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Review Article

Nonalcoholic Fatty Liver Disease and Gut-liver Axis: Role of Intestinal Microbiota and Therapeutic Mechanisms

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Abstract

The correlation between gut, secreted metabolites, and hepatic diseases has strengthened over the last decade. Interactions of intestinal permeability, gut microbes, and derived metabolites influence the development and progression of nonalcoholic fatty liver disease (NAFLD), a prevalent disease that affects more than 30% of the global population. NAFLD is now called metabolic dysfunction-associated steatotic liver disease (MASLD) to better reflect the disease process. Here, we describe mechanisms of NAFLD development, the role of gut bacteria, gut metabolites, interventions for diagnosis, and the prognosis of NAFLD. We discuss new paradigms that challenge the conventional, addressing disease etiology and translational approaches in a new dimension. Previous studies shed light on intricate relationships of the gut microbiome with the liver, or the gut-liver axis. Bidirectional communication between the gut and the liver involves exchange of metabolites, immune signaling, and inflammatory responses that has potential for novel NAFLD/nonalcoholic steatohepatitis (NASH) treatments. In this review, we propose exploring functional metagenomics to develop NAFLD diagnostic methods and risk assessment. The prospects of genetic engineering, fecal transplants, and specialized diet as targets of novel therapeutic regimes to combat NAFLD/NASH are discussed. Changes in lifestyle and diet in the population, combined with genetic predisposition, have led to an increasing number of cases of NAFLD. The microbiome responds to diet, exercise, and the environment, and can modulate NAFLD in cases with surgical impediments. It is thus vital to explore its emerging roles in human healthcare and not only liver disease.

Introduction

In 1980, Dr. Jürgen Ludwig was the first to describe nonalcoholic fatty liver disease (NAFLD).[1] As a result of severe changes in our lifestyles, NAFLD has become the most common liver condition in China and other parts of the world, with no established therapeutic interventions but only prevention in the form of lifestyle and nutrition adjustments.[2,3] Clinical symptoms of NAFLD are expected to impact around 25% of the population worldwide, making it a worldwide burden.[4,5] The disease encompasses a wide range of liver conditions, such as simple steatosis that progresses to nonalcoholic steatohepatitis (NASH), severe liver fibrosis, liver cirrhosis, and hepatocellular carcinoma (HCC).[6] Western and Eastern nations are predicted to have a two- to three-fold increase in the burden of end-stage liver disease by 2030.[5,6] Recently, using a two-stage Delphi consensus, NAFLD has been renamed metabolic dysfunction-associated steatotic liver disease (MASLD), which refers to a chronic and progressive condition that affects 30–40% of the global population and is strongly associated with features of metabolic syndrome, including obesity and type 2 diabetes mellitus.[7] MASLD is caused by accumulation of fat in the liver and includes a range of disease states, from isolated lipid accumulation or steatosis (i.e. MASL), and its active inflammatory form, metabolic dysfunction-associated steatohepatitis.[8] MASLD includes patients with hepatic steatosis along with cardiometabolic risk.

As mentioned above, many NAFLD patients have metabolic issues that further increase their risk of cardiovascular disease, diabetes, chronic renal disease, and cancer, which severely degrade health.[9] The mechanisms underlying the progression of MASLD

Keywords: Gut microbiota; NAFLD; MASLD; Metabolites; Dysbiosis; FMT.
Abbreviations: BA, bile acid; FA, fatty acid; FFA, free fatty acid; FMT, fecal microbiota transplantation; HCC, hepatocellular carcinoma; IL, interleukin; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SCFA, short-chain fatty acid.
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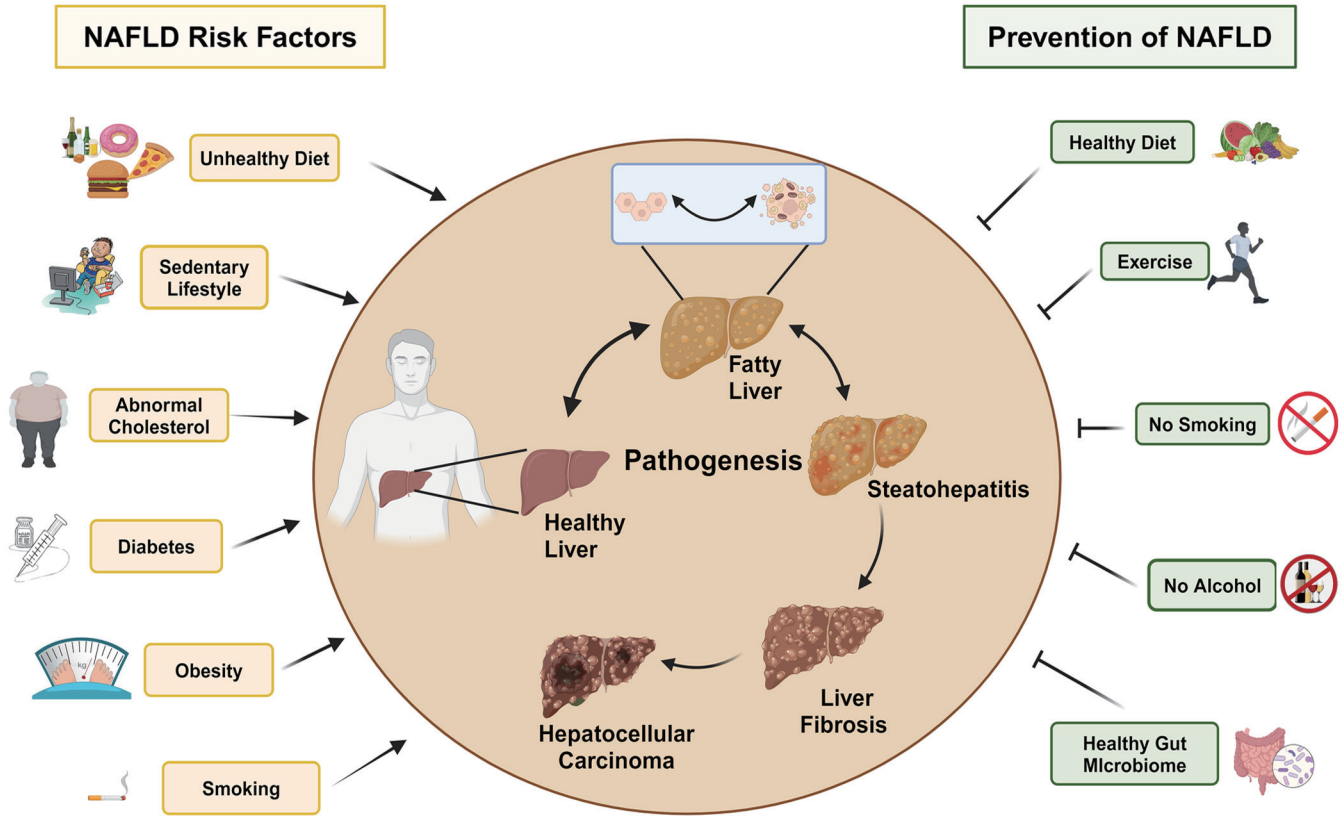

Fig. 1. Illustration of common risks and the prevention of NAFLD. NAFLD, nonalcoholic fatty liver disease.

to NASH and other severe liver disorders are largely unknown. This review explores various avenues to understand the complex interplay between intestinal microbiota and NAFLD progression.

The presence of the liver in the foregut in early development demonstrates that the gut and the liver are connected fundamentally by development stages.[7,10] Patients with NAFLD have higher levels of intestinal permeability, and it is linked with an increase in bacterial population inside the intestines.[11,12] Considering the high prevalence and morbidity of NAFLD, a better understanding of the underlying pathogenic mechanisms is essential for disease management.[13,14] This review aims to summarize significant findings on the association of the intestinal microbiota, gut-liver axis, crosstalk, and balance within the gut microbiota that in turn maintains intestinal permeability and tissue homeostasis. The goal is to present an overview depicting the impact of the intestinal microbiota on NAFLD development. The review describes recent advances in precision medicine offered by creative and emerging ideas from fecal microbiota transplantation (FMT), prebiotics, synbiotics, and probiotics. This review focuses on information that can help answer questions of the effects of alterations in microbiota composition and microbial function in NAFLD, molecular mechanisms underlying disease pathogenesis, comparative assessment of widely used diagnostic biochemical and biophysical methods, the causal relationship of gut microenvironment and progression of NAFLD, and laying the foundation for gut microbiota-targeted therapeutic regimes in NAFLD/NASH treatment. Previous reviews have discussed the role of the gut-brain axis in the onset of NAFLD, our review is focused more on the molecular mechanism of this association and investigating the key mediators of the process.

NAFLD

NAFLD is affiliated with a wide variety of liver disorders caused by lipid deposits in the hepatocytes with no causal connection to alcoholic drinks and/or drug consumption, as well as acquired or hereditary metabolic abnormalities that increase the risk of cirrhosis and HCC.[15,16] NAFLD is defined clinico-pathologically as the deposition of lipids in > 5% of hepatocytes and the exclusion of other sources of fat accumulation (Fig. 1).[17] This illness is linked to diabetes, cardiovascular disease, stroke, and liver damage. It is an implication of the hepatic metabolic syndrome that is supported by a two-hit approach in pathogenesis, as suggested and evidenced by the role of lipid peroxidation. The first hit is directed at the progression of hepatic steatosis by causing accumulation of triglycerides in hepatocytes and facilitates a second hit directed at minor and major inflammation, fibrosis, and lipoapoptosis.[18,19] Although the intrahepatic etiology is still under investigation and the interactions of immune responses are not clear, many potential pathophysiological mechanisms are proposed. It is well-established that an inflammatory cascade is activated by hepatocytic injury caused by oxidative stress and mitochondrial dysfunction. It further activates hepatic stellate cells, and infiltration of immune cells occurs as a downstream consequence that results in NASH.[20] Its prevalence is linked to obesity, insulin resistance, hypertension, hyperglycemia, and hyperlipidemia.[21] Insulin resistance and obesity contribute to chronic inflammation, NASH, and altered lipid metabolism, all of which contribute to procarcinogenic circumstances that promote HCC formation, the fifth most frequent cancer and the leading cause of death globally.[22] Type 2 diabetes occurrence signifies faster progression of NAFLD to NASH, advanced fibrosis, or cirrhosis, explaining

Table 1. Available diagnostic tools for detecting NAFLD

S. no.	Detection method	Advantage	Disadvantage	Reference
1.	Metagenomics and metabolomics	Stool specimens, easy collection, noninvasive tool in the differential diagnosis	Unsatisfactory results from long-term analysis	26
2.	Biopsy/ histopathology	Histological spectrum differentiating steatosis and fibrosis	Invasive, potentially harmful, sampling error, expensive, extreme cases lead to morbidity and mortality	27–29
3.	Liver enzymes and related scoring systems. FIB-4 index, NFS(NAFLD fibrosis score), NASH test, Fibro test, Steato test	Early detection of NAFLD, ability to grade the diseases into stages, better pathogenesis	Not sensitive for NAFLD diagnosis, validation required	30,31
4.	Liver ultrasound or ultrasonography	Noninvasive, time-saving, well tolerated	Insensitive, operator dependent, reliably diagnose NAFLD only if steatosis is >33%, less accuracy in patients of obesity and coexistent renal disease	11
5.	Magnetic resonance imaging, elastography, and magnetic resonance spectroscopy	Sufficient sensitivity, specifies the stages of the disease	Limited availability, needs expertise prescription, difficult data collection, requires spectral analysis	25,32
6.	Magnetic resonance imaging proton-density fat traction	More sensitive than liver histology, early detection	Unable to assess liver inflammation, ballooning, or the resolution of NASH	33,34
7.	Computed tomography	Sensitive techniques, easier quantification of steatosis	Radiation exposure, high cost, limited accuracy	35

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

why its treatment might prove beneficial for lowering the risks of NAFLD/NASH.[23] It is further reported that extra-hepatic cancers such as lung, breast, gynecological, or urinary system cancer are linked with NAFLD prevalence in large cohorts. Yet, the mechanism is not yet deciphered.[24] That may be because obesity and diabetes are synergistic with fatty liver pathogenesis in harming the immune system and in hindering cell signaling and affecting apoptosis, the cell cycle, and proliferation.

NAFLD nomenclature is now updated and associated to link to a state of generalized metabolic disarrangement and is therefore renamed to MASLD as a more appropriate term according to its multisystem and multifactorial characteristics, based on proven data from *in vitro* and *in vivo* research that relate NAFLD to metabolic dysfunction.[25] This undefined set of adverse conditions is characterized by hepatocellular ballooning, an increase in Mallory–Denk bodies and inflammation, glycogenated nuclei, lipogranulomas, and acidophil bodies, as indicated in Takahashi's histological research.[26] Clinical manifestations include high serum triglyceride, low serum high-density lipoprotein, and high aminotransferase, gamma-glutamyl transferase,[27] and total bile acid (BA) levels.[28] However, the enzyme activities may provide a false indication for clinical conduct; thus, liver biopsy has been deemed a reliable yet invasive approach for diagnosing the stages of steatosis and fibrosis. Ultrasound can be used as a standardized method for observing the development of simple steatosis to NASH but cannot be used to investigate occurrence.[15] Noninvasive tests for fibrosis, steatosis, and steatohepatitis, such as the Fibro-Test, Steato-Test, Nash-Test, and Acti-Test, are also in extensive use.[27] However, these tests are neither sophisticated nor completely reliable. Among studies of total antioxidant capacity, products of oxidative damage including total oxidant status and malondialdehyde, and DNA/RNA oxidative damage in human serum samples, researchers reported that advanced glycation end products were a potential noninvasive biomarker of NAFLD.[29] Magnetic resonance imaging and magnetic resonance elastography have been used for noninvasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD,[30,31] but more advancement in these imaging modalities is needed for future prospects. As a result, noninvasive approaches for early identification and treatment of progressive fibrosis are required. Table 1 depicts the various diagnostic tools available for detecting liver disease.[11,25–35]

Various mechanisms underlying development of NAFLD

The cellular and immunological mechanisms underlying the development of NAFLD toward NASH might include endoplasmic reticulum stress,[32] mitochondrial dysfunction,[33] lipotoxicity, and the release of pro-inflammatory cytokines responsible for liver inflammation, such as TNF-α, interleukin (IL)-6, leptin, and resistin in enhanced amounts and decreased secretion of adiponectin.[34,35] The molecular insights primarily suggest that the root causes are increase in fat supply or excessive adipose lipolysis as well as a reduction in fat export such as very low-density lipoprotein, a decrease in free fatty beta-oxidation and elevation in *de novo* lipogenesis, which leads to decreased insulin sensitivity, the most common manifestation of NAFLD.[36]

Effects of fatty acids (FAs)

The majority of fats are stored in hepatocytes as triglycerides, while the remaining fats are stored as a combination of free fatty acids (FFAs), triglycerides, diacylglycerol, cholesterol esters, free cholesterol, and phospholipids.[37] Insulin acts as an antagonist for lipolysis by inhibiting hormone-sensitive lipase, which controls the release of FFAs from adipose tissue, resulting in the accumulation of triglycerides.[38–40] Saturated FAs induce hepatocyte apoptosis by mediating activation of the JNK pathway.[41] TNF-α was pro-

posed to play an important role in insulin resistance[42] and was also the first pro-inflammatory cytokine discovered in adipose tissue. The sterol response element binding protein gene, which regulates lipogenesis, is upregulated when dietary fat, particularly saturated fat, is consumed.[43] When the amount of calories in our diet exceeds our liver's ability to export triglycerides, lipid droplets form in parenchymal hepatocytes, signaling the start of NAFLD.[44]

Role of insulin

The progression of NAFLD to NASH involves insulin resistance caused by aberrant insulin post-receptor signaling, which leads to dysregulated lipolysis and excessive FA delivery to the liver. FFA is a key player in NAFLD development via its role in inducement of TNF expression mediated by an activation of nuclear factor-kappa B.[45] The carbohydrate response element binding protein is activated by fructose, independent of insulin, and promotes hepatic steatosis. There is a more significant release of blood glucose by the liver as a result of increased carbohydrate consumption and decreased glucose uptake by insulin-resistant muscle and adipose tissue because a high-carbohydrate diet activates several lipogenic enzymes like acetyl CoA carboxylase and FA synthase, resulting in hyperglycemia and other health-threatening symptoms.[40]

Association between mitochondrial dysfunction and NAFLD

Mitochondrial dysfunction is a central abnormality underlying the progression from simple steatosis to steatohepatitis in NAFLD.[35] NAFLD is characterized by a metabolic infestation that often includes large, swollen, multilamellar mitochondria, often without cristae, and paracrystalline inclusion bodies.[36,46] FAs are β-oxidized in mitochondria or esterified to be excreted as very low-density lipoprotein or stored as lipid droplets.[47] When mitochondrial activity is disrupted, ATP concentrations are reduced, which causes FA metabolism to be downregulated, causing NAFLD patients to progress from steatosis to steatohepatitis.[33,48] Cell proliferation induced in NAFLD and NASH in obesity-associated HCC is promoted by elevated IL6 and TNF-β.[32] Along with hepatic stellate cells, also known as multifunctional cells of the liver, which are most closely related to immune cells, hepatic cells also play a significant role in the production of fibrogenic stimuli and reactive oxygen species,[49] which might signify the induction of mitochondria-mediated apoptosis.[50] By creating myofibroblast-like cells in the liver, reactive oxygen species' damage of the liver gradually leads to liver fibrosis. Adipokines and myokines regulate the activation and fibrosis of hepatic stellate cells. Iron accumulation catalyzes oxidative stress, which leads to fibrosis and eventually NASH, in a process known as haemochromatosis.[51] Along with anatomical changes in the liver, NAFLD patients show narrowed tight junctions and irregularly arranged microvilli, which depicts a change in the alignment of intact tight junctions and extensive microvilli in their duodenum. The structural backbone of the small intestine, occludin proteins are present in far larger quantities in healthy intestines than in NAFLD-affected counterparts.[52]

Link between BAs and NAFLD

BAs have an essential role in cholesterol homeostasis, lipid metabolism, and absorption of fat and fat-soluble vitamins. BA homeostasis disruption is another important prognostic factor of NAFLD.[53] The progression of NAFLD to HCC can be accelerated by intestinal BA deconjugation and hepatocyte exposure to more toxic BAs. In studies, increased secondary BAs, taurine, and glycine-conjugated BAs have been linked to steatohepatitis.[54] Changes in the pathway associated with the farnesoid X receptor, which plays a role in many important systems responsible for BA regulation, glucose regulation, and lipid regulation can lead to imbalances in energy balance, exacerbating inflammation and fibrosis. Cholic acid, a secondary BA, has been shown in studies to protect mice from hepatic lipogenesis by inhibiting sterol regulatory element-binding protein 1 and its target genes.[55] In human gallstone patients, chenodeoxycholic acid administration lowers the production of elevated hepatic very low-density lipoprotein and plasma triglyceride levels. Obeticholic acid (6α-ethyl-chenodeoxycholic acid), a semisynthetic form of chenodeoxycholic acid, has been shown to be very protective in obese rats in Phase-2a and Phase-2b trials. It helps reduce the risk of liver steatosis as well as fibrosis.[56,57] Intrahepatic accumulation of tauro-beta-muricholic acid, a farnesoid X receptor nuclear receptor antagonist which is involved in the regulation of BA, lipid, and glucose metabolism, showed contribution in decreasing risk to NAFLD in antibiotic and temporal treated mice by inhibiting farnesoid X receptor signaling in the intestine.[58,59] Significant decreases in serum palmitoyl-, stearoyl-, and oleoyl-lysophosphatidylcholine were detected in mice with NASH.[60]

Gut-liver axis

The gut-liver axis is the bidirectional link between the gut, its bacteria, and the liver. The gut barrier is an integral secure system with an army of tight junctional complexes. These goblet cells form the mucus layer, Paneth cells that regulate antimicrobial defense, and a network of innate and adaptive immune cells.[61] It maintains homeostasis by interacting with nuclear receptors to control metabolic activities and forming a feedback loop for BAs and antibodies via the portal circulation between the liver and the gut.[62] The gut mucosal barrier comprising intestinal epithelial cells segregating gut microbiota and host immune cells maintains gut homeostasis. The balance and smooth maintenance are due to the integrated action of the protective layer of defensins on the intraluminal surface, tight junction proteins, and gut immune cells. If the mucosal membrane is disrupted, the resulting altered intestinal permeability induces local inflammation. Bacterial products, if translocated to various cell types such as Kupfer cells, will initiate a fibrotic response resulting in harmful effects in hepatocytes and to host immunity. It also facilitates pathogen-associated molecular patterns, lipopolysaccharides, and microbiome-derived metabolites to enter the liver through the portal circulation, triggering a pro-inflammatory cascade that exacerbates hepatic inflammation.[63] IL22 is reported to regulate gut epithelial cells and, thereby, related immune functions.[64] As a result, lipopolysaccharide reduction and tight junction restoration may be effective as a treatment for reducing NAFLD and its development.[65] To gain insight into explaining the progression of NAFLD, alterations of gut bacteria abundance that are involved in NAFLD pathogenesis.

Gut microbiota

The human gut microbiome contains 10–100 trillion microorganisms, mostly bacteria, which outweigh our human cells by a factor of 10.[66] Alpha-diversity (among samples) and beta-diversity (between samples) are two types of microbiome diversity (comparison of samples from a given population).[67] The microbiome's bacterial component has received the most attention so far. *Bacteroidetes* and *Firmicutes* are the two most prevalent bacterial groups, and *Euryarchaeota* is the most common of the *Archaea*.[68] Nonbacterial species, such as resident archaeal, fungal, and viral populations,

are predicted to have roles in the microbiome, especially in their interactions with other microbiome populations. Gut colonization begins at birth, and a complex combination of dietary habits, ethnicity, and genetic variables influences microbiota composition. In humans, the gut microbiota can define the host condition, whether it is in homeostasis or illness. The gut microbiota interacts with the immune system and actively absorbs food substances into the portal and systemic circulation. Gut microbiota may affect NAFLD by improving energy production, maintaining gut permeability, regulating inflammation, modifying choline and BA metabolism, and enhancing endogenous ethanol synthesis. As a result, it may influence the host, even if it is not present, by modulating immune cells and the production of metabolites.[69] Many studies have evaluated various samples, such as fecal matter and animal tissues, to explore the roles of different bacteria in the progression of NAFLD/NASH.

The clear relationship between microorganisms and the human host makes the human a superorganism.[70] This diversity that establishes a life-long, bidirectional, symbiotic association between the gut and microorganisms is called the intestinal microbiota and is favored by the food that passes through the tract, affecting the integrity of the digestive tract and other linked systems.[71] These commensal bacteria help the host metabolize the dietary fibers that cannot be processed due to a lack of enzymes.[72] *Veillonellaceae* and *Rhinococcacea* were selected as the most representative and significant fibrosis-related bacterial taxa as shown in Table 2.[9,73-91]

Gut metabolites: keystone component

Fermentation of dietary fiber and choline yields metabolites such as short-chain fatty acids (SCFAs), including acetic acid, propionate, butyrate, and succinate, hydrogen sulfide, and other proteolytic metabolites. SCFAs mediate the regulatory effect on the gut microbiota and host inflammatory responses, such as modulating adiponectin and resistin transcriptional expression by modifying DNA methylation in obese mice.[92] Butyrate, the most potent anti-inflammatory mediator, has been shown to be effective in reducing local inflammation in the intestine and preventing the progression of inflammatory responses to the systemic circulation.[93] SCFAs enter the liver directly through the portal vein, where they help to reduce inflammation and steatosis. Though SCFAs regulate the health of visceral adipose tissue and FA, lipid, and glucose metabolism, combining their advantages while preserving intestinal homeostasis is complex, and the overall effect of SCFAs on NAFLD etiology is yet unknown.[92]

Colonic bacteria also ferment nondigestible carbohydrates to SCFAs. SCFAs have been proposed to contribute to obesity and liver steatosis as they provide approximately 10% of the daily caloric consumption and may enhance nutrient absorption by promoting the expression of glucagon-like peptides.[94] However, trimethylamine-N-oxide is only derived from gut microbial metabolism.[73] Trimethylamine-N-oxide, a gut microbe-generated metabolite produced by the flavin monooxygenase 3 produced in the liver, is detrimental to liver health. Cystathionine β-synthase/cystathionine γ-lyase regulates trans-sulphuration and desulfuration reactions in the liver, kidney, small intestine, pancreas, and brain.[74] The trans-sulphuration pathway is linked to the methionine cycle through homocysteine, a nonprotein sulfur-containing amino acid. Homocysteine is irreversibly metabolized via the trans-sulphuration pathway to support endogenous cysteine synthesis. Cystathionine β-synthase and cystathionine γ-lyase catalyze alternative desulphuration reactions in addition to the trans-sulphuration pathway.[75] H_2S is synthesized endogenously by these alternative reactions. Homocysteine and cysteine may catalyze these alternative reactions.[76,77] It has been shown that cystathionine β-synthase and cystathionine γ-lyase are highly expressed in hepatocytes, leading to their high expression in the parenchyma tissue.[78] In patients with NAFLD and its associated comorbidities, there are changes in circulating homocysteine and hydrogen sulfide levels. Homocysteine has been proposed as a risk marker for NAFLD.[79]

Gut microbiota dysbiosis

In dysbiosis, the normal flora in the gut microbiome is disturbed, resulting in increased microbial translocation and the development of alcoholic liver disease. This affects the abundance of species such as *Streptococcus*, *Shuttleworthia*, and *Rothia*.[80] Small metabolites are produced by healthy gut microbiota, including SCFAs, which provide energy to colonic epithelia. When the microbiota starts to produce toxic metabolites that interfere with the gut-liver axis and cause metabolic dysfunction, dysbiosis is confirmed, and eventually, chronic disease development occurs. In patients with NAFLD, decreased abundance of *Faecalibacterium prausnitzii* and increased abundance of *Proteobacteria*, *Escherichia coli,* and *Enterobacteriaceae* have been reported.[81] NASH patients had decreased fecal *Bacteroidetes* and increased *Clostridium coccoides*.[82] At the same time, chronic alcohol consumption can cause leaky gut and reduced gut bacterial diversity, which might be the leading cause of alcoholic liver disease.[83]

NAFLD patients had fewer *Bacteroidetes*, *Ruminococcaceae*, *Faecalibacterium prausnitzii*, and more *Prevotella*, *Porphyromas*, *Lactobacillus*, *Escherichia*, and *Streptococcus* bacteria than healthy subjects.[53,84] However, increased levels of *Veillonella*, *Megasphaera*, *Dialister*, *Atopobium*, and *Prevotella* have been observed in cirrhotic patients. Several mechanisms may contribute to NAFLD pathogenesis as a result of the influence of the gut microbiota influence, including (1) increased production and absorption of gut SCFAs, (2) altered dietary choline metabolism by the microbiota, (3) altered BA pools by the microbiota, (4) increased delivery of microbiota-derived ethanol to the liver, (5) gut permeability alterations and endotoxin release, and (6) interaction between specific diet and microbiota.[47] Chronic kidney disease may aggravate NAFLD and associated metabolic disturbances through multiple mechanisms, including altered intestinal barrier function and microbiome composition.[85] 3-phenylpropionate, a metabolite generated by anaerobic bacteria, plays a crucial part in the process.[86,87] NASH development is linked to gut microbiome-derived products of branched-chain and aromatic amino acid metabolism, such as phenylacetic acid and 3-(4-hydroxyphenyl) lactate, which are linked to insulin resistance.

Pathogen-associated molecular patterns develop when the gut microbiota is out of equilibrium (dysbiosis). Dysbiosis is also linked to increased exposure to bacterial compounds found in the intestine, such as lipopolysaccharides. Hepatic cells have a variety of cellular receptors that react to molecular pattern molecules (e.g., damage-associated molecular patterns and pathogen-associated molecular patterns), which attract neutrophils, macrophages, and other innate immune system components. Pathogen-associated molecular patterns, elevated lipopolysaccharide levels, and damage-associated molecular patterns activate Kupfer cells, which detect liver tissue injury. When Kupfer cells are activated, they release pro-inflammatory cytokines and chemotactic factors, such as the chemokine C-C motif ligand. Consequently, hepatic stellate cells are activated, which leads to the modulation of key extracellular matrix components and functional interactions with a

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Table 2. Alterations of gut bacteria abundance involved in NAFLD pathogenesis

S. no.	Genus	Phylum	Role in the progression of NAFLD/NASH	Type of sample/study	Altered abundance in NAFLD population compared with control	Reference
1.	*Blautia*	Firmicutes	Dysregulation of mucosal immunity, promotes lymphocyte activation, increases intestinal permeability	Fecal	Increase	73–75
2.	*Roseburia*	Firmicutes	Positively associated with tauro ursodeoxycholic acid and tauro chenodeoxycholic acid, reduces gut inflammation, improves intestinal barrier function, decreases intestinal fat transport	Fecal	Increase	76,77
3.	*Lactobacillus*	Firmicutes	Reduces IL17 and other angiogenesis factors, decreases pro-inflammatory (chemokine C-C motif ligand 2, CCR2, TNF) and lipopolysaccharide, increases intestinal barrier function permeability	Fecal	Increase	9,78,79
4.	*Clostridium*	Firmicutes	Modifies BAs from primary to secondary BAs	Animal models	Increase	80
5.	*Ruminococcus*	Firmicutes	Increases fibrosis	Fecal, biopsy	Increase	73
6.	*Flavonifractor*	Firmicutes	Attenuates the increase in TNF-α transcription	Animal model	Decrease	81
7.	*Coprococcus*	Firmicutes	Butyrate-producing bacteria	Fecal	Decrease	9
8.	*Prevotella*	Bacteroidetes	Reduces the Th17 polarization, and promotes differentiation of anti-inflammatory Treg/Tr1 cells in the gut	Fecal	Decrease	82
9.	*Escherichia*	Proteobacteria	Increases gut permeability	Stool study, animal model, biopsy	Decrease	83
10.	*Bifidobacterium*	Actinobacteria	Reduces lipopolysaccharide/TLR-4 axis, affects humoral and cellular inflammatory markers	Fecal	Decrease	9,84
11.	*Oscillospira*	Firmicutes	Decrease is coupled to 2-butanone upregulation	Fecal	Decrease	85
12.	*Akkermansia*		Promotes the growth of other bacteria with anti-inflammatory properties, reverses fat gain, disruptions in serum lipopolysaccharide levels and gut barrier function, and insulin resistance, increases endocannabinoids and gut peptides	Biopsy mucus layer	Decrease	86,87
13.	*Helicobacter*	Proteobacteria	Associated with tauro ursodeoxycholic acid, glycocholic acid, and tauro chenodeoxycholic acid	Ultrasonography	Increase	88
14.	*Oscillibacter*	Firmicutes	Reduces Th17 polarization, and promotes the differentiation of anti-inflammatory Treg/Tr1 cells in the gut	Fecal	Decrease	82
15.	*Erysipelotrich*	Firmicutes	Bacterial predictor of susceptibility to choline deficiency-induced fatty liver disease	Fecal, animal study	Increase	89
16.	*Klebsiella*	Proteobacteria	Predictor of susceptibility to choline deficiency-induced fatty liver disease	Fecal	Increase	90
17.	*Desulfovibrio*	Proteobacteria	Produces both H_2S and acetic acid, modulates the hepatic gene expression pattern of lipids metabolism, suppresses hepatic fatty acid synthase and CD36 protein expression	Fecal	Increase	91
18.	*Mucispirillum*	Deferribacteres	Predicts susceptibility to choline deficiency-induced fatty liver disease	Fecal	Increase	88

BA, bile acid; IL, interleukin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TLR, Toll-like receptor.

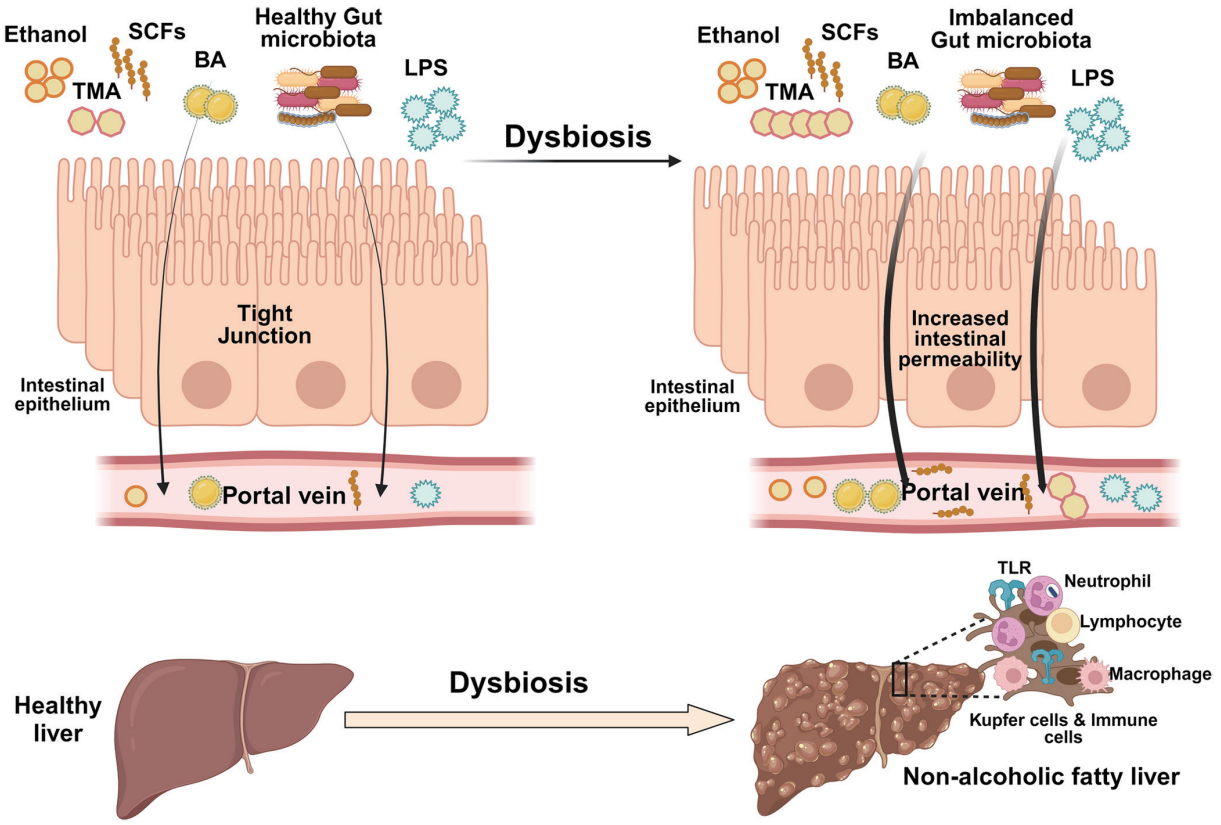

Fig. 2. Schematic representation of how the gut microbiota contributes to the development of NAFLD. In the left panel, the gut-liver axis components are functioning normally. NAFLD is depicted in the right panel. The dysbiotic microbiome, together with the changed intestinal barrier due to the malfunction of the tight junctions, facilitates the translocation of some bacterial products into the portal vein. These bacterial products interact with TLRs on the surface of the hepatic cells, which leads to inflammation and NAFLD development. NAFLD, nonalcoholic fatty liver disease; TLR, Toll-like receptor.

microRNA implicated in NAFLD fibrosis as shown in Figure 2.[88] We have highlighted various metabolites of the gut microbiota and their roles in NAFLD progression in Table 3.[88–91,95-114]

Therapeutic interventions

Gaining insights into the role of gut microbiota, microbe-associated molecular patterns, and metabolites produced by microbiota in the development of NAFLD may pave the way for innovative diagnostic and therapeutic strategies. NAFLD encompasses a diverse range of disorders, each with distinct subtypes resulting from different combinations of the aforementioned factors. Thus, it is crucial to incorporate this knowledge into both the diagnosis and treatment of NAFLD.

Currently, the diagnosis and monitoring of liver disease require a liver biopsy. Therefore, it is crucial to find reliable noninvasive methods to assess NAFLD. Recent research on gut microbiota has found that certain bacterial species and metabolites were useful as diagnostic and prognostic indicators. Loomba *et al*. have identified a panel of 37 bacterial strains from the gut microbiota that accurately diagnose advanced fibrosis in NAFLD patients. Additionally, several metabolites derived from the microbiota show promise as indicators of NAFLD. Phenylacetic acid, succinate, and 3-(4-hydroxyphenyl) lactate are among the most promising. NAFLD patients often have a decreased microbial gene richness, which affects the metabolism of aromatic and branched-chain amino acids. For example, 3-(4-hydroxyphenyl) lactate, which is associated with liver fibrosis, is a byproduct of aromatic amino acid metabolism. The level of phenylacetic acid in the blood is correlated with the severity of liver steatosis. Succinate, produced by bacteria associated with NAFLD like *Bacteroidaceae* and *Prevotella*, is elevated in feces, serum, and liver samples of NAFLD patients.[31]

On numerous levels, a comprehensive understanding of gut microbiota might be employed for therapeutic purposes, as illustrated in Figure 3. The utility of precision medicine encompassing tailored probiotics, prebiotics, synbiotics, and FMT to target dysbiosis of the gut microbiota in individual patients provides a new avenue for microbial-derived therapeutics. Another exciting prospect is the modulation of the production of beneficial metabolites and blocking the synthesis of harmful ones. FMT is emerging as a potential treatment for various gastrointestinal disorders and offers a way to restore a healthy gut microbiota composition and function in patients. FMT is a medical procedure where fecal matter from a healthy donor is transplanted into a recipient's gut to restore a healthy gut microbiome. It can help restore a balanced and diverse gut microbiota in NAFLD patients, potentially mitigating dysbiosis by the introduction of *Lactobacillus*, *Bifidobacterium*, and *Pediococcus* species.[115] FMT has been shown to enhance gut barrier function, reducing the translocation of harmful bacterial products like lipopolysaccharides into the liver and reducing inflammation.[116] FMT may influence BA composition and metabolism in the gut, which can impact liver health, inflammation, and

Table 3. Role of various metabolites in NAFLD progression

Metabolites		Role	References
Short-chain fatty acids			
1.	Propionate	Activates AMP-activated protein kinase, increases expression of the fatty acid oxidation gene, suppresses macrophage pro-inflammatory activation, inhibits isoproterenol and adenosine deaminase-stimulated lipolysis	89,90
2.	Butyrate	Activates AMPK activation, increases expression of the fatty acid oxidation gene, suppresses macrophage pro-inflammatory activation, upregulates glucagon-like peptide-1 receptor expression to improve NAFLD	94,95
3.	Acetate	Regulates hepatic lipid metabolism and insulin sensitivity via FFA receptor 2 in hepatocytes	96
Indole derivatives			
4.	Indole-3-acetic acid (IAA)	Improves lipid metabolism, insulin resistance, and inflammatory and oxidative stress	97
5.	Indole	Reduces the lipopolysaccharide-induced upregulation of -pro-inflammatory mediators	98
6.	Indican: indoxyl-3- sulfate	Reduces gut permeability in high fat diet-fed mice	99
7.	Indigo	Development of obesity, white adipose tissue, inflammation, and insulin resistance	100
8.	IPA: indole-3-propionate	Increases expression of the intestinal mucosa and tight junction proteins	101,102
9.	Ethanol	Oxidative stress and inflammation, increases gut permeability and levels of lipopolysaccharide, decreases the gut barrier	103
10.	2-butanone	Regulates insulin sensitivity	85
11.	Ceramides	Induces sterol regulatory element-binding protein regulator, increases TAG (Triacyl glycerol) synthesis and lipid droplet storage	104
Bile acids			
12.	Primary bile acids chenodeoxycholic acid, cholic acid, deoxycholic and lithocholic acid	Increases insulin sensitivity, inhibits gluconeogenesis and lipogenesis, anti-inflammatory and antifibrotic properties, regulates the gut microbiota, enhances fatty acid translocation and uptake, promotes CD36 translocation to the plasma membrane	105,106
13.	Choline	Regulates mitochondrial bioenergetics and fatty acid beta-oxidation, phosphorylcholine synthesis, loss of apoptotic mechanisms, reactive oxygen species generation, endoplasmic reticulum stress	107–109
14.	Trimethylamine N-oxide	Suppresses the BA-mediated hepatic farnesoid C receptor signaling, increases inflammatory cytokine C-C motif chemokine ligand 2 and insulin resistance	110
15.	Homocysteine	Increases hepatic oxidative stress, induces expression of inflammatory cytokines and profibrogenic factors, activates the aryl hydrocarbon receptor/CD36 pathway	111–113
16.	Serotonin	Inhibits energy expenditure of brown adipose tissue, blocks mitochondrial uncoupling protein	114

FFA, free fatty acid; NAFLD, nonalcoholic fatty liver disease.

fat accumulation in hepatocytes.[117] FMT from a healthy donor may increase the production of beneficial SCFAs in the recipient's gut. SCFAs have anti-inflammatory properties and can improve insulin sensitivity. FMT can facilitate communication between the host and the gut microbiota, leading to positive changes in metabolic pathways. Clinical trials exploring the efficacy of FMT in NAFLD patients are needed to validate its potential therapeutic role.[115] The identification of specific gut microbial markers associated with NAFLD progression could lead to the development of noninvasive diagnostic tools. These tools may rely on fecal-, blood-, or breath-based biomarkers that enable early detection and monitoring of NAFLD without the need of invasive liver biopsies. Further research on the interaction between gut microbiota and metabolites could shed light on the underlying mechanisms that drive NAFLD progression. Moreover, single beneficial strains or groups of beneficial strains (probiotics) can be introduced into the gut microbiota to restore lost functionality, while harmful or undesirable strains can be removed with antimycotics, antibiotics, or bacteriophages. Finally, microbial pathways might be targeted to minimize or prevent the formation of harmful metabolites while

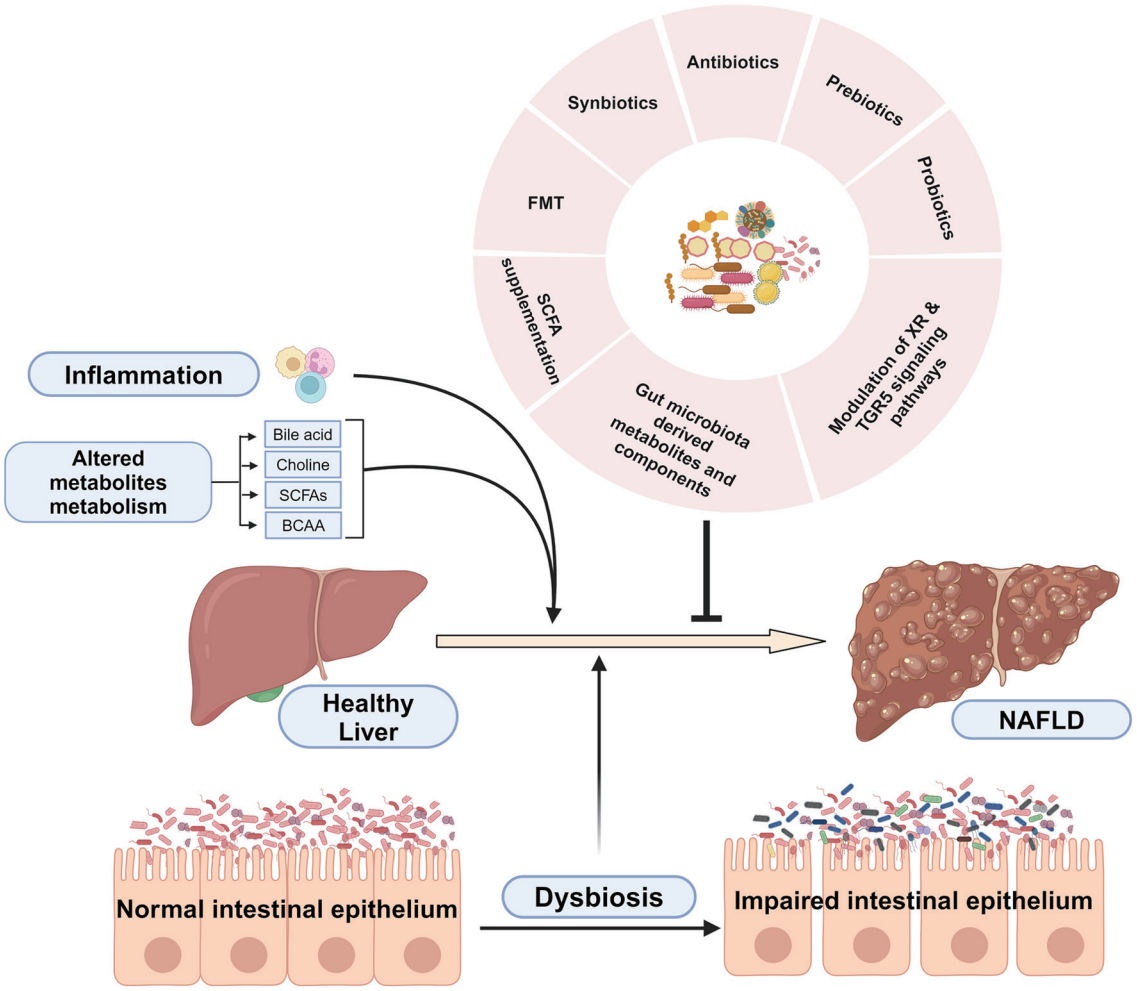

Fig. 3. Gut microbiome-centered therapeutic strategies against NAFLD. Dysbiosis promotes the process of NAFLD via multiple pathways. Gut microbiome-targeted therapeutic strategies include probiotic, prebiotic, synbiotic, and FMT that can reverse dysbiosis and mitigate the process of NAFLD. BCAA, branched-chain amino acid; FMT, fecal microbiota transplantation; NAFLD, nonalcoholic fatty liver disease; SCFA, short-chain fatty acid.

enhancing the production of beneficial ones.

FMT can reconstruct whole microbial ecosystems. Moreover, single beneficial strains or groups of beneficial strains (probiotics) can be introduced into the gut microbiota to restore lost functionality, while harmful or undesirable strains can be removed with antimycotics, antibiotics, or bacteriophages. Finally, microbial metabolic pathways might be targeted to minimize or prevent the formation of harmful metabolites while enhancing the production of beneficial ones.

Data on the efficacy of FMT in the treatment of NAFLD are scarce. FMT has been shown to be effective in treating cirrhotic individuals with hepatic encephalopathy.[118] and alcoholic hepatitis.[119] NAFLD has also been treated using prebiotics, probiotics, and synbiotics. Prebiotics are indigestible food components such as that selectively increase the development and activity of helpful gut bacteria.[120] This concept was eventually broadened to encompass fiber-based probiotics and other substrates that the host bacteria use selectively and provide health advantages. Not only indigestible carbohydrates like galacto-oligosaccharides, fructo-oligosaccharides, and trans-galacto-oligosaccharides but also other substances like polyphenols and polyunsaturated FAs that can modulate the gut microbiota are included in the new definition.[121] Probiotics are living, nonpathogenic bacteria that, when ingested, can improve the host's health. *Lactobacilli, Streptococci,* and *Bifidobacteria are* the most widely used probiotics in clinical studies.[122]

Synbiotics are a combination of probiotics and prebiotics that positively impact the host. According to animal and human trials data, synbiotics may help alleviate NAFLD-related dysbiosis and liver disease. In NAFLD patients, for example, a recent meta-analysis discovered that taking synbiotics/probiotics was linked to improvement of liver-specific indicators of hepatic stiffness, inflammation, and steatosis.[123] The therapeutic strategy of using a bacteriophage to target a specific strain, especially cytolytic *E. faecalis*, was efficacious in treating ethanol-induced liver injury in humanized mice.

Emerging therapeutic methods can change gut microbiota composition to promote the synthesis of beneficial metabolites and decrease the production of toxic metabolites. For example, 3, 3-dimethyl-1-butanol can prevent microbial trimethylamine lyases from converting dietary choline to trimethylamine. Trimethylamine is a well-known toxic metabolite that can induce inflammation in

gut, and prolonged inflammation can induce IBD and colorectal cancer.[124] Other studies have determined that increased levels of beneficial metabolites such as SCFA can improve liver steatosis. Another drug, tributyrin, which is a butyrate prodrug, is reported to protect mice from insulin resistance, obesity, and hepatic steatosis, whereas acetate and propionate supplementation prevented diet-induced weight gain, insulin resistance, and hepatic steatosis.[125] XR and TGR5 signaling pathways that modulate BA metabolism are also interesting therapeutic targets, such as obeticholic acid is shown to improve fibrosis, portal hypertension, and hepatic steatosis in animal models and improved histological features in NASH patients. In addition, fibroblast growth factor has been established as a therapeutic agent for metabolic diseases because of its role in lipid and carbohydrate metabolism. Clinical trials of fibroblast growth factor-based therapies have shown its efficacy in patients with NAFLD. These treatments contain fibroblast growth factor analogues that can reduce liver inflammation and fibrosis.[126] NGM282, counterpart of fibroblast growth factor 19 that modulates BA synthesis and glucose balance, has been identified as having the potential to reduce hepatic steatosis in NASH patients.[127] Farnesoid X receptor agonist, obeticholic acid, is a first-in-class approved agonist for noncirrhotic primary biliary cholangitis treatment; however, second-generation farnesoid X receptor agonists are in development to overcome the side effects of the first-in-class drug. For example, MET409 is a second-generation farnesoid X receptor agonist which has shown better efficacy and less side effects such as pruritus and increase in low-density lipoprotein than obeticholic acid.[128] Tropifexor and cilofexor are farnesoid X receptor agonists possessing distinct structures from obeticholic acid and MET409. A study reported that administration of 30 mg cilofexor for 12 weeks in NASH and fibrosis patients decreased liver stiffness and hepatic fat and improved liver biochemistry.[129] Additionally, under development for NAFLD treatment are specific agonists for the thyroid hormone receptor-beta, namely resmetirom and VK2809. Resmetirom is the pioneer oral, liver-targeted thyroid hormone receptor-beta 1-selective agonist. In a 36-week phase II randomized clinical study, resmetirom achieved NASH resolution in a subgroup of patients with control biopsies. Simultaneously, improvements were recorded in liver steatosis, liver stiffness, lipid serum profile, and fibrosis biomarkers like Pro-C3 and hepatic enzymes. This was coupled with a marked reduction in NAFLD activity.[130] VK2809, an alternative thyroid hormone receptor-beta agonist, undergoes hepatic metabolism through the action of CYP450 enzymes. It had a highly favorable tolerability profile, and a substantial decrease in hepatic fat was detected by magnetic resonance imaging following a 12 weeks of treatment.[131]

Conclusions

A growing body of evidence indicates that the microbiome unifies and explains the divergent findings in liver disease-related investigations. The broad interplay between the gut microbiota via specialized chemicals such as trimethylamine, acetaldehyde, and lipopolysaccharide, and the host immune system via Kupffer-cell-mediated liver inflammation is now widely accepted as playing a role in liver damage. However, we still do not completely understand the interactions between the microbiota and the liver. Many critical molecular processes in the etiology of liver disease have been elucidated primarily in animal models, notably rodents. Including the microbiome in these models will give researchers a more comprehensive picture of the liver ecosystem. Because technical heterogeneity can hide underlying biological signals in microbiome research, there is a need for uniformity in technological platforms and standardized methods so that results from diverse laboratories and model species can be replicated and confirmed. It is also crucial to find an animal model that closely resembles human illness in all physiological and metabolic aspects because studies have constantly been finding evidence of an association between NAFLD risk and extra-hepatic cancer development in both sexes. Furthermore, this review highlights the importance of placing more attention on developing biomarkers based on microbiome and metabolic profile that can diagnose the stage of NAFLD, assess the risk, and help in the selection of a specific treatment approach.

We are gradually moving away from observational studies in people as research lays the groundwork for microbiome-based treatment modalities like FMT and probiotic therapies. However, effectively translating and applying results from animal models to humans demands well-designed, large-scale clinical studies encompassing a wide range of illness etiologies and health status. We underline the necessity of concentrating on microbiome-aware initiatives to efficiently confront the socio-economic burden of this range of liver disorders as the microbiota functions in hepatic disease development, prognosis, and therapy become better understood.

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Conflict of interest

The authors declare that there are no competing interests.

Author contributions

Drafted the manuscript (AJ, AS), contributed to critical discussions (RJ, NB), and conceptualized the study and managed the manuscript process (RD, SK).

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