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REVIEW

Gut microbiota induced epigenetic modifications in the non-alcoholic fatty liver disease pathogenesis

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Abstract

Non-alcoholic fatty liver disease (NAFLD) represents a growing global health concern that can lead to liver disease and cancer. It is characterized by an excessive accumulation of fat in the liver, unrelated to excessive alcohol consumption. Studies indicate that the gut microbiota-host crosstalk may play a causal role in NAFLD pathogenesis, with epigenetic modification serving as a key mechanism for regulating this interaction. In this review, we explore how the interplay between gut microbiota and the host epigenome impacts the development of NAFLD. Specifically, we discuss how gut microbiota-derived factors, such as lipopolysaccharides (LPS) and short-chain fatty acids (SCFAs), can modulate the DNA methylation and histone acetylation of genes associated with NAFLD, subsequently affecting lipid metabolism and immune homeostasis. Although the current literature suggests a link between gut microbiota and NAFLD development, our understanding of the molecular mechanisms and signaling pathways underlying this crosstalk remains limited. Therefore, more comprehensive epigenomic and multi-omic studies, including broader clinical and animal experiments, are needed to further explore the mechanisms linking the gut microbiota

Abbreviations: AHR, aryl hydrocarbon receptor; DNMT, DNA methyltransferase; FASN, fatty acid synthase; FGF21, fibroblast growth factor 21; FMT, fecal microbiota transplantation; FXR, farnesoid X receptor; GF, germ-free; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; HFD, high-fat diet; IAA, indole acetic acid; ICA, indole-3-carboxaldehyde; IECs, intestinal epithelial cells; IL, interleukin; IPA, indole-3-propionic acid; LPS, Lipopolysaccharides; miRNAs, microRNAs; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; SCFAs, short-chain fatty acids; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; TNF-α, tumor necrosis factor alpha; VB12, vitamin B12.

Ruiqi Tang and Rongrong Liu contributed equally to this work.

to NAFLD-associated genes. These studies are anticipated to improve microbial markers based on epigenetic strategies and provide novel insights into the pathogenesis of NAFLD, ultimately addressing a significant unmet clinical need.

KEYWORDS
DNA methylation, epigenetic modification, gut microbiota-host crosstalk, histone modification, non-alcoholic fatty liver disease (NAFLD)

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent hepato-pathological disorder in which lipid content exceeds 5% of the liver weight in the absence of excessive alcohol consumption [1, 2]. From hepatic steatosis to non-alcoholic steatohepatitis (NASH), NAFLD encompasses a wide range of liver conditions, and can progress to more severe forms of liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC) [3]. NAFLD has become the leading cause of chronic liver disease globally, affecting 25%−45% of the general population [4]. NAFLD is intimately related to metabolic disorders, such as type 2 diabetes, obesity, and hypercholesterolemia [5]. The prevalence of NAFLD is estimated to be between 70% and 90% among patients with metabolic comorbidities [6]. It has been warned that the prevalence of NAFLD is predicted to rise by 21%, from 83.1 million in 2015 to 100.9 million in 2030, while NASH prevalence is predicted to increase by 63%, from 16.52 million to 27 million [7, 8]. At present, there are no Food and Drug Administration-approved effective therapeutic agents for the treatment of this disease. Patients mainly rely on lifestyle modifications and physical activity to alleviate the onset of NAFLD [9, 10]. However, the majority of NAFLD patients are unable to attain remission [11], and the survival rate of patients who progress to cirrhosis and HCC remains low.

The underlying causes of NAFLD have been widely debated over the last few years. About 20 years ago, the "two-hit" model was proposed, which has been commonly used to describe how NAFLD occurs [12]. A more comprehensive model, the updated "multiple parallel hits" model, has been proposed a decade ago [13]. In this new model, hepatic lipotoxicity and multiple hits, especially adipose tissue inflammation and gut microbiota dysbiosis, may act in parallel, finally resulting in liver inflammation in genetically predisposed individuals.

Intestinal dysbiosis is a major causative factor of NAFLD through diverse immunological, metabolic, and epigenetic mechanisms [14–16]. In patients with cirrhosis and NAFLD, the gut microbiota profile is significantly correlated with systemic inflammation and can concur in the process of HCC [17]. A recent study examined the effect of microbially-derived gut metabolites on host hepatic metabolism using a labeling strategy. The researchers identified and traced bacterial sphingolipids from the gut microbiome to the liver and established the benefit role of bacterial sphingolipids synthesized by gut symbiont Bacteroides on hepatic lipid accumulation [18].

Furthermore, epigenetic modifications play a crucial role in regulating gene expression, and their dysregulation has been linked to various diseases, including NAFLD. It is crucial to understand the underlying causes of NAFLD, such as gut microbiota dysbiosis and epigenetic modifications. The epigenetic mechanisms underlying the crosstalk between gut microbiota and abnormal gene expression in NAFLD have attracted the attention of many researchers. For example, a recent study demonstrated that gut microbiota differentially methylated genes implicated in maintaining the energy and glucose balance in adipose tissue in obese subjects [19]. Additionally, the gut microbiota regulates the expression of hepatic lipid metabolism-related genes by modulating global histone acetylation and methylation in multiple host tissues, including the liver, primarily through the synthesis of short-chain fatty acids (SCFAs) [20, 21]. Therefore, the goal of our study is to comprehensively outline of how the gut microbiota/metabolites influence epigenetic factors and their impacts on the progression of NAFLD (Figure 1).

2 | NAFLD AND GUT MICROBIOTA

Human bodies are home to 10–100 trillion microbial cells, including bacteria, archaea, fungi, and viruses. These diverse symbiotic microbial communities and their genomes, which are about 100 times more numerous than the human genome, are collectively referred to as the "microbiome" [14]. Advanced technologies, particularly next-generation sequencing-based metagenomics, have revolutionized our understanding of the microbial world, including the human microbiome. Studies have found that microbial communities are located on the skin,

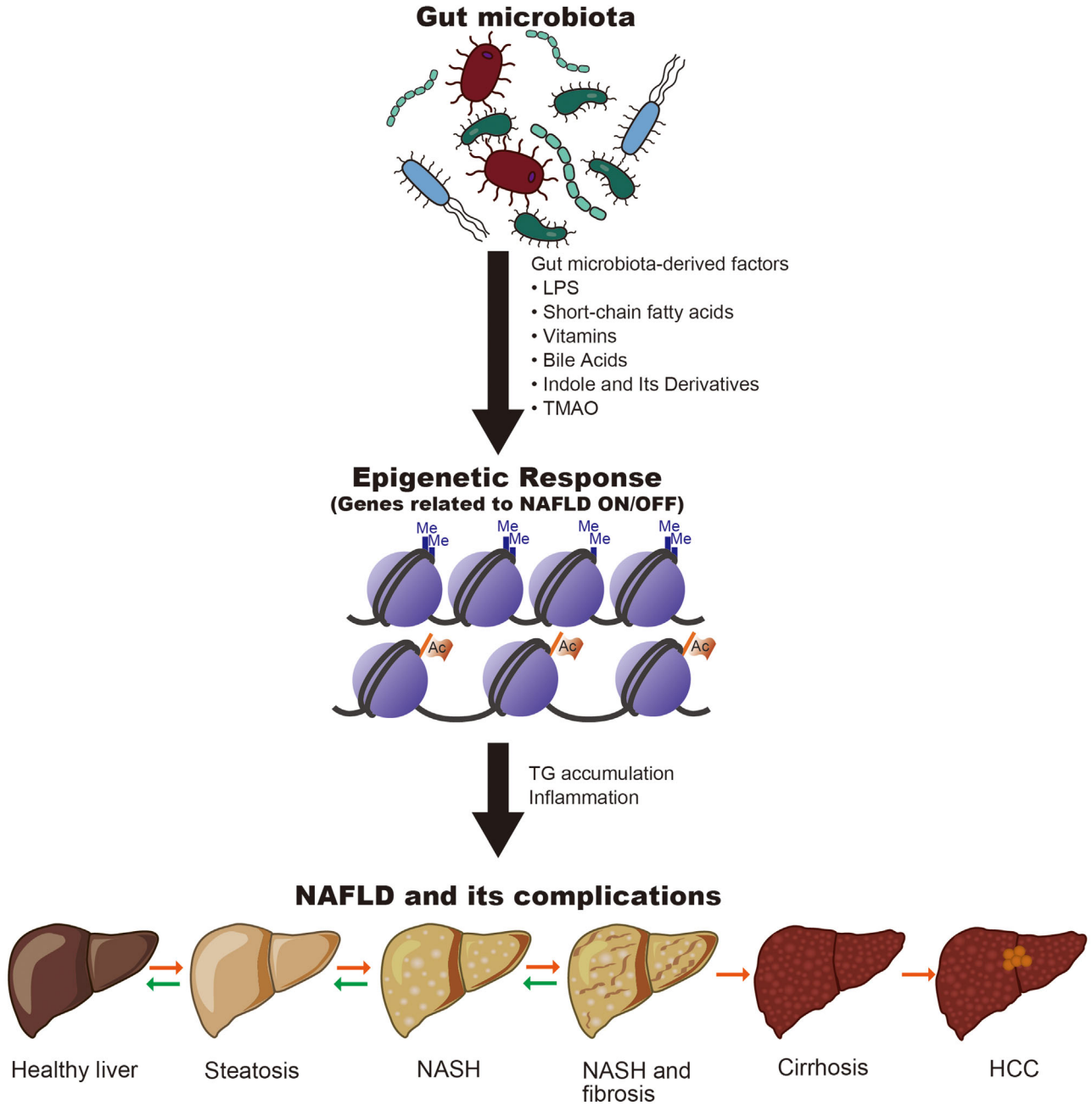

FIGURE 1 The progression of NAFLD may be facilitated by possible epigenetic changes caused further by factors derived from the gut microbiota. The NAFLD phenotype and related complications can be caused by the gut microbiota's action on the epigenetic landscape, as seen in this picture. Through epigenetic mechanisms like DNA methylation and histone acetylation, the bioactive components and metabolites of the gut microbiota can change the accessibility of chromatin. The resulting epigenetic modifications have an effect on the genome by altering gene expression patterns and thus participate in the development and progression of NAFLD and its complications.

mouth, gut, vagina, lungs, uterus, and bladder, among other mucosal surfaces of the human body [22, 23]. The microbiota-host interplay is complex and challenging to understand; however, a recent evolutionary theory states that since the earliest multicellular organisms, the microbiota and host have co-evolved, with symbionts evolving to remain competitive within the host ecosystem, while hosts evolve to keep the ecosystem under control [24].

The microbiota in the gastrointestinal tract is highly diverse, providing many unique genes that have important implications for the host [25]. The gut microbiota can help the host digest dietary fiber for nutrient uptake [26], synthesize essential metabolites to train the immune system [27], and defend against pathogens by competing for space and nutrients [28], among many other functions. However, an unbalanced gut microbiota often contributes to

metabolic disorders such as NAFLD, diabetes, and obesity [29–31].

Studies have shown that dysbiosis and metabolic dysfunction of gut microbiota are closely associated with the occurrence and development of NAFLD. Dysbiosis of gut microbiota may lead to increased gut permeability and endotoxemia, which can trigger hepatic inflammation and fibrosis [32, 33]. The gut microbiota in NAFLD is characterized by a significantly lower microbial diversity [34, 35]. Literature has identified a specific gut microbial profile in patients with NAFLD compared to normal controls [36]. Compared with healthy individuals, patients with NAFLD exhibited consistent increased Proteobacteria [37–41] at the phylum level, increased *Enterobacteriaceae* [38, 41] and decreased *Rikenellaceae* [41, 42] and *Ruminococcaceae* [38, 39, 41, 43] at the family level, and increased *Escherichia* [37, 41], *Dorea* [39, 42], *Peptoniphilus* [42, 43] and decreased *Anaerosporobacter* [43, 44], *Coprococcus* [37, 41, 43], *Eubacterium* [37, 41] and *Faecalibacterium* [41, 45] at the genus level. *Prevotella-copri* was associated with a greater risk of progression from NAFLD to NASH, which may be related to higher intestinal permeability and lower butyrate production capacity [46]. The fecal microbiome of NAFLD patients with cirrhosis had lower levels of *Akkermansia* and increased levels of *Enterobacteriaceae* and *Streptococcus* [17]. Enrichment in Bacteroides and *Ruminococcaceae* members, which were associated with systemic inflammatory and immunological indicators, was noted in patients with NAFLD-HCC [17, 47].

Fecal microbiota transplantation (FMT) experiments from NAFLD patients to rodents reproduce the liver phenotypes in patients, suggesting that gut microbiota dysbiosis has a significant causative role in the pathogenesis of NAFLD. FMT from NASH patients to germ-free (GF) mice induced hepatic steatosis and hepatic inflammation in GF mice, and these symptoms can be significantly exacerbated by a high-fat diet (HFD). In contrast, GF mice fed an HFD had only lipid accumulation and mild liver inflammation [48]. Moreover, GF mice colonized with a fecal microbiome from 2-week-old human infants of obese mothers demonstrated histological patterns common in pediatric cases of NAFLD, compared with those transferred from infants of normal-weight mothers. Furthermore, fecal microbiota obtained from obese women with hepatic steatosis induced a rapid increase in hepatic triglycerides and triggered steatosis in conventional mice fed a chow diet via FMT. A substantial link was found between the mouse phenotype and the donor's microbiome [37]. Together, these rodent studies reinforce the idea that dysbiosis of the gut microbiome leads to the onset of NAFLD.

The interaction between NAFLD and gut microbiota involves several mechanisms, including altered gut permeability, increased production of microbial metabolites, and modulation of host immune response. Dysbiosis of gut microbiota may lead to increased gut permeability, allowing translocation of bacterial components, such as lipopolysaccharides (LPS), into the circulation. LPS can activate the toll-like receptor 4 signaling pathway, triggering hepatic inflammation and fibrosis. Moreover, gut microbiota can produce various metabolites, such as SCFAs and bile acids, which can modulate host metabolism and immunity [36]. Thus, the microbiome and host crosstalk play a critical role in the NAFLD pathogenesis, making it essential to understand how the microbiome and host interact. Recent research has revealed that the gut microbiota can modulate the host's epigenetic landscape through bacterial components and microbial metabolites. Dysbiosis of gut microbiota may lead to aberrant epigenetic modifications, such as DNA methylation and histone modifications, which can affect gene expression and contribute to the pathogenesis of NAFLD [49–51]. Therefore, in this review, we will focus on the evidence suggesting that the gut microbiome influences the pathogenesis of NAFLD by manipulating epigenetic mechanisms.

3 | IMPACT OF MICROBIOTA-HOST CROSSTALK ON EPIGENETIC MODIFICATIONS IN NAFLD

Epigenetics is the study of the molecular mechanisms that dynamically and reversibly alter the structure of chromatin, without changing the DNA sequence. Dysregulation of epigenetic modifications, such as DNA methylation, histone modifications, and non-coding RNA-mediated regulation, can contribute to the development and progression of NAFLD [52]. More recently, gut microbiota have been identified as important factors that significantly influence the host epigenetic landscape in NAFLD pathogenesis [49–51, 53–56], highlighting the need for further research in this area to develop new therapeutic strategies. Gut microbiota can modulate the host's epigenetic landscape in the context of NAFLD through various mechanisms.

Firstly, gut microbiota can impact the host's epigenetic landscape by altering DNA methylation levels of specific NAFLD-associated genes. For instance, a Firmicutes-dominant gut microbiome in pregnant women has been linked to differential gene promoter methylation, which is associated with lipid metabolism and obesity [57]. Additionally, gut microbiota can maintain intestinal immunological homeostasis by epigenetically altering toll-like receptor 4 DNA methylation through recruiting DNA methyltransferase (DNMT) 3 in intestinal epithelial cells (IECs), which play a crucial role in the development of hepatic fibrosis and steatosis [49, 58]. Moreover, the

gut microbiota influences host epigenetics in NAFLD by modulating one-carbon metabolism, supplying methyl groups like folate and choline for DNA methylation. For example, in GF mice, reduced circulating levels of gut microbiota metabolite folate led to altered DNA methylation in colonic IECs, affecting crosstalk between IECs and natural killer cells, which play complex roles in NAFLD progression [53, 59]. The detailed mechanisms of how one-carbon compounds modify host epigenetics in NAFLD will be discussed in Section 4.2. Furthermore, probiotics have been shown to improve gut dysbiosis in NAFLD rats, reduce liver inflammation through adiponectin DNA methylation signaling, and modulate genetic-epigenetic networks related to hepatic stellate cell signaling, ultimately delaying or preventing NAFLD progression [55, 56].

Secondly, gut microbiota influences epigenetics and NAFLD progression by regulating histone modifications, such as acetylation and crotonylation, which are crucial for gene expression regulation [60]. By modulating these modifications, gut microbiota can impact gene expression related to lipid metabolism, inflammation, and immune response, all of which are essential factors in NAFLD development and progression [61]. Gut microbiota regulates histone modifications mainly through the production of SCFAs, such as acetate, propionate, and butyrate, which function as histone deacetylase (HDAC) inhibitors [62]. SCFAs lead to increased histone acetylation and altered gene expression, which in turn can affect the development and progression of NAFLD [63]. Furthermore, gut microbiota-induced HDAC inhibition by SCFAs can influence histone crotonylation, a recently discovered histone modification linked to gene expression activation, which may play a role in the context of NAFLD [64]. The detailed mechanisms by which gut microbiota impacts epigenetics and NAFLD progression by regulating histone modifications, primarily through SCFAs production, will be discussed further in Section 4.1.

In addition, the gut microbiota can affect host epigenetics in NAFLD through non-coding RNAs, particularly MicroRNAs (miRNAs). MiRNAs regulate gene expression post-transcriptionally and play a crucial role in regulating lipid metabolism, inflammation, and liver injury in NAFLD pathogenesis [65, 66]. Several miRNAs, such as the miR-17/92 cluster and miR-21, have been found to be elevated in patients with NAFLD and identified as potential diagnostic tools and therapeutic targets for NAFLD [67–69]. Overexpression of miR-30c-5p has been shown to reduce hepatic steatosis in db/db mice by downregulating fatty acid synthase (*FASN*) [70]. The gut microbiota can influence the regulation of these miRNAs by producing metabolites such as trimethylamine N-oxide (TMAO), which affects host miRNAs expression involved in inflammation and lipid metabolism (see more detailed information in Section 4.5) [71, 72]. Further research is needed to fully understand the interaction between the gut microbiota and host epigenetics in NAFLD through non-coding RNAs.

These studies suggest that gut microbiota play a crucial role in modulating the host's epigenetic landscape and influencing disease progression in NAFLD. Gut microbiota achieve this either through direct interactions with host cells or through the production of microbial metabolites. In conclusion, understanding the complex interactions between gut microbiota and host epigenetic modifications is critical for developing novel therapeutic strategies for NAFLD. Further research is needed to elucidate the specific mechanisms through which gut microbiota modulate the host's epigenetic landscape, including DNA methylation, histone modifications, and non-coding RNA-mediated regulation. This knowledge could potentially pave the way for the development of targeted therapies and personalized medicine approaches to manage and treat NAFLD, taking into account individual gut microbiota composition and epigenetic profiles.

4 | EPIGENETIC MODIFICATION OF HOST CELLS MEDIATED BY MICROBIAL METABOLITES IN NAFLD

An important mechanism underlying the interplay between the microbiota and the host is the production of bioactive metabolites that can be taken up by host cells and impact cellular activities [11]. This interaction has significant implications for NAFLD pathogenesis. The gut microbiota produces a variety of metabolites that can alter host metabolism, immune function, and gene expression [73]. SCFAs, for instance, have been shown to enhance insulin sensitivity and reduce inflammation, ultimately reducing the risk of developing NAFLD [74]. bile acids, another important microbial metabolite, can influence host lipid metabolism and inflammation [75]. Additionally, tryptophan and choline metabolites have been shown to contribute to the development of NAFLD by affecting lipid metabolism and inflammation [76, 77]. Studies have shown that changes in these commensal bacterial metabolites, are linked to epigenetic alterations in NAFLD pathogenesis [36, 78–80].

4.1 | SCFAs

SCFAs are major metabolites produced by the gut microbiota fermenting indigestible carbohydrates. The most prevalent SCFAs produced by gut bacteria are acetate,

propionate, and butyrate. Colonocytes primarily absorb SCFAs produced in the digestive tract via diffusion or co-transport and utilize them as energy-generating substrates [30]. SCFAs nourish intestinal cells and activate goblet cells to produce appropriate amounts of mucus and maintain the integrity of the intestinal barrier [11]. SCFAs that the colonocytes are unable to digest are delivered to the liver through the portal circulation. Moreover, certain SCFAs can enter the systemic circulation through the inferior vena cava [30]. With normal function of the gut-liver axis, SCFAs are predominantly used in gut and liver tissues.

Many studies have shown abnormal levels of SCFAs in NAFLD patients [81, 82]. NAFLD and/or NASH patients have increased concentrations of SCFAs in the feces compared with healthy individuals, and the taxonomic differences in the gut microbiota are mainly determined by the increased SCFAs-producing bacteria. The increased concentration of SCFAs correlated with immunological signatures of NAFLD disease progression [82]. A high-fiber intervention has been shown to reduced liver steatosis in NAFLD patients [81]. On the other hand, studies in rodents have suggested that SCFAs can significantly alleviate hepatic steatosis and inflammation in NASH mice [83, 84]. Thus, SCFAs play a critical role in NAFLD and can be important targets for future personalized medicine in NAFLD patients.

As inhibitors of HDACs, SCFAs play an important role in modulating immune responses by epigenetic regulating the proliferation, differentiation, and function of immune cells in NAFLD pathogenesis. For instance, SCFAs reduce tumor necrosis factor alpha (TNF-α) and interleukin (IL)−6 in monocytes and adipose tissue macrophages of obese subjects by altering free fatty acid receptor and *HDAC* expression, thereby attenuating obese systemic inflammation [85]. Studies have shown that SCFAs can upregulate IL-22 production in CD4 T cells and innate lymphoid cells through histone modification [86]. Moreover, SCFAs can inhibit the proliferation and activation of intestinal lamina propria CD4 T cells and modulate the effector function of CD8 T cells by inhibiting HDAC activity [87–89]. SCFAs can also promote the differentiation of T cells into effector T cells and regulatory T cells [90, 91]. Butyrate, a typical type of SCFAs, promotes the differentiation of induced regulatory T cells, which is associated with fatty acid oxidation [92]. In addition, SCFAs promote B10 cell generation and function by activating p38 MAPK in a manner dependent on HDAC inhibitory activity [93].

SCFAs also play a beneficial role in the process of NAFLD by epigenetically controlling the expression of hepatic lipid metabolism-related genes. Animal studies have revealed that butyrate inhibits HDAC3 and promotes the gene expression of fibroblast growth factor 21 (*FGF21*) in the liver of dietary obese C57BL/6J mice. FGF21 further mediates butyrate activity to increase fatty acid use and ketosis, which is beneficial for the treatment of NAFLD [94, 95]. Butyrate prevented the progression of steatosis to NASH in the NAFLD mouse model by inhibiting HDAC2 independently of GPR43/GPR109a to stimulate hepatic glucagon-like peptide-1 receptor expression [63]. Not only that, butyrate inhibits hepatic steatosis through liver kinase B1 induced enhancement of insulin-induced gene activity and repression of lipogenic genes in mice [96].

Furthermore, SCFAs also ameliorate NAFLD related insulin resistance and overweight through a DNA methylation-dependent epigenetic mechanism. SCFA can directly reduce the expression of *DNMT1*, *DNMT3a*, and *DNMT3b*, and inhibit the binding of these enzymes to adiponectin and resistin promoters, thereby correcting the abnormal expression of adiponectin and resistin in obesity [97].

4.2 | Vitamins

The gut microbiota is an essential source of vitamins, including vitamin B9 (folate) and vitamin B12 (VB12) [98]. This is clearly demonstrated by the need for certain B vitamins in GF animals [99]. Studies have linked vitamin deficiency with the development of NAFLD and susceptibility to more severe liver damage. Disruption of vitamins is associated with gut microbiota dysbiosis, hepatic lipotoxicity, immune system alterations, unnecessary inflammation, oxidative stress, genetic mutations, and epigenetic modifications in NAFLD [98].

Folate and VB12 play an important role in the epigenetic regulation of genes involved in NAFLD as they are methyl donors for S-adenosylmethionine synthesis [100]. Insufficient folate and VB12 levels are independent predictors of the severity of NASH histology in patients [101]. Dysregulation of these methyl donors may promote NAFLD through modulation of DNA methylation. In the mouse liver, 164 genes involved in lipid and glucose metabolism, DNA damage and repair, the onset of fibrosis, and liver tissue remodeling were mostly hypomethylated on CpG islands as a result of a methyl-deficient diet [102]. A methyl-deficient diet stimulated inflammation by enhancing the kynurenine pathway [103]. Dietary methyl-donor supplement (including folate, betaine, choline, and VB12) protects rodents from diet-induced hepatic steatosis by reversing the methylation status of genes involved in lipid metabolism, such as peroxisome proliferators-activated receptor alpha, acetyl-CoA acyltransferase and *FASN* [104–106]. VB12 oral supplementation resulted in differential CpG methylation of genes related to intestinal barrier function, fatty acid metabolism, and mitochondrial

metabolism, while suppressing the inflammatory response [107].

4.3 | Bile acids

The gut microbiota plays an important role in transforming primary bile acids into secondary bile acids. After a meal, primary bile acids synthesized in the liver are released into the duodenum, where they are subsequently converted to secondary bile acids by the gut microbiota [108, 109]. Bile acids not only control the absorption of lipids in the gut but also regulate lipogenesis in the liver [110]. Bile acids regulate host metabolism mainly through Takeda G protein-coupled receptor 5, and the nuclear receptor, farnesoid X receptor (FXR). Takeda G protein-coupled receptor 5, also known as G protein-coupled bile acid receptor 1, is a member of the membrane receptor family that induces the secretion of insulin and GLP-1,making it a key player in host energy, glucose, and lipid metabolism [36]. FXR, belonging to the family of nuclear hormone receptors, mediates antibacterial and lipid metabolism regulation [110]. Recently, the results of an 18-month interim analysis of a multicenter, randomized, placebo-controlled phase 3 trial of a strong FXR agonist, obeticholic acid, in 931 patients with biopsy-proven F2–F3 fibrosis were reported. The results showed that the obeticholic acid-treated group exhibited regression of NASH, no deterioration of fibrosis or improvement of at least one stage of fibrosis without deterioration of NASH compared to the control group [111]. Therefore, such evidence links gut microbiota-induced bile acid biotransformation to NAFLD and NASH progression [108, 112].

Studies on animals and humans have shown that bile acids can regulate the expression of lipid metabolism-related genes through epigenetic mechanisms and affect the progression of NAFLD. Multiple genes involved in bile acid homeostasis had significantly different levels of methylation, based on liver-derived methylation and expression data from three cohort studies involving 103 NAFLD and 75 non-NAFLD individuals. This reveals that NAFLD is closely related to the methylation and transcription levels of key genes involved in bile acid homeostasis [113]. Bile acids can induce FGF15/19 signaling through FXR/NR1H4 to activate Small Heterodimer Partners and recruit DNMT3A to lipogenic genes, leading to inhibition of hepatic lipogenesis via DNA methylation [114]. Furthermore, a recent study reported that the secondary bile acid taurine deoxycholic acid remodels histone modification surrounding the cell death-inducing DNA fragmentation factor-like effector A and cell-death-inducing DNA-fragmentation-factor-like effector C genes, leading to downregulation of their expression, and thereby improving the developmentally-programmed hepatic steatosis [115].

4.4 | Tryptophan metabolites

Tryptophan is produced from the degradation of dietary proteins and further catalyzed into various metabolites by tryptophanases of the gut microbiota. Tryptophan metabolites include indole, indole-3-carboxaldehyde (ICA), indole-3-propionic acid (IPA) and indole acetic acid (IAA). Tryptophan metabolites are important signaling molecules in the microbial community as well as in host-microbe interactions, and may improve NAFLD by regulating intestinal and systemic immune homeostasis and lipid metabolism through specific nuclear receptor and HDAC inhibition [116]. ICA can activate the heterologous aryl hydrocarbon receptor (AHR) to increase the expression of cytokine *IL-10*, promote the proliferation of intestinal epithelial cells and promote goblet cell differentiation [117]. IPA can also induce an IL-10R1-mediated anti-inflammatory mechanism in human and mouse intestinal epithelial cells via AHR [118]. Additionally, indole can alleviate liver inflammation and regulate cholesterol metabolism in LPS-injected mice by inhibiting key pro-inflammatory factors downstream of the NF-κB signaling pathway [119]. IAA can act on macrophages and hepatocytes to reduce hepatic lipogenesis, oxidative and inflammatory stress, and has a protective effect on liver injury in HFD-induced NAFLD mice [120]. Moreover, IAA significantly reduces pro-inflammatory cytokine production in macrophages, while attenuating lipogenesis and inflammatory responses in hepatocytes in an AHR-dependent manner [76]. Furthermore, indole-3-butyric acid derivatives are potent HDAC inhibitors that have been found to inhibit tumor growth and apoptosis in athymic mice [121].

4.5 | Choline metabolites

Trimethylamine (TMA) is produced by the gut microbiota when it metabolizes dietary choline and carnitine. TMA then reaches the liver where it is further metabolized by the hepatic enzyme hepatic flavin mono-oxygenases 3 to generate TMAO [122]. TMA and/or TMAO have been considered as novel biomarkers for early metabolic syndrome and correlated with NAFLD severity [123]. Patients with more severe NAFLD have been found to have higher TMAO but lower betaine and betaine/choline ratios in human studies [124]. A characteristic model of NAFLD has been identified in mice on a choline-deficient diet, which results in increased liver fat and altered gut

microbiota [125, 126]. Studies in mice have shown an association between elevated urinary levels of TMA and TMAO and increased severity of NAFLD [123, 127]. These findings suggest that TMA and/or TMAO play an important role in the development of NAFLD. Several studies have shown that TMAO can epigenetically modify genes associated with NAFLD. TMAO upregulates the expression of miR-17/92 cluster members in the liver and increases the expression of target genes related to atherosclerosis and inflammation [71]. TMAO alters miR-21-5p and miR-30c-5p expression in liver and macrophages, leading to induction of inflammation and release of cytokines, and regulation of cholesterol and fatty acid metabolism [72].

Table 1 details the putative relationships between host epigenetic modifications and gut microbial metabolites as well as their potential implications in the development of NAFLD.

5 | DISCUSSION

The host's physiological state is significantly affected by the complex interplay between the microbiome and its host. Current clinical and experimental investigations have uncovered the crucial role of epigenetic mechanisms in the impact of gut microbiota on the pathogenesis of NAFLD. However, research exploring the impact of gut microbiota on NAFLD pathogenesis through epigenetics faces numerous challenges.

Firstly, the interactions between the gut microbiota and the host are multifaceted, encompassing an array of signaling pathways, molecules, and biological processes. A comprehensive understanding of how gut microbiota modulate NAFLD progression through epigenetic mechanisms remains to be achieved. To address this, researchers should adopt interdisciplinary and multifaceted approaches, incorporating metagenomics, transcriptomics, metabolomics, and epigenetic techniques, to gain a more holistic and in-depth understanding of the subject.

Secondly, current research primarily relies on animal models, which may not accurately replicate the complexity of human gut microbiota and their interplay with NAFLD. Experimental conditions and dietary designs may not precisely reflect the diversity of human diets. Establishing more human-like disease models for studying NAFLD, such as "humanized" mouse models or utilizing organoids, will contribute to enhancing the accuracy and reliability of research.

Moreover, due to genetic, lifestyle, and environmental factors, there is considerable variation in gut microbiota and epigenetic features among individuals, which may affect the consistency and generalizability of research findings. Currently, studies often lack extensive samples and diverse populations for investigating the effects of individual differences on research outcomes. To gain a more comprehensive understanding of the impact of individual differences on research findings, investigators should expand sample sizes and diversity, and engage in multicenter, international collaborative studies that encompass diverse ethnicities, ages, genders, and lifestyles.

Additionally, investigations into gut microbiota and epigenetics involve extensive data sets, such as metagenomic, transcriptomic, and methylation data, requiring sophisticated computational methodologies and specialized knowledge for analysis and interpretation. Nonetheless, numerous laboratories may encounter constraints regarding analytical tools and expertise, potentially impeding research advancement. By bolstering interdisciplinary cooperation and combining proficiency in computational biology, data science, and microbiology, user-friendly analytical instruments, and platforms can be developed. This will reduce the challenges associated with data processing and analysis, thereby facilitating improved management and interpretation of substantial data quantities.

Ultimately, gut microbiota and epigenetic characteristics may undergo dynamic changes over time and space, influenced by various factors such as diet, medication, and lifestyle habits. However, most studies focus only on observations at specific time points, making it challenging to reveal the dynamic changes of gut microbiota and epigenetics in NAFLD pathogenesis. Longitudinal studies should be conducted to monitor the dynamic changes of gut microbiota and epigenetic characteristics. Furthermore, researchers need to use multiple time-point sample collection and analysis to more comprehensively explore the impact of different factors on gut microbiota and epigenetics during the pathogenesis of NAFLD.

Hence, future studies should overcome these limitations to gain a deeper understanding of how gut microbiota influence the development of NAFLD via epigenetics. By tackling these challenges, we will improve our understanding of the role of gut microbiota and epigenetics in the development of NAFLD, thus presenting new strategies and targets for the prevention and treatment of this disease.

6 | CONCLUDING REMARKS

In conclusion, the gut microbiome has been shown to have a significant impact on the development and progression of NAFLD through various components and metabolites. However, the molecular mechanisms and signaling

TABLE 1 Epigenetic mechanisms underlying epigenetic changes and biological functions induced by gut microbiota metabolites in the progression of NAFLD.

Gut microbiota metabolites	Epigenetic mechanisms	Epigenetic changes	Biological functions	References
SCFAs: Acetate, propionate, and butyrate	Inhibition of HDACs and DNMTs	Increased gene expression of anti-inflammatory factors: including *IL-10*, *IL-22*, granzyme B, *IL-17A*, *IL-17F*, retinoic acid related-orphan receptor alpha, retinoic acid related-orphan receptor gamma t, *T-bet*, interferon-γ and forkhead box P3 Decreased gene expression of pro-inflammatory cytokines: including *IL-1β*, *IL-6*, *interferon-γ*, and *TNF-α* Increased expression of genes involved in lipid metabolism: peroxisome proliferators-activated receptor alpha, *FGF21*, glucagon-like peptide-1 receptor and insulin-induced gene Decreased expression of lipogenic genes: stearoyl-CoA desaturase 1, fatty acid synthase and Sterol regulatory element-binding protein-1 Decreased the methylation in the promoter region of adiponectin and resistin	Regulation of proliferation, differentiation and function of immune cells Improvement in hepatic lipid metabolism, insulin sensitivity and weight control	[63, 85-97]
Vitamins: B9 (folate) and B12	Increased DNA methylation	Regulation of the methylation status of genes involved in intestinal barrier function, lipid, glucose and mitochondrial metabolism, DNA damage and repair, fibrogenesis and liver tissue remodeling	Regulation of lipid, glucose and energy metabolism Improvement in gut motility, liver injury and liver fibrosis	[101–107]
Bile acids: Taurine deoxycholic acid, chenodeoxycholic acid, cholic acid and deoxycholic acid	Activation of DNMT3A Dimethylation of H3K4, H3K27, or H3K36	Regulation of the methylation status of genes involved in bile acid homeostasis Increased DNA methylations of lipogenic genes Decreased expression of the enhancers of lipid droplet sizes: cell death-inducing DNA fragmentation factor-like effector A and cell-death-inducing DNA-fragmentation-factor-like effector C	Regulation of bile acid and lipid homeostasis Improvement in hepatic steatosis	[113–115]
Tryptophan metabolites: indole, IAA, ICA and IPA	Activation of AHR Inhibition of HDACs	Increased gene expression of anti-inflammatory cytokines: *IL-10* and *IL-10R1* Decreased gene expression of pro-inflammatory cytokines: nitric oxide synthase 2, *IL-1β*, C-C motif chemokine ligand 2, monocyte chemoattractant protein-1 and *TNF-α* Decreased expression of Lipogenic genes: sterol regulatory element binding transcription factor 1, acetyl-CoA carboxylase alpha and fatty acid synthase	Maintaining immune homeostasis and lipid metabolism Improvement in liver inflammation and hepatic lipogenesis	[76, 116-121]
Choline metabolites: TMA and TMAO	Upregulated expression of miR-17-5p, miR-92a-3p, miR-21-5p and miR-30c-5p	Increasing serpin family E member 1 and *IL-12A* Decreased gene expression of period circadian protein 2	Promoting atherosclerotic development and inflammation Regulation of cholesterol and fatty acid metabolism	[71, 72]

pathways underlying this complex interplay remain limited and require further investigation.

Epigenetic modifications are crucial in shaping the gut microbiome-host interaction, with the gut microbiome regulating host epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNA-mediated regulation. A more comprehensive understanding of these mechanisms could shed light on the specific role of the gut microbiome in NAFLD pathogenesis.

Studying the gut microbiome-induced epigenetic modifications in NAFLD has important realistic and clinical implications, including the development of new diagnostic biomarkers, identification of novel therapeutic targets, and personalized medicine approaches. Advanced epigenetic techniques may identify specific epigenetic modifications associated with NAFLD, providing more accurate and early disease diagnosis. Identifying specific gut microbiome metabolite-induced epigenetic modifications may lead to new biomarkers for NAFLD diagnosis and disease progression. Developing treatment strategies targeting specific gut microbiome and their metabolites could also provide more effective treatment for NAFLD patients. For example, targeting gut microbiome metabolite-induced epigenetic modifications may reduce the risk of NAFLD development and progression. Furthermore, understanding the impact of individual differences in gut microbiome composition and epigenetic modifications may help identify personalized treatment strategies for different subgroups of NAFLD patients, leading to more effective and targeted treatment.

In summary, further research into gut microbiome-induced epigenetic modifications in NAFLD could have significant clinical implications, paving the way for more effective diagnosis, treatment, and prevention of this disease.

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CONFLICTS OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Kleiner DE, Brunt EM, Natta MV, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005;41:1313-1321.
2. Zhang J, Wang C, Ji X, et al. Bile acids: a bridge linking gut microbiota and NAFLD. *Advanced Gut & Microbiome Research*. 2022;2022:4752148.
3. Younes R, Caviglia GP, Govaere O, et al. Long-term outcomes and predictive ability of non-invasive scoring systems in patients with non-alcoholic fatty liver disease. *J Hepatol*. 2021;75:786-794.
4. McPherson S, Armstrong MJ, Cobbold JF, et al. Quality standards for the management of non-alcoholic fatty liver disease (NAFLD): consensus recommendations from the British Association for the Study of the Liver and British Society of Gastroenterology NAFLD Special Interest Group. *Lancet Gastroenterol Hepatol*. 2022;7:755-769.
5. Quek J, Chan KE, Wong ZY, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2023;8:20-30.
6. Godoy-Matos AF, Júnior WSS, Valerio CM. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes. *Diabetol Metab Syndr*. 2020;12:60.
7. Estes C, Razavi H, Loomba R. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018;67:123-133.
8. Younossi ZM, Golabi P, Paik JM, et al. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*. 2023;77(4):1335-1347.
9. Trovato FM, Castrogiovanni P, Malatino L, Musumeci G. Nonalcoholic fatty liver disease (NAFLD) prevention: role of Mediterranean diet and physical activity. *Hepatobiliary Surg Nutr*. 2019;8:167-169.
10. Mungamuri SK, Vijayasarathy K. Role of the gut microbiome in nonalcoholic fatty liver disease progression. *Crit Rev Oncog*. 2020;25:57-70.
11. Chen J, Vitetta L. Gut microbiota metabolites in NAFLD pathogenesis and therapeutic implications. *Int J Mol Sci*. 2020;21:5214.
12. Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology*. 1998;114:842-845.
13. Tilg H, Adolph TE, Moschen AR. Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade. *Hepatology*. 2021;73:833-842.
14. Fellows R, Varga-Weisz P. Chromatin dynamics and histone modifications in intestinal microbiota-host crosstalk. *Mol Metab*. 2019;38:100925.
15. Chen HT, Huang HL, Li YQ, et al. Therapeutic advances in non-alcoholic fatty liver disease: a microbiota-centered view. *World J Gastroenterol*. 2020;26:1901-1911.
16. Wolfe W, Xiang Z, Yu X, et al. The challenge of applications of probiotics in gastrointestinal diseases. *Advanced Gut & Microbiome Research*. 2023:1984200.
17. Ponziani FR, Bhoori S, Castelli C, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflamma-

tion in nonalcoholic fatty liver disease. *Hepatology*. 2019;69:107-120.

18. Le HH, Lee MT, Besler KR, Johnson EL. Host hepatic metabolism is modulated by gut microbiota-derived sphingolipids. *Cell Host Microbe*. 2022;30:798-808.e797.
19. Ramos-Molina B, Sánchez-Alcoholado L, Cabrera-Mulero A, et al. Gut microbiota composition is associated with the global DNA methylation pattern in obesity. *Front Genet*. 2019;10:613.
20. Austvoll CT, Gallo V, Montag D. Health impact of the anthropocene: the complex relationship between gut microbiota, epigenetics, and human health, using obesity as an example. *Glob Health Epidemiol Genom*. 2020;5:e2.
21. Kim M, Lee HA, Cho HM, et al. Histone deacetylase inhibition attenuates hepatic steatosis in rats with experimental Cushing's syndrome. *Korean J Physiol Pharmacol*. 2018;22:23-33.
22. Pasolli E, Asnicar F, Manara S, et al. Extensive unexplored human microbiome diversity revealed by over 150,000 genomes from metagenomes spanning age, geography, and lifestyle. *Cell*. 2019;176:649-662.e620.
23. Consortium, I.H.i.R.N., The integrative human microbiome project. *nature*. 2019;569:641-648.
24. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. *Nature*. 2017;548:43-51.
25. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. *Nat Rev Gastroenterol Hepatol*. 2019;16:35-56.
26. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol*. 2021;19:55-71.
27. Wastyk HC, Fragiadakis GK, Perelman D, et al. Gut-microbiota-targeted diets modulate human immune status. *Cell*. 2021;184:4137-4153.e4114.
28. Ducarmon QR, Zwittink RD, Hornung BVH, et al. Gut microbiota and colonization resistance against bacterial enteric infection. *Microbiol Mol Biol Rev*. 2019;83:e00007-19.
29. Tilg H, Adolph TE, Dudek M, Knolle P. Non-alcoholic fatty liver disease: the interplay between metabolism, microbes and immunity. *Nat Metab*. 2021;3:1596-1607.
30. Tang R, Li L. Modulation of short-chain fatty acids as potential therapy method for type 2 diabetes mellitus. *Can J Infect Dis Med Microbiol*. 2021;2021:6632266.
31. Cuevas-Sierra A, Ramos-Lopez O, Riezu-Boj JI, et al. Diet, gut microbiota, and obesity: links with host genetics and epigenetics and potential applications. *Adv Nutr*. 2019;10:S17-S30.
32. Fang J, Yu CH, Li XJ, et al. Gut dysbiosis in nonalcoholic fatty liver disease: pathogenesis, diagnosis, and therapeutic implications. *Front Cell Infect Microbiol*. 2022;12:997018.
33. Wu J, Huang F, Ling Z, et al. Altered faecal microbiota on the expression of Th cells responses in the exacerbation of patients with hepatitis E infection. *J Viral Hepat*. 2020;27:1243-1252.
34. Lee G, You HJ, Bajaj JS, et al. Distinct signatures of gut microbiome and metabolites associated with significant fibrosis in non-obese NAFLD. *Nat Commun*. 2020;11:4982.
35. Wang T, Guo XK, Xu H. Disentangling the progression of non-alcoholic fatty liver disease in the human gut microbiota. *Front Microbiol*. 2021;12:728823.
36. Aron-Wisnewsky J, Vigliotti C, Witjes J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol*. 2020;17:279-297.
37. Hoyles L, Fernández-Real JM, Federici M, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med*. 2018;24:1070-1080.
38. Shen F, Zheng RD, Sun XQ, et al. Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int*. 2017;16:375-381.
39. Raman M, Ahmed I, Gillevet PM, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2013;11:868-875.e861-863.
40. Loomba R, Seguritan V, Li W, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. *Cell Metab*. 2017;25:1054-1062.e1055.
41. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology*. 2013;57:601-609.
42. Chierico FD, Nobili V, Vernocchi P, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology*. 2017;65:451-464.
43. Wang B, Jiang X, Cao M, et al. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. *Sci Rep*. 2016;6:32002.
44. Wong VWS, Tse CH, Lam TTY, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis–a longitudinal study. *PLoS ONE*. 2013;8:e62885.
45. Chen Y, Yang F, Lu H, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology*. 2011;54:562-572.
46. Moran-Ramos S, Cerqueda-García D, López-Contreras B, et al. A metagenomic study identifies a Prevotella-copri enriched microbial profile associated with non-alcoholic steatohepatitis in subjects with obesity. *J Gastroenterol Hepatol*. 2023;38:791-799.
47. Behary J, Amorim N, Jiang XT, et al. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun*. 2021;12:187.
48. Chiu CC, Ching YH, Li YP, et al. Nonalcoholic fatty liver disease is exacerbated in high-fat diet-fed gnotobiotic mice by colonization with the gut microbiota from patients with nonalcoholic steatohepatitis. *Nutrients*. 2017;9:1220.
49. Narabayashi H, Koma C, Nakata K, et al. Gut microbiota-dependent adaptor molecule recruits DNA methyltransferase to the TLR4 gene in colonic epithelial cells to suppress inflammatory reactions. *Front Mol Biosci*. 2022;9:1005136.
50. Jonscher KR, Abrams J, Friedman JE. Maternal diet alters trained immunity in the pathogenesis of pediatric NAFLD. *J Cell Immunol*. 2020;2:315-325.
51. Kim H, Worsley O, Yang E, et al. Persistent changes in liver methylation and microbiome composition following reversal of diet-induced non-alcoholic-fatty liver disease. *Cell Mol Life Sci*. 2019;76:4341-4354.
52. Gjorgjieva M, Sobolewski C, Dolicka D, et al. miRNAs and NAFLD: from pathophysiology to therapy. *Gut*. 2019;68:2065-2079.
53. Poupeau A, Garde C, Sulek K, et al. Genes controlling the activation of natural killer lymphocytes are epigenetically

remodeled in intestinal cells from germ-free mice. *FASEB J*. 2019;33:2719-2731.
54. Wankhade UD, Zhong Y, Kang P, et al. Enhanced offspring predisposition to steatohepatitis with maternal high-fat diet is associated with epigenetic and microbiome alterations. *PLoS ONE*. 2017;12:e0175675.
55. Zhou Y, Zheng T, Chen H, et al. Microbial intervention as a novel target in treatment of non-alcoholic fatty liver disease progression. *Cell Physiol Biochem*. 2018;51:2123-2135.
56. Hany NM, Eissa S, Basyouni M, et al. Modulation of hepatic stellate cells by Mutaflor probiotic in non-alcoholic fatty liver disease management. *J Transl Med*. 2022;20:342.
57. Kumar H, Lund R, Laiho A, et al. Gut microbiota as an epigenetic regulator: pilot study based on whole-genome methylation analysis. *mBio*. 2014;5:e02113-02114.
58. Khanmohammadi S, Kuchay MS. Toll-like receptors and metabolic (dysfunction)-associated fatty liver disease. *Pharmacol Res*. 2022;185:106507.
59. Martínez-Chantar ML, Delgado TC, Beraza N. Revisiting the role of natural killer cells in non-alcoholic fatty liver disease. *Front Immunol*. 2021;12:640869.
60. Li D, Li Y, Yang S, et al. Diet-gut microbiota-epigenetics in metabolic diseases: from mechanisms to therapeutics. *Biomed Pharmacother*. 2022;153:113290.
61. Liu Q, Liu S, Chen L, et al. Role and effective therapeutic target of gut microbiota in NAFLD/NASH. *Exp Ther Med*. 2019;18:1935-1944.
62. Liu P, Wang Y, Yang G, et al. The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol Res*. 2021;165: 105420.
63. Zhou D, Chen YW, Zhao ZH, et al. Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Exp Mol Med*. 2018;50:1-12.
64. Fellows R, Denizot J, Stellato C, et al. Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. *Nat Commun*. 2018;9:105.
65. Atic AI, Thiele M, Munk A, Dalgaard LT. Circulating miRNAs associated with nonalcoholic fatty liver disease. *Am J Physiol Cell Physiol*. 2023;324:C588-c602.
66. Hochreuter MY, Dall M, Treebak JT, Barrès R. MicroRNAs in non-alcoholic fatty liver disease: progress and perspectives. *Mol Metab*. 2022;65:101581.
67. Gong R, Lv X, Liu F. MiRNA-17 encoded by the miR-17-92 cluster increases the potential for steatosis in hepatoma cells by targeting CYP7A1. *Cell Mol Biol Lett*. 2018;23:16.
68. Lin H, Mercer KE, Ou X, et al. Circulating microRNAs are associated with metabolic markers in adolescents with hepatosteatosis. *Front Endocrinol (Lausanne)*. 2022;13:856973.
69. Kim TH, Lee Y, Lee YS, et al. Circulating miRNA is a useful diagnostic biomarker for nonalcoholic steatohepatitis in nonalcoholic fatty liver disease. *Sci Rep*. 2021;11:14639.
70. Fan J, Li H, Nie X, et al. MiR-30c-5p ameliorates hepatic steatosis in leptin receptor-deficient (db/db) mice via downregulating FASN. *Oncotarget*. 2017;8:13450-13463.
71. Díez-Ricote L, Ruiz-Valderrey P, Micó V, et al. TMAO upregulates members of the miR-17/92 cluster and impacts targets associated with atherosclerosis. *Int J Mol Sci*. 2022;23:12107.
72. Díez-Ricote L, Ruiz-Valderrey P, Micó V, et al. nTrimethylamine -Oxide (TMAO) modulates the expression of cardiovascular disease-related microRNAs and their targets. *Int J Mol Sci*. 2021;22:11145.
73. Ji Y, Yin Y, Li Z, Zhang W. Gut microbiota-derived components and metabolites in the progression of non-alcoholic fatty liver disease (NAFLD). *Nutrients*. 2019;11:1712.
74. Huang W, Man Y, Gao C, et al. Short-chain fatty acids ameliorate diabetic nephropathy via GPR43-mediated inhibition of oxidative stress and NF-κB signaling. *Oxid Med Cell Longev*. 2020;2020:4074832.
75. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology*. 2017;152:1679-1694.e1673.
76. Krishnan S, Ding Y, Saedi N, et al. Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. *Cell Rep*. 2018;23:1099-1111.
77. Yang G, Zhang X. Trimethylamine N-oxide promotes hyperlipidemia acute pancreatitis via inflammatory response. *Can J Physiol Pharmacol*. 2022;100:61-67.
78. Gu C, Zhou Z, Yu Z, et al. The microbiota and it's correlation with metabolites in the gut of mice with nonalcoholic fatty liver disease. *Front Cell Infect Microbiol*. 2022;12:870785.
79. Barchetta I, Cimini FA, Cavallo MG. Vitamin D and metabolic dysfunction-associated fatty liver disease (MAFLD): an update. *Nutrients*. 2020;12:3302.
80. Adams LA, Wang Z, Liddle C, et al. Bile acids associate with specific gut microbiota, low-level alcohol consumption and liver fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int*. 2020;40:1356-1365.
81. Stachowska E, Maciejewska-Markiewicz D, Palma J, et al. Precision nutrition in NAFLD: effects of a high-fiber intervention on the serum metabolome of NAFD patients-a pilot study. *Nutrients*. 2022;14:5355.
82. Rau M, Rehman A, Dittrich M, et al. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J*. 2018;6:1496-1507.
83. Deng M, Qu F, Chen L, et al. SCFAs alleviated steatosis and inflammation in mice with NASH induced by MCD. *J Endocrinol*. 2020;245:425-437.
84. Liu L, Fu Q, Li T, et al. Gut microbiota and butyrate contribute to nonalcoholic fatty liver disease in premenopause due to estrogen deficiency. *PLoS ONE*. 2022;17:e0262855.
85. Eslick S, Williams EJ, Berthon BS, et al. Weight loss and short-chain fatty acids reduce systemic inflammation in monocytes and adipose tissue macrophages from obese subjects. *Nutrients*. 2022;14:765.
86. Yang W, Yu T, Huang X, et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat Commun*. 2020;11:4457.
87. Kibbie JJ, Dillon SM, Thompson TA, et al. Butyrate directly decreases human gut lamina propria CD4 T cell function through histone deacetylase (HDAC) inhibition and GPR43 signaling. *Immunobiology*. 2021;226:152126.
88. Luu M, Weigand K, Wedi F, et al. Regulation of the effector function of CD8 T cells by gut microbiota-derived metabolite butyrate. *Sci Rep*. 2018;8:14430.

89. Schiweck C, Thanarajah SE, Aichholzer M, et al. Regulation of CD4(+) and CD8(+) T cell biology by short-chain fatty acids and its relevance for autoimmune pathology. *Int J Mol Sci*. 2022;23:8272.
90. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341:569-573.
91. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504:451-455.
92. Hao F, Tian M, Zhang X, et al. Butyrate enhances CPT1A activity to promote fatty acid oxidation and iTreg differentiation. *Proc Natl Acad Sci U S A*. 2021;118:e2014681118.
93. Zou F, Qiu Y, Huang Y, et al. Effects of short-chain fatty acids in inhibiting HDAC and activating p38 MAPK are critical for promoting B10 cell generation and function. *Cell Death Dis*. 2021;12:582.
94. Li H, Gao Z, Zhang J, et al. Sodium butyrate stimulates expression of fibroblast growth factor 21 in liver by inhibition of histone deacetylase 3. *Diabetes*. 2012;61:797-806.
95. Lin D, Sun Q, Liu Z, et al. Gut microbiota and bile acids partially mediate the improvement of fibroblast growth factor 21 on methionine-choline-deficient diet-induced non-alcoholic fatty liver disease mice. *Free Radic Biol Med*. 2023;195:199-218.
96. Zhao ZH, Wang ZX, Zhou D, et al. Sodium butyrate supplementation inhibits hepatic steatosis by stimulating liver kinase B1 and insulin-induced gene. *Cell Mol Gastroenterol Hepatol*. 2021;12:857-871.
97. Lu Y, Fan C, Liang A, et al. Effects of SCFA on the DNA methylation pattern of adiponectin and resistin in high-fat-diet-induced obese male mice. *Br J Nutr*. 2018;120:385-392.
98. Abe RAM, Masroor A, Khorochkov A, et al. The role of vitamins in non-alcoholic fatty liver disease: a systematic review. *Cureus*. 2021;13:e16855.
99. Rossi M, Amaretti A, Raimondi S. Folate production by probiotic bacteria. *Nutrients*. 2011;3:118-134.
100. Srivastava S, Singh A, Sandeep K, Yadav D. Epigenetic regulation of gut microbial dysbiosis. *Indian J Microbiol*. 2021;61:125-129.
101. Mahamid M, Mahroum N, Bragazzi NL, et al. Folate and B12 levels correlate with histological severity in NASH patients. *Nutrients*. 2018;10:440.
102. Tryndyak VP, Han T, Muskhelishvili L, et al. Coupling global methylation and gene expression profiles reveal key pathophysiological events in liver injury induced by a methyl-deficient diet. *Mol Nutr Food Res*. 2011;55:411-418.
103. Tillmann S, Awwad HM, MacPherson CW, et al. The kynurenine pathway is upregulated by methyl-deficient diet and changes are averted by probiotics. *Mol Nutr Food Res*. 2021;65:e2100078.
104. Sun C, Fan JG, Qiao L. Potential epigenetic mechanism in non-alcoholic Fatty liver disease. *Int J Mol Sci*. 2015;16:5161-5179.
105. Chen S, Yang M, Wang R, et al. Suppression of high-fat-diet-induced obesity in mice by dietary folic acid supplementation is linked to changes in gut microbiota. *Eur J Nutr*. 2022;61:2015-2031.
106. Hu H, Tan L, Li X, et al. Betaine reduces lipid anabolism and promotes lipid transport in mice fed a high-fat diet by influencing intestinal protein expression. *Foods*. 2022;11:2421.
107. Ge Y, Zadeh M, Mohamadzadeh M. Vitamin B12 regulates the transcriptional, metabolic, and epigenetic programing in human ileal epithelial cells. *Nutrients*. 2022;14:2825.
108. Fuchs CD, Trauner M. Role of bile acids and their receptors in gastrointestinal and hepatic pathophysiology. *Nat Rev Gastroenterol Hepatol*. 2022;19:432-450.
109. Gottlieb A, Canbay A. Why bile acids are so important in non-alcoholic fatty liver disease (NAFLD) progression. *Cells*. 2019;8:1358.
110. Clifford BL, Sedgeman LR, Williams KJ, et al. FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. *Cell Metab*. 2021;33:1671-1684.e1674.
111. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet*. 2019;394:2184-2196.
112. Eslam M, Alvani R, Shiha G. Obeticholic acid: towards first approval for NASH. *Lancet*. 2019;394:2131-2133.
113. Schiöth HB, Boström A, Murphy SK, et al. A targeted analysis reveals relevant shifts in the methylation and transcription of genes responsible for bile acid homeostasis and drug metabolism in non-alcoholic fatty liver disease. *Bmc Genom*. 2016;17:462.
114. Kim YC, Seok S, Zhang Y, et al. Intestinal FGF15/19 physiologically repress hepatic lipogenesis in the late fed-state by activating SHP and DNMT3A. *Nat Commun*. 2020;11:5969.
115. Urmi JF, Itoh H, Muramatsu-Kato K, et al. Plasticity of histone modifications around Cidea and Cidec genes with secondary bile in the amelioration of developmentally-programmed hepatic steatosis. *Sci Rep*. 2019;9:17100.
116. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. *Nat Commun*. 2018;9:3294.
117. Powell DN, Swimm A, Sonowal R, et al. Indoles from the commensal microbiota act via the AHR and IL-10 to tune the cellular composition of the colonic epithelium during aging. *Proc Natl Acad Sci U S A*. 2020;117:21519-21526.
118. Alexeev EE, Lanis JM, Kao DJ, et al. Microbiota-derived indole metabolites promote human and murine intestinal homeostasis through regulation of interleukin-10 receptor. *Am J Pathol*. 2018;188:1183-1194.
119. Beaumont M, Neyrinck AM, Olivares M, et al. The gut microbiota metabolite indole alleviates liver inflammation in mice. *FASEB J*. 2018;32:fj201800544.
120. Ji Y, Gao Y, Chen H, et al. Indole-3-acetic acid alleviates non-alcoholic fatty liver disease in mice via attenuation of hepatic lipogenesis, and oxidative and inflammatory stress. *Nutrients*. 2019;11:2062.
121. Chen Y, Zhang L, Zhang L, et al. Discovery of indole-3-butyric acid derivatives as potent histone deacetylase inhibitors. *J Enzyme Inhib Med Chem*. 2021;36:425-436.
122. Agus A, Clément K, Sokol H. Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut*. 2021;70:1174-1182.
123. Barrea L, Annunziata G, Muscogiuri G, et al. Trimethylamine-N-oxide (TMAO) as novel potential biomarker of early predictors of metabolic syndrome. *Nutrients*. 2018;10:1971.
124. Chen YM, Liu Y, Zhou RF, et al. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep*. 2016;6:19076.

125. Alshawsh MA, Alsalahi A, Alshehade SA, et al. A comparison of the gene expression profiles of non-alcoholic fatty liver disease between animal models of a high-fat diet and methionine-choline-deficient diet. *Molecules*. 2022;27:858.
126. Wortmann SB, Mayr JA. Choline-related-inherited metabolic diseases-a mini review. *J Inherit Metab Dis*. 2019;42:237-242.
127. León-Mimila P, Villamil-Ramírez H, Li XS, et al. Trimethylamine N-oxide levels are associated with NASH in obese subjects with type 2 diabetes. *Diabetes Metab*. 2021;47:101183.

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