce Nonalcoholic Fatty Liver Disease Progression in Rats: New Insight into the Probiotics for the Gut-Liver Axis. PLoS ONE. 2013; 8:e63388. [PubMed: 23696823]
70. Jin CJ, Sellmann C, Engstler AJ, Ziegenhardt D, Bergheim I. Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH). Br J Nutr. 2015; 114:1745–1755. [PubMed: 26450277]
71. Ma YY, et al. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol. 2013; 19:6911–6918. [PubMed: 24187469]
72. Neuschwander-Tetri BA, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet Lond Engl. 2015; 385:956–965.
73. Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology. 1995; 108:218–224. [PubMed: 7806045]
74. Zhou Z, et al. A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. Am J Pathol. 2003; 163:1137–1146. [PubMed: 12937155]
75. Kirpich IA, et al. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol Fayettev N. 2008; 42:675–682.
76. Stadlbauer V, et al. Effect of probiotic treatment on deranged neutrophil function and cytokine responses in patients with compensated alcoholic cirrhosis. J Hepatol. 2008; 48:945–951. [PubMed: 18433921]

77. Chen P, et al. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver injury in mice. Gastroenterology. 2015; 148:203–214.e16. [PubMed: 25239591]

78. Boursier J, et al. The severity of NAFLD is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatol Baltim Md. 2015; doi: 10.1002/hep.28356

79. Das K, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. Hepatol Baltim Md. 2010; 51:1593–1602.

80. Caporaso JG, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7:335–336. [PubMed: 20383131]

81. Schloss PD, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009; 75:7537–7541. [PubMed: 19801464]

82. White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol. 2009; 5:e1000352. [PubMed: 19360128]

83. Cole JR, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014; 42:D633–642. [PubMed: 24288368]

84. Segata N, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011; 12:R60. [PubMed: 21702898]

85. https://mbac.gmu.edu/galaxy.

86. Langille MGI, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013; 31:814–821. [PubMed: 23975157]

87. Abubucker S, et al. Metabolic reconstruction for metagenomic data and its application to the human microbiome. PLoS Comput Biol. 2012; 8:e1002358. [PubMed: 22719234]

88. Segata N, et al. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat Methods. 2012; 9:811–814. [PubMed: 22688413]

89. Treangen TJ, et al. MetAMOS: a metagenomic assembly and analysis pipeline for AMOS. Genome Biol. 2011; 12:1–25.

90. Michail S, et al. Altered gut microbial energy and metabolism in children with non-alcoholic fatty liver disease. FEMS Microbiol Ecol. 2015; 91:1–9. [PubMed: 25764541]

91. Raman M, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2013; 11:868–875. e1–3.

92. Matsushita N, et al. Effect of Lipopolysaccharide on the Progression of Non-Alcoholic Fatty Liver Disease in High Caloric Diet-Fed Mice. Scand J Immunol. 2015; doi: 10.1111/sji.12397

93. Fouts DE, Torralba M, Nelson KE, Brenner DA, Schnabl B. Bacterial translocation and changes in the intestinal microbiome in mouse models of liver disease. J Hepatol. 2012; 56:1283–1292. [PubMed: 22326468]

94. Okubo H, et al. Mosapride citrate improves nonalcoholic steatohepatitis with increased fecal lactic acid bacteria and plasma glucagon-like peptide-1 level in a rodent model. Am J Physiol Gastrointest Liver Physiol. 2015; 308:G151–158. [PubMed: 25428903]

95. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. Hepatology. 2015; 61:883–894. [PubMed: 25251280]

Table 1

Strategies for Analyzing the Microbiota

Name	Input	Output	Primary Features	Analysis
Quantitative Insights into Microbial Ecology (QIIME) [80]	16S RNA Raw Sequence Data (FASTA Sequences)	**i.** Clustered Operational Taxonomy Units (OTU) File **ii.** Taxonomy File **iii.** Biological Observation Matrix (BIOM) File **iv.** Newick Formatted Tree File	Tool for performing Microbial Community Analysis	Sequence Alignments, Clustering, Alpha Diversity Analysis, Network Analysis, Beta Diversity Analysis, PCO, UNIFRAC, Heat map
Mothur [81]	16S RNA Raw Sequence Data (FASTA)	**i.** Clustered Operational Taxonomy Units (OTU) File **ii.** Taxonomy File **iii.** Biological Observation Matrix (BIOM) File **iv.** Newick Formatted Tree File	Tool for performing Microbial Community Analysis	Sequence Alignments, Clustering, Alpha Diversity Analysis, Network Analysis, Beta Diversity Analysis, PCO, UNIFRAC, Heat map
Metastats [82]	Feature Abundance Matrix (tabular format)	**i.** P-value, Q-value and Variance	Detection of differentially abundant features (taxa, pathways, subsystems, etc.) between clinical meta-genomic datasets.	Non parametric t-test with false discovery rates
Ribosomal Database Project (RDP) [83]	16S RNA Raw Sequence Data (FASTA)	Abundance Table, Bayesian Probabilities (Taxa information, Probabilities)	Assignment of Relative Abundance to sequences	Bayesian Probability of genera abundance
LEfSE [84]	Feature Abundance Matrix (tabular format)	Linear Discriminant Scores, Histogram, Cladograms	Detection of differential features	Kruskal Wallis Test Wilcoxon Test Linear Discriminant Analysis
Correlation Analysis [85]	Feature Abundance Matrix (tabular format)	Correlation Network (Matrix format)		Spearman Correlation
PICRUSt [86]	16S RNA Raw Sequence Data (FASTA)	KEGG Pathway scores, COG scores	Prediction of KEGG pathways	Gene content Prediction
HUMAnN [87]	Raw Sequence Data (FASTA)	KEGG Pathway scores	Prediction of KEGG pathways	Gene content Prediction
MetaPhlAn [88]	Raw Sequence Data (FASTA), CDC calls (the list of gene or hypothetical gene start and end nucleotide positions), taxonomic classification of the genomes		Profiling microbial communities	Estimation of the relative abundance of microbial cells, Identify microbes populating a microbial community
MetAMOS [89]	Raw sequence data	**i.** FASTA sequence of the contigs, scaffolds, or variant	Metagenomic de novo assembly	Classification methods, de novo assembly

Name	Input	Output	Primary Features	Analysis
		i. motifs belonging to specified taxonomic levels ii. Collection of all unclassified or potentially novel contigs contained in the assembly iii. HTML report with detailed assembly statistics and summary charts.		

Table 2

Clinical Studies of ALD and NASH

Study	Comparison	Sequencing Technique, Sample Source	Phylum	Class	Order	Family	Genus
Healthy (n = 26) NAFLD (n = 13)	Healthy children vs NAFLD [90]	Ion One Touch System Stool Samples	Actinobacteria	Actinobacteridae ↓ Coriobacteridae ↓			
			Bacteroidetes	Bacteroidia ↓			
			Firmicutes	Bacilli ↓, Clostridia ↑, Erysipelotrichi ↑			Prevotella ↓
			Fusobacteria	Fusobacteria ↓			
			Lentisphaerae	Lentisphaeria [NA]			
			Proteobacteria	Alphaproteobacteria ↑, Betaproteobacteria ↑, Deltaproteobacteria↑, Epsilonproteobacteria↓, Gammaproteobacteria↓,			
			Verrucomicrobia	Verrucomicrobiae↑			
Healthy (n=30) NAFLD (n=30)	Healthy vs NAFLD[91]	16s rRNA gene pyrosequencing Stool sample	Firmicutes	Bacilli Clostridia	Lactobacillales Clostridiales	Lactobacillaceae ↓ Lachnospiraceae↓	Lactobacillus ↓, Robinsoniella↓, Roseburia↓, Dorea↓
				Clostridia	Clostridiales	Ruminococaceae ↑, Oscillospiraceae	Oscillibacter ↑
No NASH (n=22) NASH(n=35)	No NASH vs NASH [78]	16s rRNA gene MiSeq Stool samples	Actinobacteria ↑			Bifidobacteriaceae ↓	Bifidobacterium ↓
			Bacteriodetes ↑			Bacteroidaceae ↓ Porphyromonadaceae ↑ Prevotellaceae ↑ Rikenellaceae ↑ Paraprevotellaceae ↓	Bacteriodetes ↓ Parabacteriodes ↑, Prevotella ↑
			Firmicutes ↓			Clostridiales ↑ Lachnospiraceae ↓, Ruminococcaceae ↑, Veillonellaceae ↓, Erysipelotrichaceae ↓	Blautia ↓ Ruminococcus ↓
			Proteobacteria ↑			Alcaligenaceae ↑, Enterobacteriaceae ↑	Megasphaera [na] Sutterella ↑
Healthy (n=25) Cirrhotic patients (alcoholic = 43, not alcoholic = 170)	Control patients vs Alcoholic cirrhotic patients [58]	16s rRNA gene pyrosequencing Stool samples	Firmicutes	Clostridia	Clostridiales	Family XIV Incertae Sedis ↑, Lachnospiraceae↑, Ruminococcaceae ↑	
			Proteobacteria	Gammabacteria	Enterobacteriales Oceanospirillales	Enterobacteriaceae ↓, Holomonadaceae ↓	

Study	Comparison	Sequencing Technique, Sample Source	Phylum	Class	Order	Family	Genus
Healthy (n=18) Alcoholics without cirrhosis (n=29) Alcoholics with cirrhosis (n=19)	Healthy vs alcoholic cirrhotic patients [48]	16s rRNA gene pyrosequencing Sigmoid, Mucosal biopsy	Bacteriodetes	Bacteroidia	Bacteroidales	Baceteroidaceae ↑	
Nonalcoholic patients (n=5) Alcoholic patients with cirrhosis, patients with active alcohol abuse (n=5)	Nonalcoholic cirrhotic patients vs alcoholic cirrhotic patients [55]	16s rRNA gene pyrosequencing Stool samples	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae ↑	
			Firmicutes	Negativicutes	Selenomonadales	Veilonellaceae ↓	

Comparison of condition- A vs condition B. ↑ indicates higher in Condition A relative to condition B, ↓ indicates decrease in Condition A relative to Condition B, na, no significant

Table 3

Studies of the Microbiota in Animal Models of NASH and ALD

Study	Comparison	Sequencing Technology, Sample Source	Phylum	Class	Order	Family	Genus
Maintenance food fed mice, High calorie diet-fed mice.	Healthy vs High Fat Diet [92]	16s rRNA gene pyrosequencing Stool Samples				Bacteroidaceae ↓, Peptostreptococcaceae ↓, Erysipelotrichaceae ↓, Lachnospiraceae ↑	
Healthy mice, CCL4-induced liver injury mice	Healthy vs Liver Injury Mice [93]	16s rRNA gene pyrosequencing Cecal Contents, Mucosa	Firmicutes ↓				Lactobacillus ↓, Dorea ↓, Lachnospiraceae Incertae Sedis ↓
			Actinobacteria ↓				Coriobacteriaceae ↓
Mice fed choline - deficent or methionine-choline deficient diets (n = 12)	Normal choline diet mice vs Methionine-choline deficient Mice, induced NASH [94]	Quantitative reverse transcription PCR Stool Samples					Clostridium coccoides ↑, C. leptum subgroup ↓, Bacteroides fragilies ↓, Bifidobacterium ↑, Prevotella ↑, L. gasseri subgroup ↑, L. reuteri subgroup ↑, L. ruminis subgroup ↑, Enterobacteriaceae ↓, Enterococcus ↓
Healthy Mice (n=8), Mice fed alcohol (n=8)	Healthy vs Alcohol [53]	16s rRNA gene 454 FLX-Titanium Stool Samples	Bacteriodetes				Bacteroides ↑, Parabacteroides↑, Tannerella↑, Hallella↑
			Firmicutes				Lachnospiraceae other↑, Ruminococcaceae Incertae Sedis↑, Ruminococcaceae other↑, Aerococcus↓, Listeria ↓, Clostridiales other ↓, Allobaculum ↓, Lactobacillus ↓
			Actinobacteria				Corynebacterium ↓
			Proteobacteria				Alcaligenes ↓
Healthy mice (n=3), Mice fed alcohol (n=3)	Healthy vs Alcohol [51]	16s rRNA gene pyrosequencing, Cecum samples	Bacteroidetes ↓	Bacteroidia ↓	Bacteroidales	Bacteroidaceae	Bacteroides spp. ↓
			Firmicutes ↑	Bacilli	Lactobacillales ↑	Enterococcaceae	Enterococcus spp. ↓
						Lactobacillaceae ↑	Lactobacillus spp. ↑ Pediococcus spp. ↑
						Leuconostocaceae	Leuconostoc spp. ↑
						Streptococcaceae	Lactococcus spp. ↑
				Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae ↓	
			Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkesrmansia muciniphila ↓

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Study	Comparison	Sequencing Technology, Sample Source	Phylum	Class	Order	Family	Genus
Healthy mice, Mice fed alcohol (n =2 to 7)	Healthy vs Alcohol [95]	16s rRNA gene pyrosequencing. Cecum Samples	Bacteroidetes ↓				
			Firmicutes ↑	Bacilli	Lactobacilla	Lactobacillaceae	L rhamnosus ↑ Lactobacillus spp. ↑
NASH, Mice fed a high-fat diet	Healthy vs NASH [36]	16s rRNA pyrosequencing				Porphyromonadaceae ↓	

Comparison of condition- A vs condition B. ↑ indicates higher in Condition A relative to condition B, ↓ indicates decrease in Condition A relative to Condition B, na, no significant