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Role of gut microbiota in diabetes mellitus and tumor immunity

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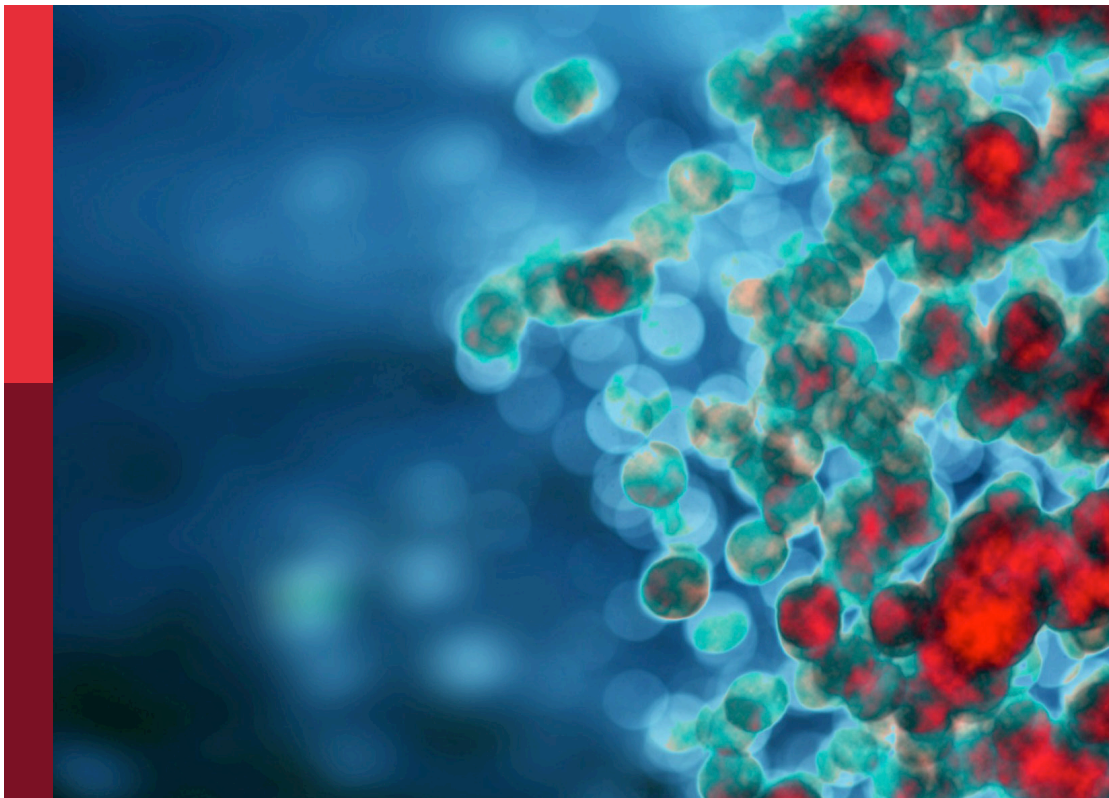

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Role of gut microbiota in diabetes mellitus and tumor immunity

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Editorial: Role of gut microbiota in diabetes mellitus and tumor immunity

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Editorial on the Research Topic
Role of gut microbiota in diabetes mellitus and tumor immunity

Gut microecology is an important component of human health and environment. It's composed of gut microbiota, gut epithelial cells, immune system, which forms gut mucosal barrier and act in energy metabolism. Gut microbiota plays an important role in the proper functioning of human organisms, it coevolves and symbiosis with humans by combating pathogenic bacteria, assisting in nutrient digestion, maintaining the integrity of the gut epithelia, and promoting immunological development. The steady state of the gut microbiota is closely related to human health and both external and genetic factors affect its composition and function. Thus, gut microbiota-mediated immunomodulatory effects play an important role in diabetes mellitus and tumor immunity. However, the causal relationship between an altered gut microbiota composition and diabetes mellitus or tumor immunity remains to be elucidated. Host immune responses are an extremely complex process and an in-depth exploration of the gut microbiota-host-disease relationship will be helpful to identify potential therapeutic targets for diabetes mellitus and cancer. We encourage the exploration of the etiology and factors influencing diabetes mellitus and tumor immunity from a gut microecological perspective with the aim of addressing these challenging questions. In this Research Topic, we aimed to explore gut microbiota profiles and the specific processes by which bacterial components, metabolites, and other mediators of gut microbiota interact with the host to affect the immunity of patients with diabetes mellitus or cancer.

This Research Topic included correlation analysis of gut microbiota composition with diabetes mellitus and tumor immunity. This review confirmed the differences in gut microbiota between healthy individuals and diabetic patients (Ye et al.). In addition, the differences in gut microbiota between type 1 diabetes mellitus and type 2 diabetes mellitus patients and the role of gut microbiota imbalance in the pathogenesis of diabetes patients, are discussed, especially with regard to the incidence of leaky gut syndrome, immune

dysfunction, and metabolic disorders. They also summarized the progress made in developing microbial therapies to prevent and treat diabetes, especially the current status and application prospects of fecal microbiota transplantation in diabetes. Liu et al. expanded our understanding of the relationship between gut microbiota, an immune checkpoint inhibitor (ICI) response, and immune-related adverse events (irAE) occurrence. They studied the gut microbiota of patients who experienced a series of irAE in different cancers, particularly lung cancer; 1) with and without irAE; 2) with different severity of irAE; 3) with differences in microbiota composition between patients with and without irAE associated with colitis. Moreover, they explored the causal relationship between microbiota composition and immune-related colitis. Subsequently, their assessment of colitis and dynamic microbiota analysis led to more fundamental questions such as whether differences in microbiota cause immune-related colitis and whether immune-related colitis disrupts the gut microbiota. Huang et al. sequenced the ulcerated and intact skin of patients with diabetes and found that skin had significantly higher bacterial richness and diversity than diabetic foot wounds. The microecological balance of the skin was disrupted, and pathogenic bacteria replaced the original microbiota. It is suggested that commensal bacteria of intact skin may be important in maintaining microecological balance and preventing the development of diabetic skin ulcers.

This special issue also discussed gut microbiota-associated molecules that can affect host immune responses; Bao et al. reviewed such molecules from the perspective of traditional Chinese medicine (TCM), explaining why it can be a treasure trove of potential probiotics, and may shed light on the mechanisms of action underlying TCM successes in disease treatment. To further investigate the effect of TCM on different levels of gut microbial abundance. Lin et al. described gut microbiota characteristics of patients who were first diagnosed with diffuse large B-cell lymphoma (DLBCL), aiming to explore the correlation between immune indicators affecting patients and to clarify the role of the microecosystem-immune axis in the occurrence and development of DLBCL. In the future, it may be possible to improve the immune function of DLBCL patients by regulating the gut microbiota, increasing therapeutic efficacy and improving patient survival rates. Moreover, their study also revealed a correlation between changes in microbiota structure and host immunity, and this research suggests a better understanding of the specific mechanisms underlying the development of differential and dominant microbiota in DLBCL in future studies to identify new disease biomarkers and develop new therapeutic strategies.

This Research Topic also discussed the mechanisms whereby specific gut microbiota can affect the development of diabetes mellitus and tumor immunity; Yan et al. established and validated a diagnosis model based on microbial amplicon sequence variants markers for light chain(AL) amyloidosis in China, and initially explored the important role of gut microbiota in other types of amyloidosis. Their characterization of gut microbial communities in AL amyloidosis patients revealed that alterations in the abundance of certain gut microbial species may play a crucial role in regulating metabolic function and inflammatory responses. They further revealed the association between baseline gut microbiota and disease severity, response to treatment, and prognosis. Subsequently, they suggested that the effect of the gut microbiota on clonal plasma cell proliferation may be the focus of future studies. To further investigate this relationship between gut microbiome, immunity, and complications in diabetics, these authors established a diabetic cornea wound healing model in rodents (Bu et al.). They demonstrated that alterations in microvascular complications such as diabetic keratopathy and immune responses may potentially correlate with alterations in gut microbiota composition in diabetic patients. They were pleasantly surprised to find that these metabolic effects could be transferred to healthy lean individuals using a fecal transplant technique to produce a similar state of insulin resistance. Thus, the gut microbiota may be an important target in the treatment of metabolic syndrome and type 2 diabetes mellitus. The meta-analysis by Yan et al. showed differences in enterobacteriaceae in gestational diabetes mellitus (GDM) and non-GDM populations, and explored inflammation and possible immune mechanisms associated with the pathophysiology of this disease. The results showed specific changes in the composition of the gut microbiota in GDM patients. This suggests that we can provide bacterial targets for the prevention or treatment of GDM by rebuilding homeostasis of the gut microbiota.

The final contribution to this Research Topic centers on the molecular mechanisms whereby bacterial components, metabolites, and other mediators of gut microbiota, interact with the host immune system; Mao et al. used gut microbiota as a target to investigate differences in gut microbiota diversity between diabetic kidney disease (DKD) patients and non-DKD patients. Furthermore, the combined application of metagenomic and metabolomic approaches verified that a large number of metabolites produced by gut microbiota and circulating metabolites are key signaling molecules and substrates involved in metabolic reactions in the progression of DKD. In a surprising result, they confirmed the existence of an enteric-renal axis. The exploration of the molecular mechanism or pathway involved in DKD may be beneficial to the development of individualized prevention and treatment options. It is well known that short-chain fatty acids (SCFAs), a type of saturated fatty acid, are produced by the fermentation of the gut microbiome. Tao et al. discussed the role of gut microbiota imbalance in the pathogenesis of diabetic nephropathy under the influence of SCFAs. At the same time, it may also underlie immune response by gut microbiota imbalance in diabetic patients, which damages the structure and function of the kidney. They also discussed the role of SCFAs in regulating energy metabolism, the inhibition of inflammation, and oxidative stress, and the regulation immune response. Thus, they emphasize that SCFAs may be valuable in the diagnosis and treatment of DKD.

The importance of the gut microbiota is gradually being recognized by the field of microbiology due to the huge diversity

seen in both its quantity and composition, and its ability to regulate metabolic organs. The reviews and original research in the present collection expand our understanding of the role of the gut microbiota and its metabolites as a critical endocrine organ, with a particular focus on the relationship between the gut microbiota and host diseases. We anticipate that future clinical validation of these findings and more detailed stratified analyses will identify novel biomarkers and disease-causing mechanisms that may permit prophylactic and/or therapeutic intervention.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Rapid microbiological diagnosis based on 16S rRNA gene sequencing: A comparison of bacterial composition in diabetic foot infections and contralateral intact skin

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Diabetic foot infections (DFIs) represent a frequent complication of diabetes and a major cause of amputations. This study aimed to evaluate the utility of 16S rRNA gene sequencing for the rapid microbiological diagnosis of DFIs and to consistently characterize the microbiome of chronic diabetic foot ulcers (DFUs) and intact skin. Wound samples were collected by ulcer swabbing and tissue biopsy, and paired swabs of intact skin were collected from 10 patients with DFIs (five were moderately infected, and the other five were severely infected). Samples were analyzed by conventional culture and using Personal Genome Machine (PGM) 16S rRNA sequencing technology. The results showed that PGM technology detected significantly more bacterial genera (66.1 vs. 1.5 per wound sample, p <0.001); more obligate anaerobes (52.5 vs. 0%, p <0.001) and more polymicrobial infections (100.0 vs. 55.0%, p <0.01) than conventional cultures. There was no statistically significant difference in bacterial richness, diversity or composition between the wound swabs and tissues (p >0.05). The bacterial community on intact skin was significantly more diverse than that in DFUs (Chao1 value, p <0.05; Shannon index value, p <0.001). Gram-positive bacteria (67.6%) and aerobes (59.2%) were predominant in contralateral intact skin, while Gram-negative bacteria (63.3%) and obligate anaerobes (50.6%) were the most ubiquitous in DFUs. The most differentially abundant taxon in skin was *Bacillales*, while *Bacteroidia* was the bacterial taxon most representative of DFUs. Moreover, *Fusobacterium* (ρ=0.80, p <0.01) and *Proteus* (ρ=0.78, p <0.01) were significantly correlated with the duration of DFIs. In conclusion, PGM 16S rRNA sequencing technology could be a potentially useful technique for the rapid microbiological diagnosis of DFIs. Wound swabbing may be sufficient for sampling bacterial pathogens in DFIs compared with biopsy which is an invasive technique. The empirical use of broad-spectrum antibiotics covering Gram-negative obligate anaerobes should be considered for the treatment of moderate or severe DFIs.

KEYWORDS

16S rRNA gene sequencing, personal genome machine, diabetic foot infections, anaerobes, tissue biopsy, swab

Introduction

An estimated 537 million adults aged 20–79 years worldwide have diabetes, including 140.9 million adults in China (Sun et al., 2022). Diabetic foot ulcers (DFUs) represent a serious complication of diabetes, with a reported annual incidence of 1.5–16.6% (roughly 8.3% in China) and an estimated lifetime incidence of 15–25% (Singh et al., 2005; Jiang et al., 2015; Zhang et al., 2017). It is estimated that every 20 s, a lower limb is amputated due to diabetes somewhere in the world, and nearly 90% of these amputations occur in association with infections (Lavery et al., 2006, 2007; Bakker et al., 2016). Consequently, causative organisms must be reliably diagnosed and promptly controlled. Nevertheless, existing clinical microbiology methods lack the capacity to rapidly and comprehensively diagnose complex pathogenic microorganisms in DFUs.

Traditionally, the composition of the wound microbiota has been defined using culture-based methods. However, as previous studies have indicated, conventional culture suffers from several limitations: it is time consuming (requiring 3–5 days on average), only approximately 2% of all known bacteria can be cultured in the laboratory and culture-based techniques may not necessarily reveal the most abundant or clinically important organisms *in vivo* (Rowan, 2004; Forward, 2006; Petti et al., 2006a; Dowd et al., 2008; Grice and Segre, 2011; Tuttle et al., 2011; Dunyach-Remy et al., 2014). The development of molecular techniques to identify and quantify microbial organisms has revolutionized our view of the microbial world. 16S rRNA gene sequencing may provide more definitive taxonomic classification than culture-based approaches for many organisms while also proving less time consuming and labor intensive (Petti et al., 2006b; Salipante et al., 2013). Ion Torrent Personal Genome Machine (PGM) sequencing is a cost effective and time saving technique. It has been demonstrated to be sufficiently rapid and accurate for bacterial species identification from cystic fibrosis sputum samples and periodontitis saliva samples (Sebastian et al., 2012; Salipante et al., 2013). However, the PGM technique has not yet been applied to diabetic foot infections (DFIs). Therefore, we explored the feasibility of using PGM technology for the rapid diagnosis of bacterial pathogens in infected diabetic foot wounds.

In addition, the reliability of different sampling techniques for the microbiological diagnosis of DFIs has been disputed (Pellizzer et al., 2001; Gjødsbøl et al., 2011; Lipsky et al., 2013). Most researchers consider tissue biopsy to be the most reliable sampling technique for the identification of pathogens in DFIs, but swabbing is more widely applied in clinical practice because it is easy to perform and noninvasive (Bowler et al., 2001; Gjødsbøl et al., 2011; Lipsky et al., 2013, 2020). Thus, we compared swab and tissue specimens for the microbiological diagnosis of DFIs to evaluate the necessity of performing biopsies.

Furthermore, previous studies have indicated that skin supports the growth of commensal bacteria, which directly and indirectly protect hosts from pathogenic bacteria (Chiller et al., 2001; Cogen et al., 2008). As an attempt to find a novel target for microecological prevention and to consistently characterize the microbiome of chronic DFUs, the differences in bacterial community composition between wounds and intact skin were analyzed. Moreover, Angela Oates et al. proposed that contralateral intact skin samples may provide insight into the microbial composition of skin prior to wounding because of high levels of conservation between contralateral skin sites within individuals (Oates et al., 2012). Based on this, correlation analysis of the microbiome between DFUs and contralateral intact skin was performed within individuals to provide insights into whether DFUs are vulnerable to opportunistic pathogens from skin prior to wounding.

Materials and methods

Patients

A total of 10 patients with DFIs were recruited for this study. All patients agreed to participate in this study and provided written consent. The study inclusion criteria were as follows: (1) a current or previous diagnosis of type 2 diabetes; (2) age $\geq$ 18 years; (3) the presence of a foot ulcer, which is defined as a break in the skin of the foot that involves at minimum the epidermis and part of the dermis (Van Netten et al., 2020); and (4) clinically infected DFUs, which were diagnosed based on the presence of at least two of the following symptoms: local swelling or induration, >0.5 cm of erythema around the wound, local tenderness or pain, local warmth, and purulent discharge. The severity of DFIs was graded according to the infection part of the PEDIS classification proposed by the IWGDF (Lipsky et al., 2020; Monteiro-Soares et al., 2020): grade 1 wounds were uninfected; grade 2 wounds were mildly infected, involving only the skin or subcutaneous tissue, and any erythema present extended <2 cm around the wound; grade 3 lesions were moderately infected, involving erythema extending $\geq$2 cm from the wound margin, and/or tissue deeper than the skin and subcutaneous tissues (e.g., bone, joint, tendon, and muscle); and grade 4 wounds were severely infected, including any foot infection with systemic inflammatory response syndrome. The study exclusion criteria were as follows: (1) receiving systemic or topical antimicrobials 2 weeks prior to this study; and (2) refusal or inability to tolerate debridement, e.g.,

those with severe coagulopathy, peripheral artery disease, or cardiopulmonary insufficiency.

Sample collection

No antimicrobial agent (e.g., alcohol or iodine) or antiseptic was introduced into the wound before specimen collection. After debridement, two specimens were collected from the same area of each wound by swabbing the wound with the Levine technique (Rondas et al., 2013; S group, labeled S1, S2…S9, S10) and by taking a deep tissue biopsy (T group, labeled T1, T2…T9, T10). The Levine method involves rotating the swab over a 1 cm^2 area of the wound for 5 s, and applying sufficient pressure to exude and collect fluid from the tissue onto the swab. Meanwhile, the area of intact contralateral skin was sampled by swabbing the skin with a cotton swab that was moistened with sterile saline (Oates et al., 2012; C group, labeled C1, C2…C9, C10). All samples were placed into sterile transport containers and transported to the laboratory within 30 min. One swab and one biopsy from the same wound were aerobically and anaerobically cultured using standard procedures. The remaining samples were frozen at −80°C until DNA extraction was performed.

DNA extraction and PCR amplification of 16S rRNA genes

Genomic DNA was extracted from tissue and swab samples by using the QIAamp DNA Mini Kit (Qiagen). The V1–V2 hypervariable region of the 16S rRNA gene was amplified as described in the literature (Noah et al., 2008; Anne Han et al., 2011; Salipante et al., 2013). The PCR products were run on an agarose gel and purified using the AxyPrepDNA Gel Extraction Kit (Axygen). After being extracted from the gel and having their presence confirmed by further agarose gel electrophoresis, the products were quantified. Equal quantities of all samples were pooled for sequencing. Emulsion PCR was performed using the Ion Xpress Template kit V2.0 (Life Technologies) according to the instructions described in the user guide provided by the manufacturer (Sebastian et al., 2012).

Sequencing data processing

Sequencing was carried out on the Ion Torrent PGM system (Life Technologies) using 318 chips (Salipante et al., 2013). After sequencing, the data were optimized and denoised. The resulting sequences met the following criteria: (1) contained the reverse primer and the barcode; (2) ≥ 150 bp in length; (3) no ambiguous bases; (4) homopolymers <8 bp; and (5) average quality score > 25 (Wang et al., 2015). Subsequently, we clustered the sequences into 97% similarity operational taxonomic units (OTUs) as a basis for further analysis. Finally, each processed 16S rRNA gene sequence was aligned with the SILVA rRNA database, to identify the most similar bacterial taxon with more than 80% confidence.

Statistical analysis

Variables were compared using Student's *t*-test (to compare normally distributed quantitative variables between two groups), one-way ANOVA (to compare normally distributed quantitative variables among three groups), or the χ^2 test (to compare categorical variables). Spearman correlation analysis was used to analyze the correlations between the genera and clinical indicators of DFI patients. A p value of 5% was considered statistically significant ($p < 0.05$).

Results

Clinical data and conventional culture results

Ten patients (seven males and three females) with DFIs were enrolled in this study. Among the 10 DFUs, 5 (50%) were moderately infected, and the other 5 (50%) were severely infected. A total of 14 isolates from six genera were cultured from wound swabs, while 17 isolates from 10 genera were cultured from deep tissue specimens. The most frequently occurring genera in swab specimens and tissue specimens were *Streptococcus* (28.6%) and *Enterococcus* (23.5%), respectively. There were no obligate anaerobes (Table 1).

Composition of bacterial communities determined by 16S rRNA sequencing

The dominant genera that had a relative abundance >1% are indicated in Figure 1. In contralateral intact skin, Gram-positive bacteria and aerobes were predominant, accounting for 67.6 and 59.2%, respectively. In diabetic foot wounds, Gram-negative bacteria (56.8% in the S Group, 63.3% in the T Group) and obligate anaerobes (54.3% in the S Group, 50.6% in the T Group) were the most ubiquitous. No significant differences in the bacterial composition of DFUs or skin were observed between females and males [permutational multivariate analysis of variance (PERMANOVA), $p > 0.05$].

Staphylococcus had the greatest relative abundance in contralateral intact skin (41.3%), followed by *Corynebacterium* (16.5%), *Stenotrophomonas* (6.1%), and *Kocuria* (6.0%). In diabetic foot wounds, *Prevotella* (15.5, 11.7%) was the most abundant genus in both swab and deep tissue samples, followed by *Corynebacterium* (11.9, 11.6%), *Bacteroides* (10.0, 10.9%), and *Stenotrophomonas* (6.4, 7.6%). All wound samples showed polymicrobial infections using 16S rRNA sequencing, and Gram-negative obligate anaerobes (including *Prevotella*, *Bacteroides*, *Fusobacterium*, and *Porphyromonas*) predominated in 70% of wound swabs and 50% of wound tissue samples.

TABLE 1 Clinical data and conventional culture results.

Characteristics of patients and wounds						Conventional culture	
Patients	**Age**	**Gender**	**Diabetes duration (years)**	**Ulcer duration (days)**	**PEDIS Grade**	**Swab (S Group)**	**Tissue (T Group)**
1	65	Female	7	90	3	*Streptococcus anginosus*	*Stenotrophomonas maltophilia* *Staphylococcus aureus* *Enterococcus faecalis*
2	72	Male	16	30	3	*Staphylococcus haemolyticus* *Streptococcus agalactiae*	*Enterococcus faecalis*
3	43	Male	3	30	4	*Corynebacterium*	*Corynebacterium* *Serratia marcescens*
4	39	Male	1	30	4	*Enterobacter aerogenes*	*Enterobacter aerogenes* *Citrobacter freundii*
5	68	Male	10	30	3	*Corynebacterium striatum* *Enterococcus faecalis*	*Staphylococcus haemolyticus* *Morganella morganii*
6	53	Male	8	60	3	Negative	Negative
7	62	Male	16	120	4	*Staphylococcus aureus* *Proteus mirabilis*	*Escherichia coli* *Proteus mirabilis*
8	62	Female	0.5	10	4	*Enterococcus avium* *Streptococcus agalactiae*	*Enterococcus avium* *Enterococcus faecalis* *Escherichia coli*
9	70	Female	8	30	4	*Staphylococcus aureus*	*Staphylococcus aureus*
10	52	Male	10	90	3	*Proteus vulgaris* *Streptococcus agalactiae*	*Proteus vulgaris*

Comparison of microbiological diagnosis between conventional culture and 16S rRNA sequencing.

16S rRNA sequencing technology detected significantly more bacterial genera (an average of 66.1 vs. 1.5 per wound sample, $p < 0.001$), more obligate anaerobes (52.5 vs. 0%, $p < 0.001$), more Gram-negative bacteria (60.0 vs. 38.7%, $p < 0.05$), and more polymicrobial infections (100.0 vs. 55.0%, $p < 0.01$) than traditional bacterial culture (Figure 2). The sequencing results contained 93.5% of genera identified by conventional culture in this study. However, 70% of the most dominant pathogens (highest abundance) diagnosed by 16S rRNA sequencing were in disagreement with conventional culture results. In addition, the bacterial culture result for DFI was negative in Patient 6, but 16S rRNA sequencing found that *Bacteroides* (Gram-negative obligate anaerobe) was dominant.

Concordance between wound swabs and tissues analyzed by 16S rRNA sequencing

There was no significant difference in bacterial richness or diversity between the S and T groups at the number of observed genera, Chao1 value or Shannon index value ($p > 0.05$). Subsequently, principal coordinate analysis indicated that wound swabs and tissues had similar bacterial communities (PERMANOVA, $p > 0.05$; Figure 3). Hierarchical clustering also showed that the compositions of the samples from the S Group and T Group from the same patient were similar, with noticeably closer evolutionary relationships than samples from separate patients (Figure 1). A subsequent correlation analysis revealed strong similarity between wound swabs and tissue samples within an individual, which was significantly higher than the similarity between individuals (the average correlation coefficient was 0.84 vs. 0.19, $p < 0.001$; Figure 4).

Microbiome correlation between skin and DFUs

According to the hierarchical clustering dendrogram, there was no strong correlation of the microbiome between healthy skin and wounds within an individual in 80% of the patients; the exceptions were patients 3 and 9 (Figure 1). A subsequent correlation analysis (Figure 4) indicated significant intra-individual positive correlations between skin and DFIs in patients 3 and 9 (correlation coefficients of 0.74 and 0.95, respectively), while weak positive correlations between the paired skin and wounds were observed in the other patients (mean: 0.18). The average correlation coefficient of the C and T groups within an individual was 0.31, whereas the correlation between individuals was 0.22; the difference was not statistically significant ($p > 0.05$).

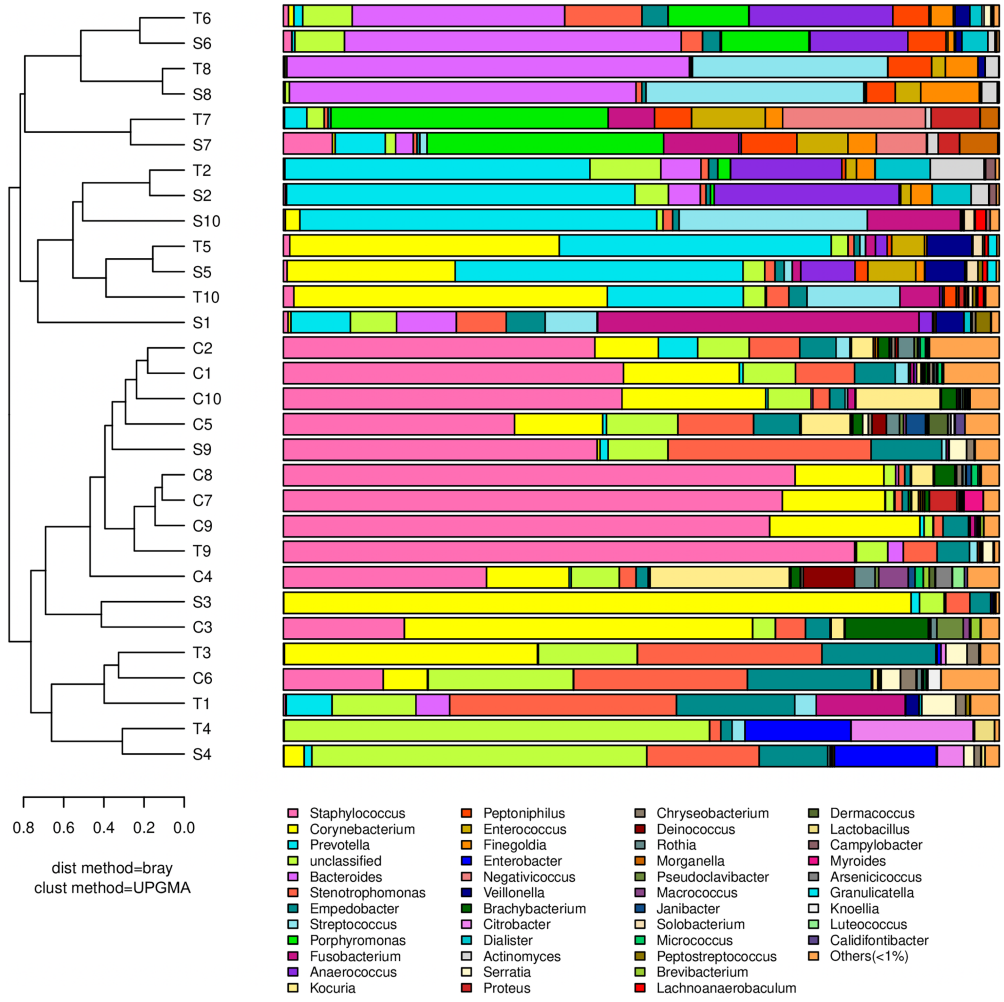

FIGURE 1
A dendrogram and histogram of the bacterial composition at the genus level. The dendrograms were constructed based on the unweighted pair group method with the arithmetic mean (UPGMA). The bacterial compositions of samples are more similar to each other with closer evolutionary relationships. The histogram visualizes the relative abundance of each sample at the genus level.

Differences in the microbiome between DFUs and intact skin by using 16S rRNA sequencing

The bacterial richness and diversity of contralateral skin were significantly higher than those of diabetic foot wounds (including the S group and T group) in terms of the number of observed genera ($p < 0.001$), Chao1 value ($p < 0.05$), and Shannon index value ($p < 0.001$). The principal coordinate analysis indicated a significant difference in bacterial communities between diabetic foot wounds and skin (PERMANOVA, $p < 0.01$; Figure 3).

Linear discriminant analysis (LDA) effect size (LEfSe) was used to describe the effect sizes of differences in the microbiota composition between intact skin and diabetic foot wounds (Figure 5). At the phylum level, *Bacteroidetes* (LDA score = 4.05, $p < 0.01$) was enriched in DFIs, whereas *Actinobacteria* (LDA score = 3.91, $p < 0.05$) was mostly enriched in intact skin. At the genus level, no taxa in diabetic foot wounds reached the minimum LDA score, while *Staphylococcus* (LDA score = 4.21, $p < 0.01$), *Kocuria* (LDA score = 3.41, $p < 0.001$), and *Brachybacterium* (LDA score = 2.98, $p < 0.001$) were the most differentially abundant bacterial taxa in intact skin.

Correlations between genera in DFI wounds and clinical parameters

Prevotella ($\rho = 0.80$, $p < 0.01$) and *Porphyromonas* ($\rho = 0.76$, $p < 0.05$) were significantly correlated with the duration of diabetes (DM_y). *Fusobacterium* ($\rho = 0.80$, $p < 0.01$) and *Proteus* ($\rho = 0.78$, $p < 0.01$) were significantly correlated with the duration of DFIs (DFI_d). *Rothia* was positively correlated with the PEDIS grade, and the genera with the greatest negative correlations with PEDIS grade were *Lachnoanaerobaculum* ($\rho = 0.83$, $p < 0.01$) and *Prevotella* ($\rho = 0.80$, $p < 0.01$; Figure 6).

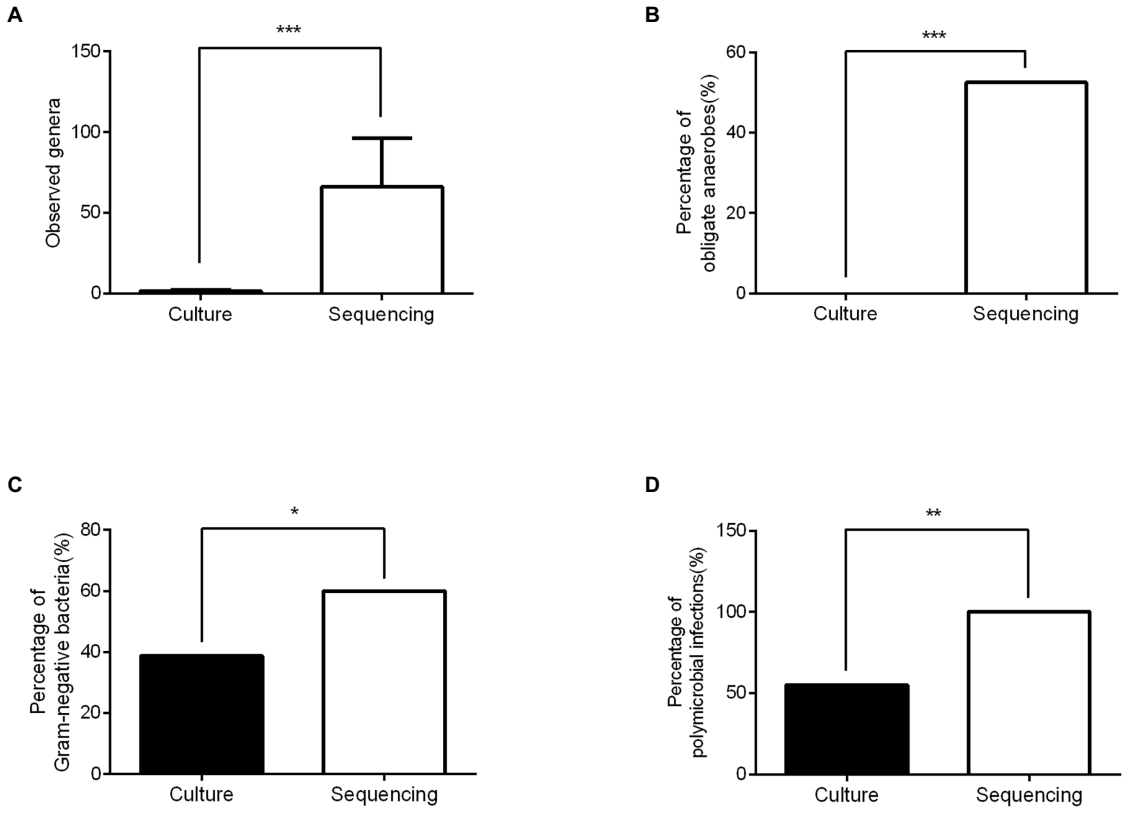

FIGURE 2
Comparison of the microbiological diagnosis between conventional culture and 16SrRNA sequencing. **(A)** The numbers of genera identified in the sequencing group were significantly higher than those in the culture group. **(B)** Significantly more obligate anaerobes were detected in the sequencing group than in the culture group. **(C)** The percentage of Gram-negative bacteria in the sequencing group was higher than that in the culture group. **(D)** More polymicrobial infections were detected in the sequencing group than in the culture group. ***p <0.001, ** p <0.01, and *p <0.05.

Discussion

A rapid and accurate technique for identifying pathogenic bacteria from infected diabetic foot wounds would be of great value in helping clinicians quickly select targeted antibiotic treatments. The results of our study highlight PGM sequencing as a promising tool for the bacteriological diagnosis of DFIs. By comparing the results of PGM sequencing and conventional culture, we identified the following advantages of 16S rRNA sequencing for identifying pathogenic bacteria in DFIs: (1) fast speed (2.3–7.3 h of running time, total process <24 h) leads to rapid diagnosis of the bacterial community, which enables the informed clinical selection of targeted antibiotics in a timely manner. (2) 16S rRNA sequencing technology detected significantly more bacterial genera and more polymicrobial infections than conventional culture. Cultures may greatly underestimate the diversity and richness of the microbiome in DFIs. (3) PGM technology is not restricted by culture conditions and can identify bacteria that have fastidious requirements for their growth environment. In this study, 16S rRNA sequencing detected that Gram-negative bacteria and obligate anaerobes were predominant in DFIs. However, the prevalence of anaerobes may

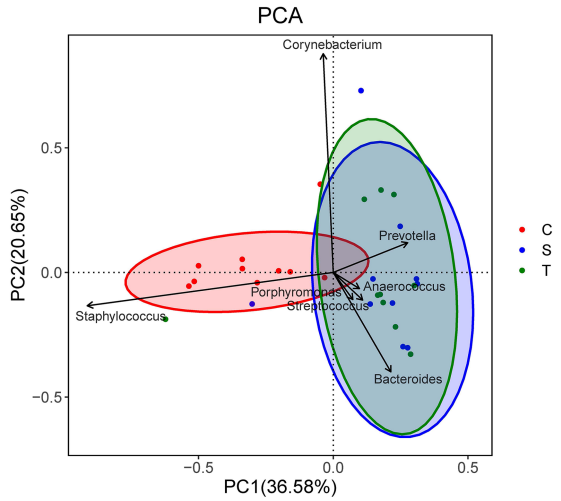

FIGURE 3
Principal component analysis (PCA) of the three groups. Samples that are more similar to each other should appear closer together along the corresponding axis reflecting the variation among all samples. Coordinates for the genera arrows are correlations with each axis, and only the seven most important (based on the magnitude of correlations) genera are shown.

be underrepresented by conventional culturing, while overestimating the abundance of Gram-positive aerobes (such as *Staphylococci*, *Streptococcus,* etc.) which grow more easily in ordinary culture medium. (4) Theoretically, 16S rRNA sequencing may better reflect the true bacterial community of the samples *in vivo* than traditional culture. Some bacteria grow more rapidly than others during the culture procedure and may come to dominate the composition of the culture; thus, the results of culture may not reflect the true bacterial composition within the sample (Moon et al., 2021). In contrast, 16S rRNA sequencing identifies the pathogenic bacteria of DFIs by processing and analyzing the genomic DNA of samples directly. Thus, the sequencing results can be largely protected from influence by the *in vitro* environment. This may be one of the reasons for the low consistency of microbiological results between conventional culture and 16S rRNA sequencing. Based on these results, PGM 16S rRNA gene sequencing may be able to increase the reliability and speed of identification of bacterial communities in wounds to provide a basis for timely and efficient antibiotic selection for patients with DFIs.

The International Working Group has proposed that clinicians should obtain cultures from a tissue specimen rather than from a swab for DFIs (Bowler et al., 2001; Lipsky et al., 2020). However, some researchers have proposed that it is sufficient to use swabbing instead of the more invasive procedure of tissue biopsy to identify pathogens, as swabs can recover high relative abundances of known and potential genera (Gjødsbøl et al., 2011; Travis et al., 2020). In our study, the sequencing results showed no significant differences in the richness, diversity, or composition of bacterial communities between the two different specimen types. The results were consistent with those of some early studies in DFUs and other chronic wounds (Pellizzer et al., 2001; Gjødsbøl et al., 2011). However, other studies showed that the quantity of pathogens or composition of bacterial communities identified by swabbing significantly differ from those obtained by tissue biopsy in DFUs (Dunyach-Remy et al., 2014; Nelson et al., 2016; Travis et al., 2020). This discrepancy might be related to the cleansing or debridement step performed before specimen collection and the Levine swabbing technique used in our study, which may reduce superficial contamination and facilitate access to exudate fluid from deeper in the wound bed by swabbing.

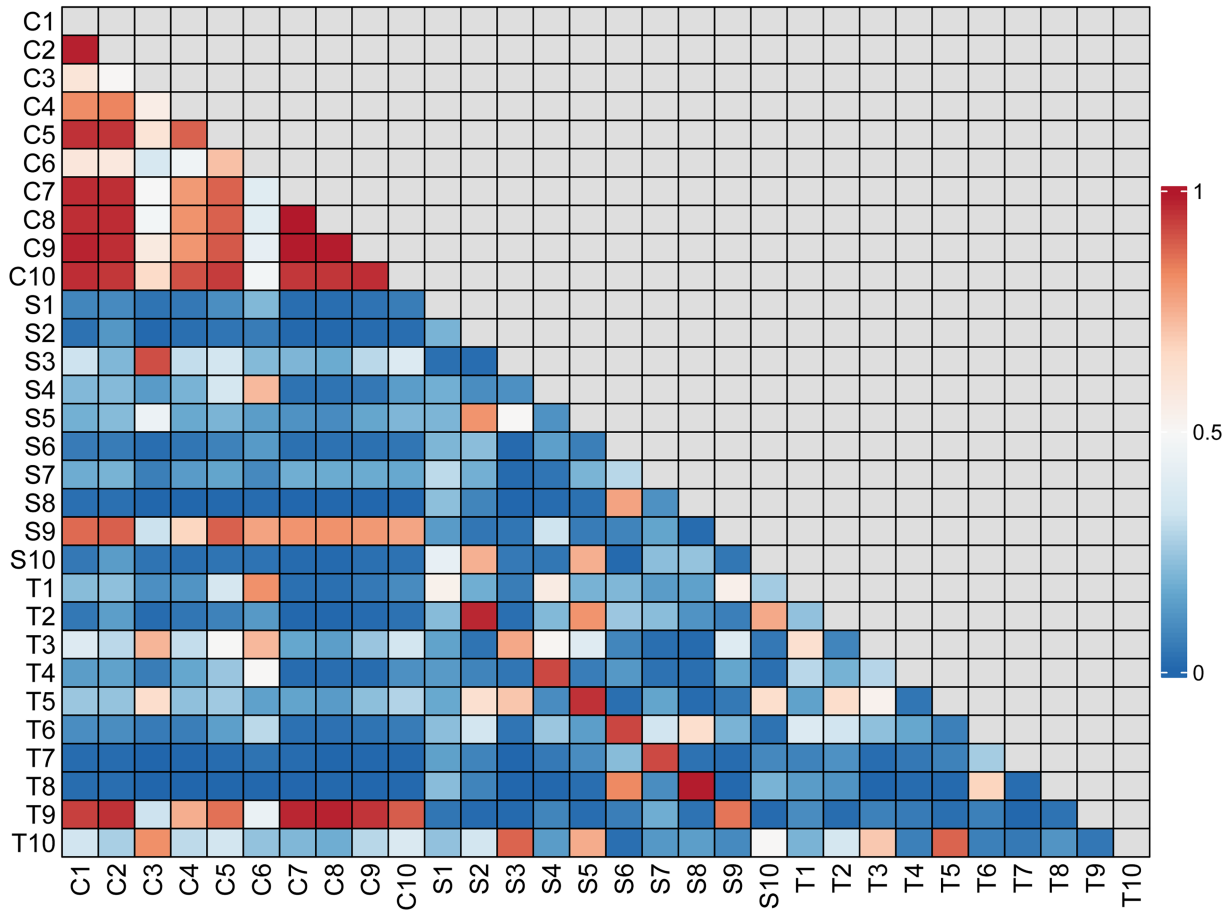

FIGURE 4
Correlation matrix for all samples. The correlation coefficient values are indicated by different shades of color according to the scale to the right of the matrix. The scale ranges from 0 to 1, where 1 represents the most similar samples.

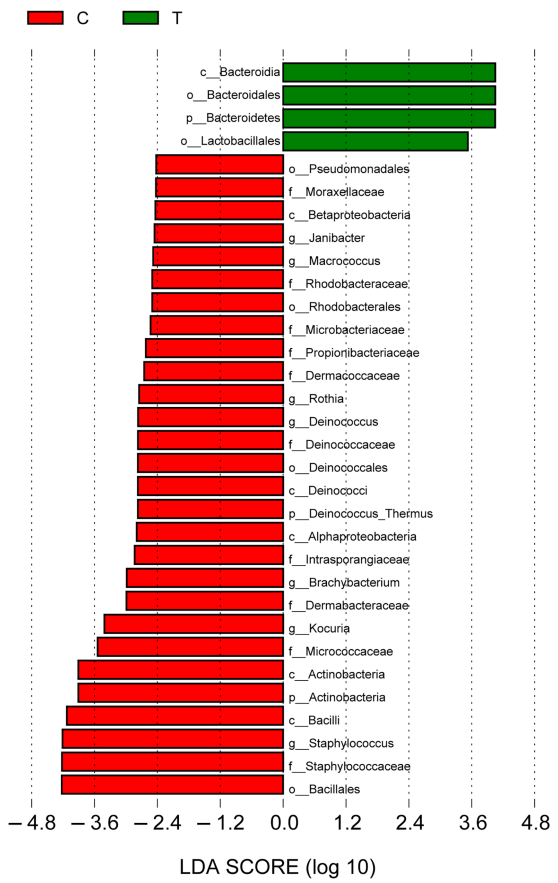

FIGURE 5
Linear discriminant analysis (LDA) effect size results for the bacterial communities of intact skin and wounds. Linear discriminant analysis indicating differentially abundant taxa between skin and wounds. Only taxa with an LDA score>2.4 and p<0.05 are shown. p_: phylum, c_: class, o_: order, f_: family, and g_: genus.

In addition, the distribution of organisms within the wounds is patchy, which may lead to different results of bacterial communities between swab and tissue samples. In the present study, we collected swab samples and paired tissue biopsy samples from the same area of each wound, while J. Travis et al. collected tissue samples from an unswabbed area (Travis et al., 2020), and Frank et al. collected swab samples covering a greater wound surface area than the tissue biopsy (Frank et al., 2009). Biopsy to obtain tissue specimens is more invasive than swabbing and may cause damage to surrounding tissues and blood vessels. In addition, biopsy requires a skilled clinician and usually needs to be performed under local anesthesia (Gjødsbøl et al., 2011; Travis et al., 2020). Our study showed that by ulcer swabbing using the Levine technique, which is less invasive and easier to perform than biopsy, followed by 16S rRNA gene sequencing may be suitable for sampling bacterial pathogens in DFIs.

In this study, 16S rRNA gene sequencing results indicated that Gram-positive bacteria and aerobes were predominant in the intact skin contralateral to DFUs, while Gram-negative bacteria and obligate anaerobes were the most ubiquitous in infected diabetic foot wounds. Some earlier studies have also shown that anaerobic bacteria are the most prevalent pathogens in chronic wounds (Urbancic-Rovan and Gubina, 2000; Howell-Jones et al., 2005; Dowd et al., 2008). At the genus level, *Staphylococcus*, *Corynebacterium*, *Stenotrophomonas*, and *Kocuria* had the greatest relative abundance in skin, while *Prevotella*, *Corynebacterium*, *Bacteroides*, and *Stenotrophomonas* were the most abundant genera in DFUs. Notably, there are some dominant genera (such as *Corynebacterium*, *Stenotrophomonas*, etc.) shared between contralateral skin and wounds within an individual; these genera are believed to be opportunistic pathogens from skin. Further analysis indicated that patient 3 and patient 9, who were infected predominantly with *Corynebacterium* and *Staphylococcus,* respectively, showed strong intra-individual positive correlations between skin and DFI microbiome composition, reflecting the principle that opportunistic pathogens from skin sometimes may result in DFIs. However, the correlation coefficients of paired and unpaired skin/wound samples were not significantly different; thus, it cannot be concluded that an individual wound should be more similar to the corresponding skin than to any other DFI patient's skin. This may be due to the high similarity of skin microbiomes between individuals with DFIs. Further research should be performed to compare the microbiomes of intact foot skin among people with diabetes, people with DFIs, and healthy people to better understand the correlation of the microbiome between DFIs and corresponding skin. Our study suggests that clinicians should focus on treatments that target Gram-negative obligate anaerobes and opportunistic pathogens in moderate or severe DFIs when selecting an empirical antibiotic therapy.

Our study also found that skin had significantly higher bacterial richness and diversity than diabetic foot wounds, indicating that the microecological balance of skin would be destroyed and that pathogenic bacteria would predominate instead of resident flora when the skin of diabetic patients had an ulcer. These results were consistent with the results reported by Scot E. Dowd et al. (Gontcharova et al., 2009). LEfSe analyses were performed, emphasizing statistical significance, biological consistency, and effect relevance (Segata et al., 2011; Pang et al., 2020), with the aim of identifying differential biomarkers that explain most of the effect differentiating phenotypes of DFIs and intact skin. The results demonstrated that *Bacteroidia*, *Bacteroidales*, *Bacteroidetes*, and *Lactobacillales* represented bacterial taxa in diabetic foot wounds, but no taxa at the family or genus level were consistently present because of the dispersive distribution of bacterial families and genera in DFIs. Further correlation analysis indicated that *Prevotella* and *Porphyromonas* were positively correlated with the duration of diabetes. *Fusobacterium* and *Proteus* were positively correlated with the duration of DFIs. The results were partially consistent with a previous study, reporting that the DFU duration was positively correlated with *Proteobacteria*, and the ulcer depth was associated with the abundance of anaerobic bacteria (Gardner et al., 2013).

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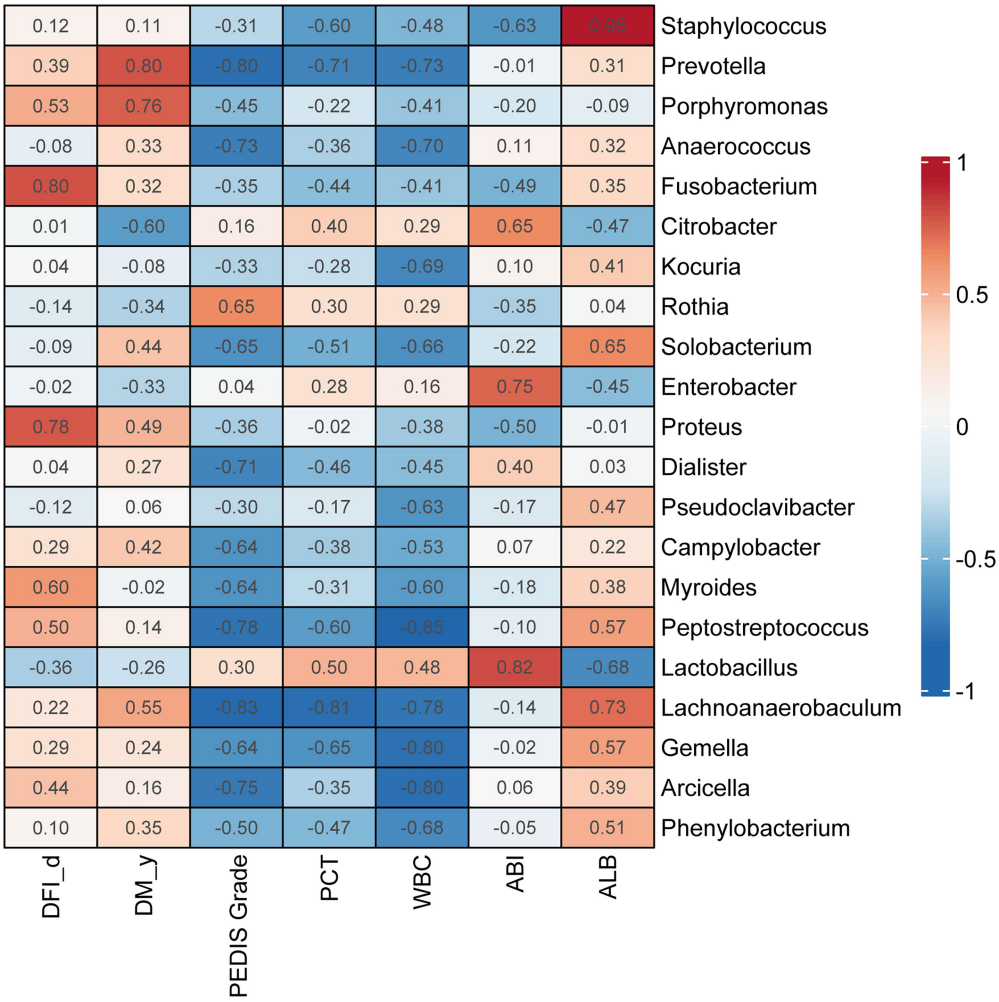

FIGURE 6
Correlations between genera in DFI wounds and clinical parameters. Correlation coefficients are marked in the heatmap. Red indicates a positive correlation with each index, and blue indicates a negative correlation with each index. DM _y: duration of diabetes (years), DFI _d: duration of diabetic foot infection (days).

These results further emphasized the importance of performing anaerobic culture and using broad-spectrum empirical antibiotics covering anaerobes and *Proteus* for patients with chronic DFIs, which is easily ignored in clinical practice. In the present study, all samples were aerobically and anaerobically cultured. However, we did not use a special anaerobic container to transport the swab or tissue specimens in our hospital, which may be one potential explanation for the negative results of aerobic culture. On the other hand, the most representative bacterial taxa in intact skin included *Bacillales*, *Staphylococcaceae*, *Staphylococcus*, *Bacilli*, and *Actinobacteria*. Among them, previous research (Grice and Segre, 2011) has shown that *Staphylococcus epidermidis* (belonging to *Staphylococcus*, *Staphylococcaceae*, *Bacillales*, *Bacilli*, and *Firmicutes*) can bind keratinocyte receptors and inhibit the adherence of virulent *Staphylococcus aureus*. *Propionibacterium acnes* (belonging to *Propionibacterium*, *Propionibacteriaceae*, and *Actinobacteria*) can release fatty acids from lipid breakdown, acidifying the milieu, and inhibiting the growth of *Streptococcus pyogenes*. These symbiotic bacteria exist on the skin surface to maintain microecological balance, indicating that maintaining microecological balance may be an important way to prevent the occurrence of skin ulcers in diabetes. Moreover, there may be some "probiotics" that could promote wound healing at the skin surface. However, further study is required to prove this speculation.

In summary, PGM 16S rRNA sequencing technology is less time-consuming and a potential technique for the rapid microbiological diagnosis of DFIs. Ulcer swabbing which is relatively noninvasive and easy to perform, may be a suitable method for sampling bacterial pathogens in DFIs using the Levine technique, followed by 16S rRNA sequencing. Moreover, Gram-negative obligate anaerobes play a crucial role in DFIs, and opportunistic pathogens from the skin can lead to wound infections. The empirical use of broad-spectrum antibiotics

covering Gram-negative obligate anaerobes should be considered for the treatment of moderate or severe DFIs.

Limitations

The major limitations of this study include the small number of included patients and the fact that all DFUs were classified as PEDIS grade 3 or 4. Because of these limitations, our study did not analyze the bacterial communities in wounds at varying depths. In addition, some defects in PGM 16S rRNA gene sequencing technology have been noted: (1) potential bias in estimates of bacterial diversity may exist because many copies of the 16S rRNA gene are present in some species; (2) the selection of the 16S rRNA gene amplification area may affect the identification of bacterial species; and (3) the specific information at the species level cannot be obtained accurately because of the limited information available from selective amplification of the target gene fragment, and bacteria were only able to be identified to the genus level in this study. Despite the shortcomings of 16S rRNA gene sequencing technology, this technology has great potential for clinical microbiological diagnosis and research on microbial diversity with the development of new molecular biological techniques.

Data availability statement

Raw sequence reads for this project have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA862325.

Ethics statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Nanfang Hospital (No. NFEC-2015-104). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YH, ZX, and YC contributed equally to this study. YH performed the laboratory experiments and was responsible for writing the original draft. YC and ZX analyzed the data and reviewed the initial draft. MZ and YX performed the literature search and designed the study. YJ and XL collected the specimens. FG and YF designed the study and provided a critical review of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Anne Han, M. D., Zenilman, J. M., Ms, J. H. M., Shirtliff, M. E., Agostinho, A., James, G., et al. (2011). The importance of a multifaceted approach to characterizing the microbial flora of chronic wounds. *Wound Repair Regen.* 19, 532–541. doi: 10.1111/j.1524-475X.2011.00720.x

Bakker, K., Apelqvist, J., Lipsky, B. A., and Van Netten, J. J. (2016). The 2015 IWGDF guidance documents on prevention and management of foot problems in diabetes: development of an evidence-based global consensus. *Diabetes Metab. Res. Rev.* 32, 2–6. doi: 10.1002/dmrr.2694

Bowler, P. G., Duerden, B. I., and Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clin. Microbiol. Rev.* 14, 244–269. doi: 10.1128/CMR.14.2.244-269.2001

Chiller, K., Selkin, B. A., and Murakawa, G. J. (2001). Skin microflora and bacterial infections of the skin. *J. Investig. Dermatol. Symp. Proc.* 6, 170–174. doi: 10.1046/j.0022-202x.2001.00043.x

Cogen, A. L., Nizet, V., and Gallo, R. L. (2008). Skin microbiota: a source of disease or defence? *Br. J. Dermatol.* 158, 442–455. doi: 10.1111/j.1365-2133.2008.08437.x

Dowd, S. E., Sun, Y., Secor, P. R., Rhoads, D. D., Wolcott, B. M., James, G. A., et al. (2008). Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol 8:43. *BMC Microbiol.* 8:43. doi: 10.1186/1471-2180-8-43

Dunyach-Remy, C., Cadiere, A., Richard, J. L., Schuldiner, S., Bayle, S., Roig, B., et al. (2014). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE): a promising tool to diagnose bacterial infections in diabetic foot ulcers. *Diabetes Metab.* 40, 476–480. doi: 10.1016/j.diabet.2014.03.002

Forward, K. R. (2006). An evaluation of extended incubation time with blind subculture of blood cultures in patients with suspected endocarditis. *Canad. J. Infect. Dis. Med. Microbiol.* 17, 186–188. doi: 10.1155/2006/284019

Frank, D. N., Wysocki, A., Specht-Glick, D. D., Rooney, A., Feldman, R. A., St, A. A., et al. (2009). Microbial diversity in chronic open wounds. *Wound Repair Regen.* 17, 163–172. doi: 10.1111/j.1524-475X.2009.00472.x.doi:10.1111/j.1524-475x.2009.00472.x

Gardner, S. E., Hillis, S. L., Heilmann, K., Segre, J. A., and Grice, E. A. (2013). The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. *Diabetes* 62, 923–930. doi: 10.2337/db12-0771

Gjødsbøl, K., Skindersoe, M. E., Christensen, J. J., Karlsmark, T., Bo, J., Jensen, A. M., et al. (2011). No need for biopsies: comparison of three sample

techniques for wound microbiota determination. *Int. Wound J.* 9, 295–302. doi: 10.1111/j.1742-481X.2011.00883.x

Gontcharova, V., Youn, E., Sun, Y., Wolcott, R. D., and Dowd, S. E. (2009). A comparison of bacterial composition in diabetic ulcers and contralateral intact skin. *Open Microbiol. J.* 4, 8–19. doi: 10.2174/1874285801004010008

Grice, E. A. and Segre, J. A. (2011). The skin microbiome. *Nat. Rev. Microbiol.* 9, 244–253. doi: 10.1038/nrmicro2537

Howell-Jones, R. S., Wilson, M. J., Hill, K. E., Howard, A. J., Price, P. E., and Thomas, D. W. (2005). A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. *J. Antimicrob. Chemother.* 55, 143–149. doi: 10.1093/jac/dkh513

Jiang, Y., Wang, X., Xia, L., Fu, X., Xu, Z., Ran, X., et al. (2015). A cohort study of diabetic patients and diabetic foot ulceration patients in China. *Wound Repair Regen.* 23, 222–230. doi: 10.1111/wrr.12263

Lavery, L. A., Armstrong, D. G., Murdoch, D. P., Peters, E. J., and Lipsky, B. A. (2007). Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. *Clin. Infect. Dis.* 44, 562–565. doi: 10.1086/511036

Lavery, L. A., Armstrong, D. G., Wunderlich, R. P., Mohler, M. J., Wendel, C. S., and Lipsky, B. A. (2006). Risk factors for foot infections in individuals with diabetes. *Diabetes Care* 29, 1288–1293. doi: 10.2337/dc05-2425

Lipsky, B. A., Berendt, A. R., Cornia, P. B., Pile, J. C., Peters, E. J., Armstrong, D. G., et al. (2013). 2012 infectious diseases society of america clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *J. Am. Podiatr. Med. Assoc.* 103, 2–7. doi: 10.1093/cid/cis346

Lipsky, B. A., Senneville, É., Abbas, Z. G., Aragón-Sánchez, J., Diggle, M., Embil, J. M., et al. (2020). Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). *Diabetes Metab. Res. Rev.* 36:e3280. doi: 10.1002/dmrr.3280

Monteiro-Soares, M., Russell, D., Boyko, E. J., Jeffcoate, W., Mills, J. L., Morbach, S., et al. (2020). Guidelines on the classification of diabetic foot ulcers (IWGDF 2019). *Diabetes Metab. Res. Rev.* 36:e3273. doi: 10.1002/dmrr.3273

Moon, J., Kim, N., Han, S. L., Lee, S. T., and Chu, K. (2021). Nanopore 16S amplicon sequencing enhances the understanding of pathogens in medically intractable diabetic foot infections. *Diabetes* 70, 1357–1371. doi: 10.2337/db20-0907

Nelson, E. A., Wright-Hughes, A., Brown, S., Lipsky, B. A., and Nixon, J. (2016). Concordance in diabetic foot ulceration: a cross-sectional study of agreement between wound swabbing and tissue sampling in infected ulcers. *Health Technol. Assess.* 20, 1–176. doi: 10.3310/hta20820

Noah, F., Micah, H., Lauber, C. L., and Rob, K. (2008). The influence of sex, handedness, and washing on the diversity of hand surface bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17994–17999. doi: 10.1073/pnas.0807920105

Oates, A., Bowling, F. L., Boulton, A. J., and McBain, A. J. (2012). Molecular and culture-based assessment of the microbial diversity of diabetic chronic foot wounds and contralateral skin sites. *J. Clin. Microbiol.* 50, 2263–2271. doi: 10.1128/JCM.06599-11

Pang, M., Zhu, M., Lei, X., Chen, C., and Cheng, B. (2020). Changes in foot skin microbiome of patients with diabetes mellitus using high-throughput 16S rRNA gene sequencing: a case control study from a single center. *Med. Sci. Monit.* 26:e921440. doi: 10.12659/MSM.921440

Pellizzer, G., Strazzabosco, M., Presi, S., Furlan, F., Lora, L., Benedetti, P., et al. (2001). Deep tissue biopsy vs. superficial swab culture monitoring in the microbiological assessment of limb-threatening diabetic foot infection. *Diabet. Med.* 18, 822–827. doi: 10.1046/j.1464-5491.2001.00584.x

Petti, C. A., Bhally, H. S., Weinstein, M. P., Kim, J., Teresa, W. L., Barth, R., et al. (2006a). Utility of extended blood culture incubation for isolation of Haemophilus, Actinobacillus, Cardiobacterium, Eikenella, and Kingella organisms: a retrospective multicenter evaluation. *J. Clin. Microbiol.* 44, 257–259. doi: 10.1128/JCM.44.1.257-259.2006

Petti, C. A., Polage, C. R., and Schreckenberger, P. (2006b). The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. *J. Clin. Microbiol.* 43, 6123–6125. doi: 10.1128/JCM.43.12.6123-6125.2005

Rondas, A. A. L. M., Schols, J. M. G. A., Halfens, R. J. G., and Stobberingh, E. E. (2013). Swab versus biopsy for the diagnosis of chronic infected wounds. *Adv. Skin Wound Care* 26, 211–219. doi: 10.1097/01.ASW.0000428984.58483.aa

Rowan, N. J. (2004). Viable but non-culturable forms of food and waterborne bacteria: quo Vadis? *Trends Food Sci. Technol.* 15, 462–467. doi: 10.1016/j.tifs.2004.02.009

Salipante, S. J., Sengupta, D. J., Christopher, R., Gina, C., Jessica, S., Sims, E. H., et al. (2013). Rapid 16S rRNA next-generation sequencing of polymicrobial clinical samples for diagnosis of complex bacterial infections. *PLoS One* 8:e65226. doi: 10.1371/journal.pone.0065226

Sebastian, J., Karola, P., Rafael, S., Inga, H., Benjamin, E., Alexander, G., et al. (2012). Bacterial community shift in treated periodontitis patients revealed by ion torrent 16S rRNA gene amplicon sequencing. *PLoS One* 7:e41606. doi: 10.1371/journal.pone.0041606

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. doi: 10.1186/gb-2011-12-6-r60

Singh, N., Armstrong, D. G., and Lipsky, B. A. (2005). Preventing foot ulcers in patients with diabetes. *JAMA* 293, 217–228. doi: 10.1001/jama.293.2.217

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., et al. (2022). IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res. Clin. Pract.* 183:109119. doi: 10.1016/j.diabres.2021.109119

Travis, J., Malone, M., Hu, H., Baten, A., and Benkendorff, K. (2020). The microbiome of diabetic foot ulcers: a comparison of swab and tissue biopsy wound sampling techniques using 16S rRNA gene sequencing. *BMC Microbiol.* 20:163. doi: 10.1186/s12866-020-01843-2

Tuttle, M. S., Eliot, M., Pranab, M., Hu, F. Z., Rachael, M. K., Ehrlich, G. D., et al. (2011). Characterization of bacterial communities in venous insufficiency wounds by use of conventional culture and molecular diagnostic methods. *Work. Papers.* 49, 3812–3819. doi: 10.1128/JCM.00847-11

Urbancic-Rovan, V., and Gubina, M. (2000). Bacteria in superficial diabetic foot ulcers. *Diabet. Med. J. Br. Diabet. Assoc.* 17, 814–815. doi: 10.1046/j.1464-5491.2000.00374-2.x

Van Netten, J. J., Bus, S. A., Apelqvist, J., Lipsky, B. A., Hinchliffe, R. J., Game, F., et al. (2020). Definitions and criteria for diabetic foot disease. *Diabetes Metab. Res. Rev.* 36:e3268. doi: 10.1002/dmrr.3268

Wang, N. F., Zhang, T., Zhang, F., Wang, E. T., He, J. F., Ding, H., et al. (2015). Diversity and structure of soil bacterial communities in the Fildes region (maritime Antarctica) as revealed by 454 pyrosequencing. *Front. Microbiol.* 6:1188. doi: 10.3389/fmicb.2015.01188

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D., and Bi, Y. (2017). Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis (†). *Ann. Med.* 49, 106–116. doi: 10.1080/07853890.2016.1231932

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Alterations of the gut microbiota in patients with immunoglobulin light chain amyloidosis

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Background: Emerging evidence revealed that gut microbial dysbiosis is implicated in the development of plasma cell dyscrasias and amyloid deposition diseases, but no data are available on the relationship between gut microbiota and immunoglobulin light chain (AL) amyloidosis.

Methods: To characterize the gut microbiota in patients with AL amyloidosis, we collected fecal samples from patients with AL amyloidosis (n=27) and age-, gender-, and BMI-matched healthy controls (n=27), and conducted 16S rRNA MiSeq sequencing and amplicon sequence variants (ASV)-based analysis.

Results: There were significant differences in gut microbial communities between the two groups. At the phylum level, the abundance of *Actinobacteriota* and *Verrucomicrobiota* was significantly higher, while *Bacteroidota* reduced remarkably in patients with AL amyloidosis. At the genus level, 17 genera, including *Bifidobacterium*, *Akkermansia, and Streptococcus* were enriched, while only 4 genera including *Faecalibacterium*, *Tyzzerella*, *Pseudomonas*, and *Anaerostignum* decreased evidently in patients with AL amyloidosis. Notably, 5 optimal ASV-based microbial markers were identified as the diagnostic model of AL amyloidosis and the AUC value of the train set and the test set was 0.8549 (95% CI 0.7310-0.9789) and 0.8025 (95% CI 0.5771-1), respectively. With a median follow-up of 19.0 months, further subgroup analysis also demonstrated some key gut microbial markers were related to disease severity, treatment response, and even prognosis of patients with AL amyloidosis.

Conclusions: For the first time, we demonstrated the alterations of gut microbiota in AL amyloidosis and successfully established and validated the microbial-based diagnostic model, which boosted more studies about microbe-based strategies for diagnosis and treatment in patients with AL amyloidosis in the future.

KEYWORDS

AL amyloidosis, amyloid, gut dysbiosis, gut microbiota, immunoglobulin light chain amyloidosis

1 Introduction

Immunoglobulin light chain (AL) amyloidosis is the most common type of systemic amyloidosis, triggered by an underlying plasma cell dyscrasia and involves increased production and release of free immunoglobulin light chains, which form insoluble amyloid fibrils that are deposited in organs (1, 2). The incidence of AL amyloidosis is only 12 cases per million persons per year (3) and advanced multiorgan involvement often leads to delayed diagnosis and poor prognosis (4). Over the last decade, the advent of another plasma cell dyscrasia, multiple myeloma (MM)–derived novel agents such as proteasome inhibitors and daratumumab, has improved the longevity of most AL amyloidosis patients (5, 6). However, several issues remain unaddressed, such as a suboptimal hematological complete response rate, high early mortality, various outcomes depending on the extent and severity of organ damage, and frequently observed treatment-related toxic effects (7). In response to these existing problems, researches are mounting to identify factors that are responsible for the onset and progression of AL amyloidosis and determine the response to treatment in individual patients.

Recently, regarding the relationship between plasma cell dyscrasias and gut microbiota, several studies highlighted an intimate and intricate interaction between gut microbiota and MM (8–10). For example, *Prevotella heparinolytica* was found to promote the progression of MM in Vk*MYC mice by stimulating the differentiation of Th17 cells that inhabited the gut and migrated to the bone marrow (10). Jian and his colleagues (8) demonstrated that nitrogen-cycle bacteria such as *Klebsiella* and *Streptococcus* were considerably abundant in MM, which was probably due to the excessive accumulation of blood urea, and the altered gut microbiota, in turn, contributed to the malignant progression of MM. In addition, gut dysbiosis was observed in recipients of autologous hematopoietic cell transplantation (auto-HCT), which is the first-line therapy in MM or AL amyloidosis patients, and reduced peri-engraftment diversity in fecal samples is linked to poorer overall and progression-free survival in auto-HCT patients (11). Furthermore, the butyrate-produced bacteria *Eubacterium hallii* was more abundant in MM patients with minimal residual disease $(MRD)^-$ compared to MRD^+ patients, which indicated an association between microbial community and treatment responses in MM patients (12).

The important role of gut microbiota in other types of amyloidosis was also preliminarily explored. Among patients with Familial Mediterranean fever (FMF), AA amyloidosis (AAA) was found to have two increased operational taxonomic units (OTUs) in the gut microbiota, and increased indoleamine 2,3-dioxygenase (IDO) activity and higher adiponectin levels (13). Additionally, the serum amyloid-associated (SAA) protein could function as an opsonin, and intestinal epithelial cells could synthesize it in response to the gut microbiota (14, 15). Furthermore, AAA can be induced by a high-fat diet in a mouse model overexpressing hepatic SAA (16), and numerous bacteria can release amyloid-enhancing factors that may be transmitted by ingestion and cross species barriers (17–20). In the autopsy series, approximately all patients with amyloidosis had digestive system involvement (21), and evidence of gut microbiota involvement in the etiology of another amyloid deposition disease, Alzheimer's disease, is accumulating both in animal models and humans (22–25).

Based on these findings, gut microbiota seems to be a potential candidate for both synthesis of amyloid-enhancing factors and the overproduction of amyloid-related protein in amyloidosis diseases. However, to our best knowledge, no data are available on the relationship between gut microbiota and AL amyloidosis. In this study, we intended to fill this gap in knowledge by characterizing the gut microbial community in patients with AL amyloidosis, identifying specific microbial markers and validating their diagnostic efficacy, and further conducting a preliminary exploration of the relationship between baseline gut microbiota and disease severity, treatment response, and prognosis of AL amyloidosis.

2 Materials and methods

2.1 Research subjects

The research was conducted in accordance with the PRoBE design concept (prospective specimen collection and retrospective blinded evaluation) (26). This study was approved by the Ethics Committee of Xijing Hospital of the Fourth military medical university (KY20192070), and the participants provided their written informed consent to participate in this study.

Patients who were newly diagnosed with AL amyloidosis between July 2018 and February 2021 in Xijing hospital (Xi'an, China) were screened. The inclusion criteria included patients: I) were biopsy-proven primary systemic AL amyloidosis; II) ≥18 years of age. The exclusion criteria were patients: I) had digestive diseases or systemic diseases such as diabetes and hypertension; II) have been treated with chemotherapy; III) used antibiotics or probiotics within 3 months before sampling; IV) in the period of pregnancy.

Healthy volunteers who were age-, gender-, and body mass index (BMI)-matched with AL amyloidosis patients from the physical examination center of Xijing hospital were enrolled. The inclusion criteria were individuals: I) had a normal value of kidney and liver function, routine blood, feces, and urine tests, fasting blood glucose, blood lipids, and blood pressure; and II) ≥18 years of age. The exclusion criteria were individuals: I) administered antibiotics or probiotics three months before enrollment; II) had a history of any chronic diseases or acute infection.

2.2 Clinical data collection

The AL amyloidosis patient registration form was designed to record the demographic and clinical characteristics at kidney biopsy including gender, age, height, weight, blood pressure, medical history, pathological results, biochemical indexes, and therapies. All indicators above were uniformly tested and issued by the Laboratory Department of Xijing Hospital and extracted from the patient medical record system. Clinical organ involvement was assessed according to international consensus criteria (27), and the Mayo 2012 staging system was used for stratification (28).

2.3 Follow-up and survival outcomes

The final follow-up date was March 31, 2022. Overall survival (OS) was defined as the time from diagnosis to death or the last follow-up. Patients who were alive at the last follow-up were censored at that date. Progression-free survival (PFS) was defined as the period from treatment initiation to disease progression, relapse, or death from any cause.

2.4 Therapeutic strategy and response evaluation

All patients received subcutaneous bortezomib at a dose of 1.3 mg per square meter of body-surface area, and dexamethasone at a dose of 40 mg orally or intravenously once weekly for 28 days each cycle. The assessment of hematological and organ response was performed according to the validated response criteria published by the International Society of Amyloidosis (29, 30). A ≥25% eGFR decrease was considered as the criterion for renal progression. All response assessment was performed on an intent-to-treat (ITT) basis.

2.5 Fecal samples collection and bacterial taxon identification

Each individual provided a fresh tail stool sample at 06:30–08:30 hours. Fecal samples were quickly placed in sterile specimen tubes, and transferred to a -80°C cryogenic refrigerator for further analysis. DNA extraction was carried out as previously described by our team (31).

2.6 Polymerase chain reaction (PCR), miseq sequencing, and sequence data processing

DNA libraries were constructed, and the sequencing was performed on an Illumina MiSeq platform by Shanghai Mobio Biomedical Technology Co. Ltd., China. The 16S ribosomal RNA (rRNA) gene sequence that targeted the V3-V4 region was applied to identify the bacterial taxon, which was detailed described in a previously published article by our team (31). Original Illumina read data for all samples were stored in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA825339 and PRJNA574226.

2.7 Bioinformatics and statistical analysis

Amplicon sequence variants (ASVs) were identified with the DADA2 algorithm. The representative sequences for each ASV were annotated using the SILVA reference database (SSU138). QIIME feature classifier was used for species annotation, which was the species annotation plug-in of the QIIME 2 analysis

process, and adopted the classify-sklearn algorithm. Alpha diversity metrics (ACE estimator, Chao 1 estimator, Shannon-Wiener diversity index and Simpson diversity index) were assessed by using Mothur v1.42.1. The non-parametric Mann-Whitney U test was used to test for significant differences between two groups. A comparison of multiple groups was done using a nonparametric Kruskal-Wallis test. Bray-Curtis, weighted UniFrac, unweighted UniFrac, and Jaccard-binary dissimilarity were calculated in QIIME. Principal coordinate analysis (PCoA) plots and PERMANOVA which were used to test for statistical significance between the groups using 10,000 permutations were generated in R (version 3.6.0) package vegan 2.5-7. The linear discriminant analysis (LDA) effect size (LEfSe) (32) was used to detect taxa with differential abundance among groups (lefse 1.1, https://github.com/SegataLab/lefse). A heatmap plot of the key ASVs identified by random forest models was generated by using the 'pheatmap' package of the R program. Probability of disease (POD) index was defined as the ratio between the number of randomly generated decision trees that predicted sample as "AL amyloidosis" and that of healthy controls. The identified optimal set of ASVs was finally used for the calculation of POD index for both the training and the testing cohort. A receiver operating characteristics (ROC) analysis was performed to measure the quality of the classification models by the R software package pROC. PICRUSt2 v2.4.1(https://github.com/picrust/picrust2/wiki) (33) was used to predict functional abundances in the Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database based on 16S rRNA gene sequences. Spearman's rank correlation was performed to explore correlations between ASVs and the clinical characteristics of patients with AL amyloidosis.

3 Result

3.1 Baseline clinical characteristics of patients with AL amyloidosis and healthy controls

We recruited 27 patients with AL amyloidosis and 27 healthy controls (HCs). Process and flow chart of this study were demonstrated in Figure 1. The baseline clinical characteristics of these two groups were shown in Table 1. Age, gender, and BMI were matched between these two groups. There was also no significant difference between two groups at serum creatinine level, while albumin was considerably lower in patients with AL amyloidosis than that in healthy controls. The median baseline difference between the involved and uninvolved free light chain (dFLC) levels was 142 mg/L (range, 15 to 1201), and the median proteinuria was 2780 mg/24 h (range, 102 to 7905). A total of 18 patients (66.7%) had two or more organs involved; 81.48% of patients had kidney involvement, and 62.96% had heart involvement. The majority of patients (96.29%) were classified as lambda isotype and approximately half of patients (48.15%) had a Mayo 2012 stage of III or higher.

3.2 Data quality and changes in gut microbiota diversity of AL amyloidosis

The rarefaction curve revealed the number of ASVs of each sample at different sequencing quantities, which indicated that the amount of sequencing data is large enough to reflect the vast

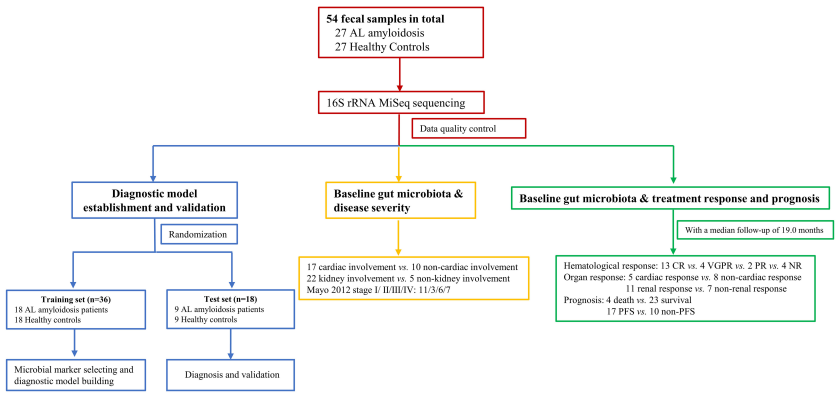

FIGURE 1
Study design and flow diagram. A total of 54 fecal samples from 27 AL amyloidosis patients and 27 healthy controls were collected. Firstly, we characterized the gut microbiota in patients with AL amyloidosis compared with age-, gender-, and BMI-matched HCs. Secondly, a diagnostic model was established and validated to identify the optimal microbial markers for AL amyloidosis. Furthermore, after a median follow-up of 19.0 months, subgroup analysis also demonstrated some key gut microbial markers were related to organ involvement, mayo 2012 stages, hematological response, organ response, and even prognosis of patients with AL amyloidosis. AL amyloidosis, immunoglobulin Light Chain Amyloidosis; BMI, body mass index; HCs, healthy controls; CR, complete response; VGPR, very good partial response; PR, patial response; NR, no response; PFS, progression-free survival.

TABLE 1 Demographic and clinical characteristics of the participants at baseline.

Characteristic	AL amyloidosis (n=27)	Healthy control (n=27)	*P* value
Age, mean ± SD, yr	56.78 ± 8.95	53.04 ± 7.17	0.096
Gender (Male/Female)	19/8	14/13	0.264
BMI, mean ± SD, kg/m^2	22.83 ± 3.77	24.39 ± 1.68	0.057
Albumin, mean ± SD, g/L	29.19 ± 8.12	46.78 ± 3.20	<0.001
Serum Creatinine, mean ± SD, umol/L	73.30 ± 21.49	76.78 ± 10.17	0.451
24-h urinary protein, median (range), mg	2780 (102-7905)	—	
ALP, median (range), IU/L	68 (35-373)	—	
Lambda isotype, no. (%)	26 (96.29%)	—	
dFLC, median (range), mg/L	142 (15-1201)	—	
Involved organs, no. (%)		—	
Kidney	22 (81.48%)		
Heart	17 (62.96%)		
Liver	2 (7.41%)		
Other	21 (77.78%)		
NT-proBNP, median (range), ng/L	890 (30-15398)	—	
cTnT, median (range), ng/L	0.029 (0.004-0.168)	—	
IVST, mean ± SD, cm	13.67 ± 4.67	—	
LVEF, mean ± SD, %	56.74 ± 5.22	—	
Mayo 2012 stage, no. (%)		—	
I	11 (40.74%)		
II	3 (11.11%)		
III	6 (22.22%)		
IV	7 (25.93%)		

BMI, body mass index; ALP, alkaline phosphatase; dFLC, difference in the involved to uninvolved free light chain; NT-proBNP, N-terminal pro-B type natriuretic peptide; cTnT, Troponin T; IVST, inter-ventricular septum thickness; EF, LVEF, left ventricular ejection fraction.

majority of microbial information in the samples when the curve approaches plateau (Figure 2A). In total, 3,629,337 usable raw reads were obtained from 54 stool samples. After quality filtering and assembly of overlapping paired-end reads, 1,892,625 high-quality reads were generated and 976 ASVs were obtained. The average number of sequences per sample was 67,209 ± 20,151 (range 35,925–120,795, Supplementary Table 1). A total of 729 ASVs were shared among the two groups according to the Venn diagram, while 168 ASVs were unique for AL amyloidosis, and 79 ASVs were specific for HCs (Figure 2B). No significant differences in community richness (estimated by Chao and ACE indices) and diversity (measured by Shannon and Simpson indices) were observed between AL amyloidosis and healthy controls (Supplementary Table 2). The gut microbial communities in patients with AL amyloidosis and the HCs were clustered separately, indicating a marked differentiation in taxonomic composition based on Jaccard-binary distances (Adonis, p = 0.0155, Figure 2C; Supplementary Table 3). In the ANOSIM analysis based on Jaccard-binary distances (Figure 2D; Supplementary Table 3), the difference between the two groups was significantly greater than that within the groups, further confirming the value of comparison between these two groups (R = 0.055, p = 0.0162).

3.3 Changes in gut microbiota at the phylum and the genus levels in AL amyloidosis

Based on the taxonomic classification of the ASVs, the relative microbial abundance of 54 samples at different levels (phylum, class, order, family, and genus) was identified. The phylum and genus levels of fecal microbial composition in each sample from two groups were shown in Supplementary Figures 1A, B.

At the phylum level, *Firmicutes*, *Bacteroidota*, and *Proteobacteria* were the three dominant populations in two groups, accounting for up to 90% of sequences on average (Figure 3A). Compared with HCs, *Actinobacteriota* and *Verrucomicrobiota* were enriched (3.22% *vs.* 1.15%, 2.20% *vs.* 0.52%, respectively) in patients with AL amyloidosis, while *Bacteroidota* was considerably reduced in patients with AL amyloidosis (31.20% *vs.* 42.85%, all $p < 0.05$, Figure 3B; Supplementary Table 4).

At the genus level, *Bacteroides*, *Faecalibacterium*, and *Escherichia-Shigella* in AL amyloidosis, while *Bacteroides*, *Faecalibacterium*, and *Prevotella* in the HCs, were three dominant populations in two groups, each accounting for up to 5% of the sequences on average (Figure 3C). By comparison, we observed that a total of 21 genera had significantly different

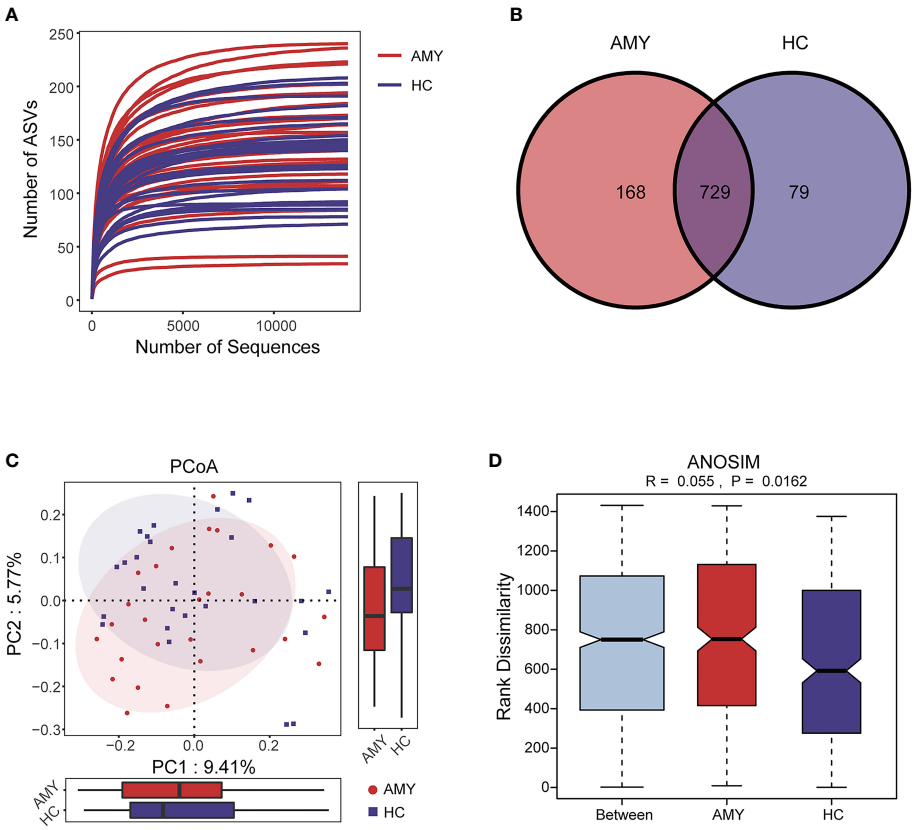

FIGURE 2
Data quality and gut microbiota diversity in patients with AL amyloidosis and HCs. **(A)** The rarefaction curve revealed the number of ASVs of each sample at different sequencing quantities. **(B)** The Venn diagram displaying overlap between the two groups showed that 729 of the 976 ASVs were shared between the AMY and HCs. A total of 168 of the 976 ASVs were unique to AMY. **(C)** β diversity based on PCoA analysis (Jaccard-binary distances) showed the fecal microbial communities in patients with AMY group and the HCs were clustered separately, indicating a good differentiation in taxonomic composition (Supplementary Table 3). **(D)** ANOSIM based on Jaccard-binary distances also showed that there were significant differences between the two groups (R = 0.055, p = 0.0162, Supplementary Table 3). AMY, patients with AL amyloidosis; HCs, healthy controls; ASVs, amplicon sequence variants; PCoA, principal coordinate analysis.

abundance between the two groups, and the relative abundance of the top 8 genera was presented in Figure 3D. Specifically, six genera, namely, *Bifidobacterium*, *[Eubacterium]_coprostanoligenes_group*, *Akkermansia*, *Enterobacteriaceae_unclassified*, *Streptococcus*, and *Collinsella* were enriched in the AL amyloidosis group (all $p < 0.05$), whereas *Faecalibacterium* and *Tyzzerella* were remarkably decreased in AL amyloidosis group (all $p < 0.05$, Figure 3D; Supplementary Table 5).

3.4 Phylogenetic profiles of the gut microbial communities in AL amyloidosis

In addition to the differences at the above different levels, we analyzed gut microbiota using the LEfSe approach to identify the specific taxa associated with AL amyloidosis. The histogram of LDA value distribution (Figure 4A) revealed that 35 microbial biomarkers clearly distinguished patients with AL amyloidosis and HCs (LDA > 2.5, all $p < 0.05$, Supplementary Table 6). Meanwhile, a cladogram (Figure 4B) depicting the fecal microbial structure and prevalent bacteria revealed the significant alterations in taxa between AL amyloidosis patients and HCs. Compared with the healthy group, patients with AL amyloidosis had a markedly higher abundance of several bacterial taxon chains within the phylum *Verrucomicrobiota* and *Actinobacteriota*. Specifically, *p-Verrucomicrobiota.c-Verrucomicrobiae.o-Verrucomicrobiales.f-Akkermansiaceae.g-Akkermansia*, *p-Actinobacteriota.c-Actinobacteria.o-Bifidobacteriales.f-Bifidobacteriaceae.g-Bifidobacterium*, *p-Actinobacteriota.c-Coriobacteriia.o-Coriobacteriales.f-Coriobacteriaceae.g-Collinsella*, as well as genus *Eggerthella* of family *Eggerthellaceae* in phylum *Bacteroidota*, were enriched in AL amyloidosis. Whereas in phyla *Bacteroidota* and *Proteobacteria*, two taxon clades, *p-Bacteroidota.c-Bacteroidia.o-Bacteroidales* and *o-Pseudomonadales.f-Pseudomonadaceae.g-Pseudomonas*, were substantially less abundant in AL amyloidosis patients (LDA > 2.5, all $p < 0.05$).

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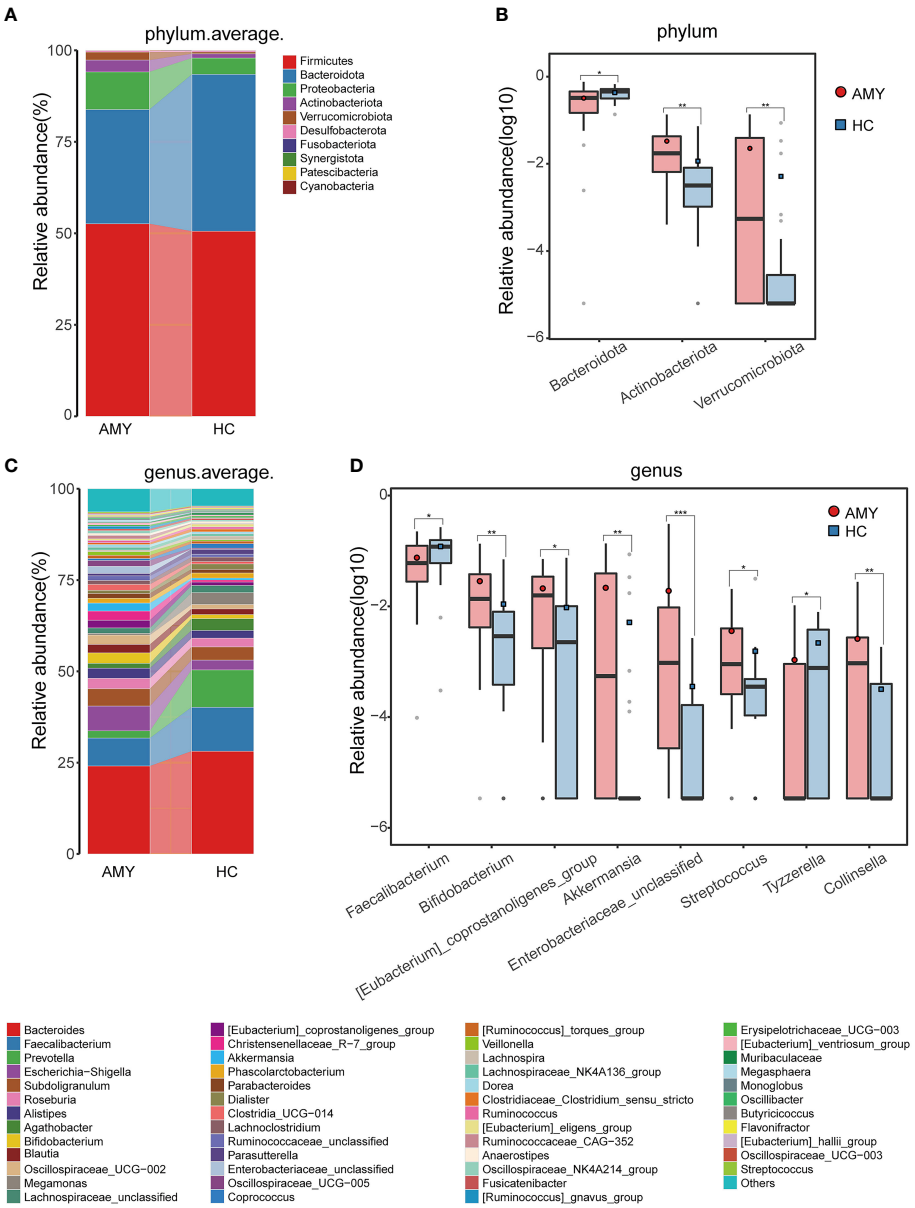

FIGURE 3
Composition and comparison of gut microbiomes in AMY (n = 27) and HCs (n = 27). Composition of the gut microbiota at the **(A)** phylum and **(C)** genus levels in AMY versus HCs. The significant different microbial community at the phylum level **(B)** and genus level **(D)** in AMY and HCs. The average abundance of each bacterium is depicted as the mean ± SE. P-values were calculated by a Wilcoxon rank-sum test and are shown in the Supplementary Tables 4, 5. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. AMY, patients with AL amyloidosis; HCs, healthy controls.

3.5 Microbial functional alteration in AL amyloidosis

To elucidate the functional and metabolic alterations of gut microbiota between AL amyloidosis and HC groups, PICRUSt2 analysis was used to predict functional abundances based on 16S rRNA gene sequences. We observed a substantial increase in functional abundance of 7 KEGG pathways in patients with AL amyloidosis compared with HCs, including one pathway at level 2, metabolism of other amino acids, and 6 pathways at level 3, namely, retinol metabolism, glutathione metabolism, naphthalene degradation, taurine and hypotaurine metabolism, zeatin biosynthesis, and apoptosis. Whereas, the genes of four pathways including metabolism of terpenoids and polyketides at level 2, and biosynthesis of ansamycins, synthesis and degradation of ketone bodies, glycerolipid metabolism at level

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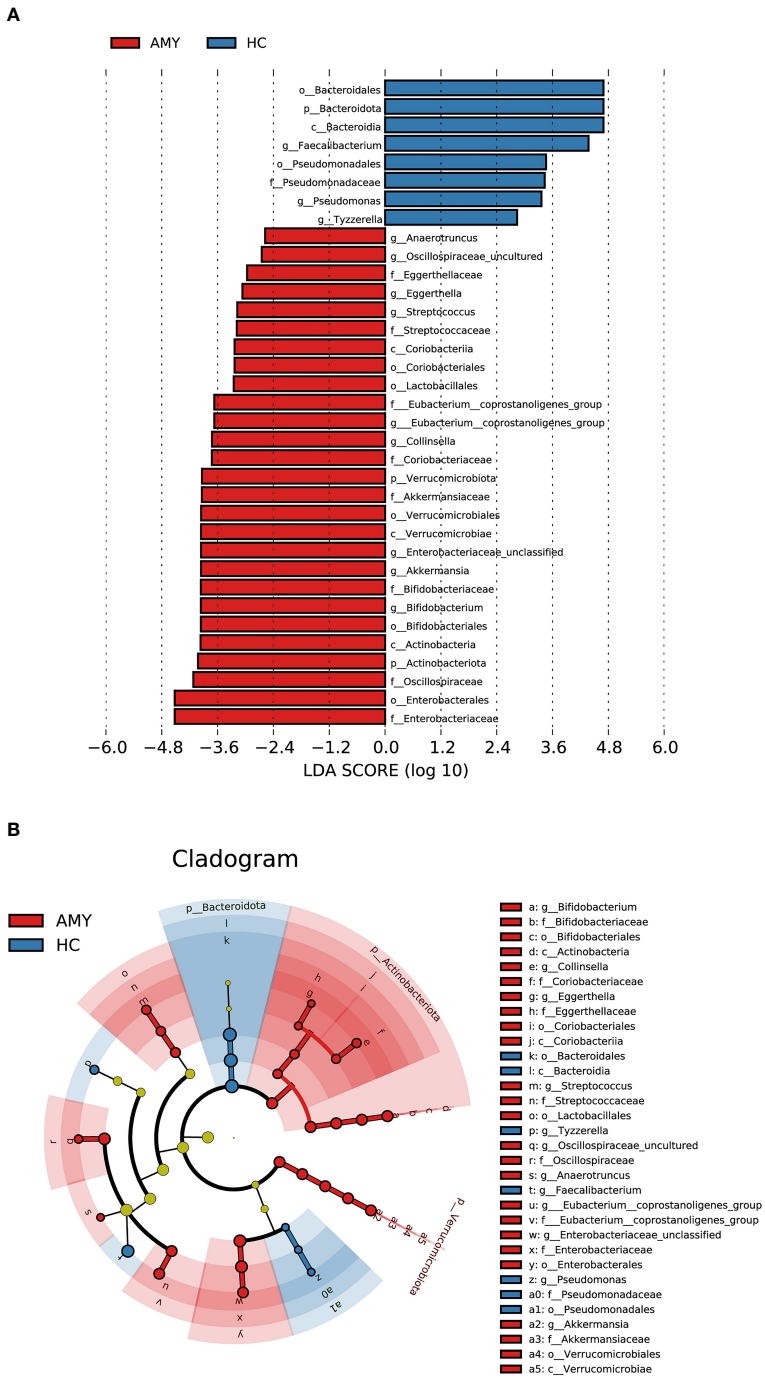

FIGURE 4
LEfSe analysis based on ASV characterizations of gut microbiota in AMY (n = 27) and HCs (n = 27). **(A)** Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between AMY (red) and HCs (blue). LDA scores on a log10 scale are indicated at the bottom. The significance of the microbial marker increases with the LDA score. **(B)** Cladogram generated by the LEfSe method showed the phylogenetic distribution of gut microbiomes associated with AMY and HCs. Nodes in red indicate taxa that were enriched in AMY compared to those in HCs, while nodes in blue indicate taxa that were enriched in HCs compared to those in AMY. Only the taxa having a $p < 0.05$ (Wilcoxon rank-sum test) and LDA >2.5 are shown in the figure legend (Supplementary Table 6). AMY, patients with AL amyloidosis; HCs, healthy controls; ASVs, amplicon sequence variants; LEfSe, linear discriminant analysis effect size; LDA, linear discriminant analysis; *p*, phylum; *c*, class; *o*, order; *f*, family; *g*, genus.

3 were significantly decreased in AL amyloidosis patients (all $p < 0.05$, LDA > 2.5, Figures 5A, B, and Supplementary Table 7).

3.6 Correlations between significantly different ASVs and clinical characteristics in AL amyloidosis

To explore correlations between ASVs and the clinical characteristics of patients with AL amyloidosis, Spearman's rank correlation was performed. The oblique triangle heatmap indicated a taxon-taxon correlation between gut microbiota and clinical parameters. A total of 17 solid lines represented strong correlations ($p < 0.01$) between 9 ASVs and 7 clinical parameters and may be the focus of research (Supplementary Table 8; Figure 6A). For example, ASV605 (*Streptococcus*) was negatively correlated with systolic blood pressure (SBP) and positively correlated with ASV244 (*Christensenellaceae_R-7_group*), ASV496 (*Marvinbryantia*) and ASV638 (*Bifidobacterium*). In the genus *Christensenellaceae_R-7_group*, ASV518 and ASV895 were negatively correlated with albumin (ALB) and total protein (TP), and ASV518 was also positively correlated with age.

As for AL amyloidosis-unique clinical parameters, a significant positive correlation existed between mayo 2012 stage and ASV649 (*Alistipes*) (ρ=-0.38, $p = 0.049$), and ASV167 (*Bacteroides*) (ρ=-0.61, $p < 0.001$). Moreover, lambda

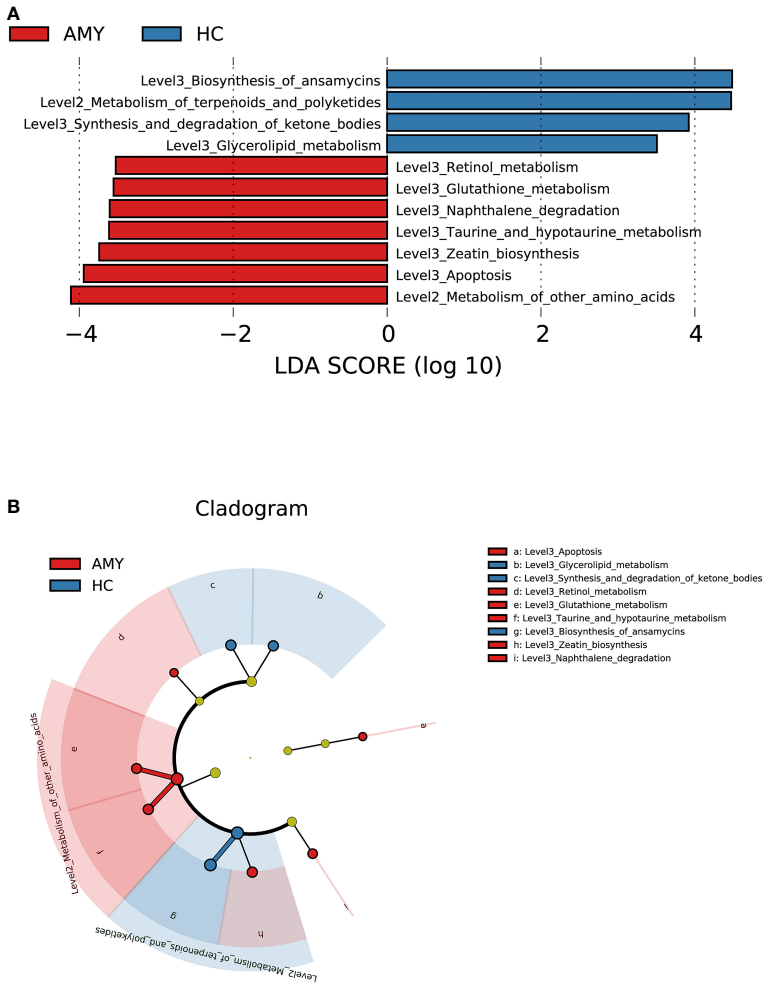

FIGURE 5
Prediction for differential functional and metabolic alterations of gut microbiota in AL amyloidosis by PICRUSt2 analysis. **(A)** Histogram of LDA scores calculated for selected KEGG pathways showed significant differences in gene functions between AMY (red) and HCs (blue). **(B)** Cladogram generated by the LEfSe method showed the phylogenetic distribution of differential gene functions in AMY and HCs. The default criteria LDA > 2.5 and $p < 0.05$ indicate different KEGG pathways and a higher abundance in one group than in the other (Supplementary Table 7). AMY, patients with AL amyloidosis; HCs, healthy controls; LEfSe, linear discriminant analysis effect size; LDA, linear discriminant analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes.

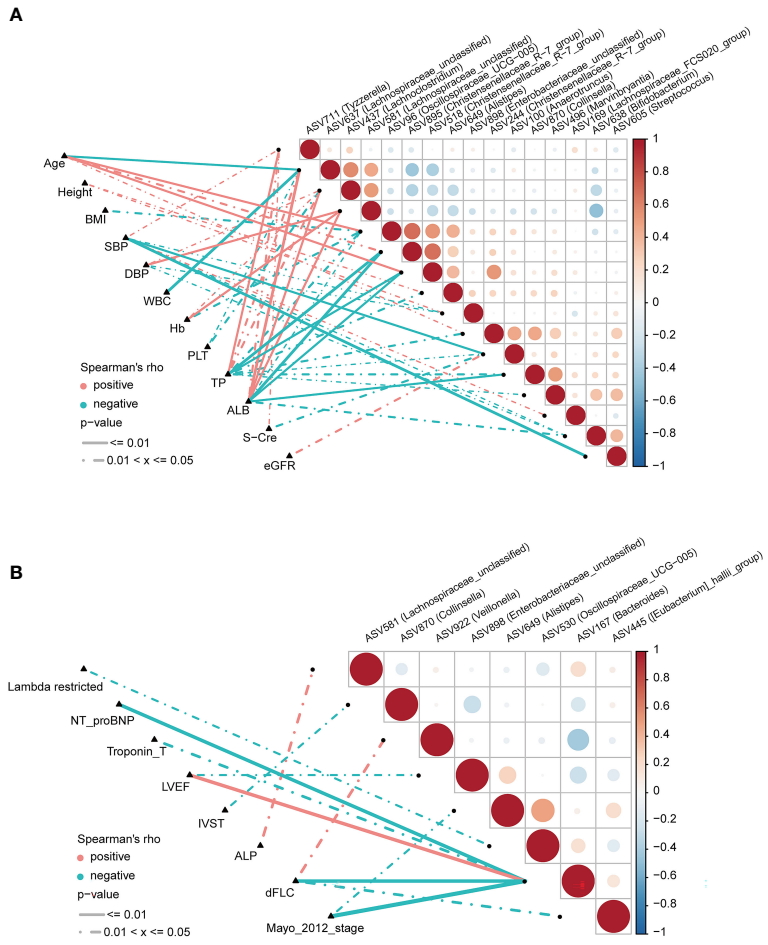

FIGURE 6
Correlation analysis of differential ASVs and clinical characteristics of AL amyloidosis patients by Spearman's rank test. The oblique triangle heatmap indicated a taxon-taxon correlation between fecal microbiota and clinical parameters in **(A)** both AMY and HCs and **(B)** only AMY group (Supplementary Tables 8, 9). Positive values (red) indicate positive correlations. Negative values (blue) indicate inverse correlations. Solid lines represent $p \leq 0.01$. Dotted lines represent $0.01 < p \leq 0.05$. AMY, patients with AL amyloidosis; HCs, healthy controls; ASVs, amplicon sequence variants. BMI, body mass index; dFLC, difference in the involved to uninvolved free light chain; DBP, diastolic blood pressure; SBP, systolic blood pressure; WBC, white blood cell; TP, total protein; ALB, albumin; Hb, hemoglobin; PLT, platelet; S-cre, serum creatinine; eGFR, estimated glomerular filtration rate; ALP, alkaline phosphatase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; LVEF, left ventricular ejection fraction; IVST, interventricular septal thickness.

restricted had a negative correlation with ASV530 (*Oscillospiraceae_UCG-005*) (ρ=-0.39, $p = 0.04$). Significant positive correlations also existed between dFLC and ASV922 (*Veillonella*) (ρ=0.42, $p = 0.027$), and between ALP and ASV581 (*Lachnospiraceae_unclassified*) (ρ=0.40, $p = 0.037$, Supplementary Table 9; Figure 6B).

3.7 Identification and validation of ASV-based markers in the diagnosis of AL amyloidosis

According to the above significant difference in gut microbiota between AL amyloidosis and HCs, we assessed the potential use of gut microbiota-based signatures in the diagnosis of AL amyloidosis. A total of 63 ASVs were used for random forest modeling and biomarker selection, which were significantly different between the two groups (Mann-Whitney U test, $p < 0.05$) and their corresponding abundance was more than 0.1% in any sample. A five-fold cross-validation curve of the random forest model revealed that the 5 ASV-based markers were identified as the optimal marker set (Figure 7A). The relative abundance of the 5 ASV-based markers in each sample was presented in Supplementary Table 10. In the stochastic decision forest model, the distribution of ASV importance was demonstrated by the mean decrease in accuracy and mean decrease in the Gini coefficient (Figure 7B).

In the training phase, the probability of disease (POD) value was significantly increased in the AL amyloidosis samples compared with the control samples (p = 1.3×10^{-4}, Figure 7C; Supplementary Table 11). The POD index achieved an AUC value of 0.8549 with 95% CI of 0.731 to 0.9789 between AL amyloidosis and HCs cohorts (p = 0.0001, Figure 7D).

In the validation phase, 9 HCs and 9 AL amyloidosis were used to validate the diagnostic efficacy of the POD for AL

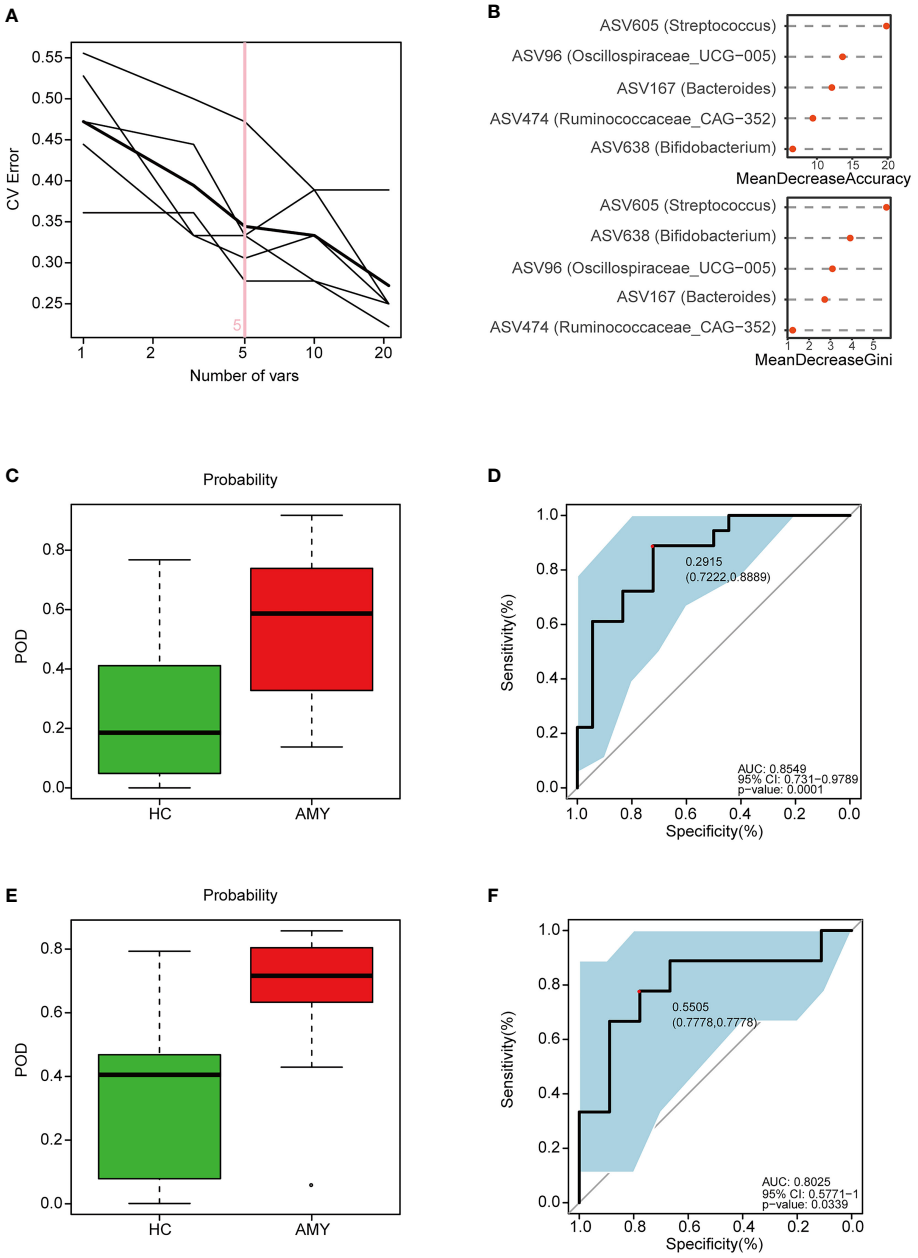

FIGURE 7
Identification and validation of microbial ASV-based markers of AL amyloidosis by random forest models. A total of 63 ASVs were used for random forest modeling and biomarker selection, which were significantly different between the two groups (Mann-Whitney U test, p < 0.05) and their corresponding abundance was more than 0.1% in any sample. **(A)** 5 ASVs were selected by random forest models as the optimal AL amyloidosis biomarkers (Supplementary Table 10). **(B)** Importance distribution map of the selected microbial markers in the model. **(C)** POD was significantly higher in AMY than HCs in the training set (Supplementary Table 11). **(D)** The POD index had an AUC = 0.8549 with a 95% CI = 0.731- 0.9789 between AMY and HCs in the training set. **(E)** POD was significantly higher in Eps than HCs in the test set (Supplementary Table 12). **(F)** The POD index had an AUC = 0.8025 with a 95% CI = 0.57771-1 between AMY and HCs in the test set. AMY, patients with AL amyloidosis; HCs, healthy controls; ASVs, amplicon sequence variants; POD, probability of disease; AUC, area under the curve.

amyloidosis. Each POD of each patient was estimated and the relevant results are shown in the online Supplementary Table 12. The average POD value was significantly higher in the 9 patients with AL amyloidosis than that in 9 controls (p = 0.034, Figure 7E), and the POD attained an AUC value of 0.8025 (95% CI 0.5771-1, p = 0.0339) between AL amyloidosis and controls (Figure 7F).

3.8 Association between baseline gut microbiota and disease severity, treatment response, and prognosis in AL amyloidosis

Subgroup analysis was further done to reveal the association between the characteristics of gut microbiota at baseline and disease severity, treatment response, and prognosis in 27 patients with AL amyloidosis. All the following subgroups were matched between age, gender, and BMI (Supplementary Table 13).

3.8.1 Association between baseline gut microbiota and heart involvement in AL amyloidosis

Although patients with or without heart involvement did not have significant differences in the alpha and beta diversity in the baseline gut microbiota, they had abundance differences regarding some specific bacterial taxons. Compared with 10 patients without heart involvement, 17 patients with cardiac involvement had significantly enriched abundance of a bacterial taxon in the baseline gut microbiota, *o-Enterobacterales. f-Enterobacteriaceae* in phylum *Proteobacteria* (LDA > 2.5, p < 0.05, Figure 8A). While one bacterial taxon, *o-Monoglobales.f-Monoglobaceae.g-Monoglobus* was decreased in patients with cardiac involvement (in phylum *Firmicutes*, LDA > 2.5, p < 0.05, Figure 8A; Supplementary Table 14).

3.8.2 Association between baseline gut microbiota and kidney involvement in AL amyloidosis

Compared with 5 patients without kidney involvement, 22 patients with kidney involvement had a higher abundance of three bacterial taxon clades in phylum *Firmicutes*, *o-Clostridiales.f-Clostridiaceae.g-Clostridiaceae_Clostridium_sensu_stricto, o-Monoglobales.f-Monoglobaceae.g-Monoglobus*, and *o-Peptostreptococcales Tissierellales.f-Peptostreptococcaceae*, and two main decreased abundance of bacterial taxon clades, *p-Desulfobacterota.c-Desulfovibrionia.o-Desulfovibrionales.f-Desulfovibrionaceae.g-Bilophila*, and *p-Synergistota.c-Synergistia.o-Synergistales.f-Synergistaceae* (LDA > 2.5, all p < 0.05, Figure 8B; Supplementary Table 14).

3.8.3 Association between baseline gut microbiota and Mayo 2012 staging system in AL amyloidosis

In phylum *Proteobacteria*, order *Burkholderiales* and genus *Parasutterella* were enriched in patients with Mayo 2012 stage I. Two bacterial taxon chains were abundant in patients with Mayo 2012 stage II, *o-Actinomycetales.f-Actinomycetaceae.g-Actinomyces* and *o-Peptostreptococcales Tissierellales.f-Peptostreptococcaceae.g-Romboutsia*. Genus *Cloacibacillus* in phylum *Synergistota* was found enriched in patients with Mayo 2012 stage III (LDA > 2.5, all p < 0.05, Figure 8C). Patients with Mayo 2012 stage III/IV had a higher abundance of genus *Escherichia_Shigella* and *Ruminococcaceae_UBA1819*, while genus *Oscillospiraceae_UCG_003* and *Parasutterella* were decreased compared with patients with Mayo 2012 stage I/II (Supplementary Figure 2; Supplementary Table 14).

3.8.4 Association between baseline gut microbiota and hematological response in AL amyloidosis

With a median follow-up of 19.0 months (range, 4.0 to 67.0), 19 (82.6%) of 23 patients had a hematologic response, including complete response (CR, 13 patients), very good partial response, (VGPR, 4 patients), partial response (PR, 2 patients) and 4 patients could not be evaluated because of incomplete data. According to the PCoA analysis based on weighted UniFrac distances, the microbial composition of CR patients was significantly distinct from VGPR patients (Adonis test, p = 0.0132, Supplementary Figure 3A). By applying LEfSe analysis (LDA > 2.5, Supplementary Figure 3B; Supplementary Table 14), we found one taxonomic chain, *o-Monoglobales.f-Monoglobaceae.g-Monoglobus* in phylum *Firmicutes* as well as genera *Eggerthella* and *Eubacterium hallii group* were specifically enriched in CR patients. In addition, two taxonomic chains, *p-Synergistota.c-Synergistia.o-Synergistales.f-Synergistaceae.g-Pyramidobacter* and *c-Firmicutes_Incertae_Sedis.o-Firmicutes_Incertae_Sedis_DTU014.f-Firmicutes Incertae Sedis DTU014.g-Firmicutes Incertae Sedis DTU014* were significantly enriched in VGPR patients, as well as genera *Eisenbergiella, Negativibacillus of* phylum *Firmicutes*. In phylum *Firmicutes*, we also found genera *Dorea* and *Epulopiscium* were abundant in PR patients.

Patients were further divided into two groups based on whether they had CR or VGPR. Interestingly, consistent with the findings above in CR patients, a higher abundance of bacterial taxon clade, *o-Monoglobales.f-Monoglobaceae.g-Monoglobus*, and genera *Eggerthella* and *Eubacterium hallii group* were still enriched in 13 CR patients compared with 10 non-CR patients (Figure 9A). Genus *Eggerthella* was also enriched in 17 patients with VGPR or better compared with 6 patients less than VGPR (Supplementary Figure 3C; Supplementary Table 14).

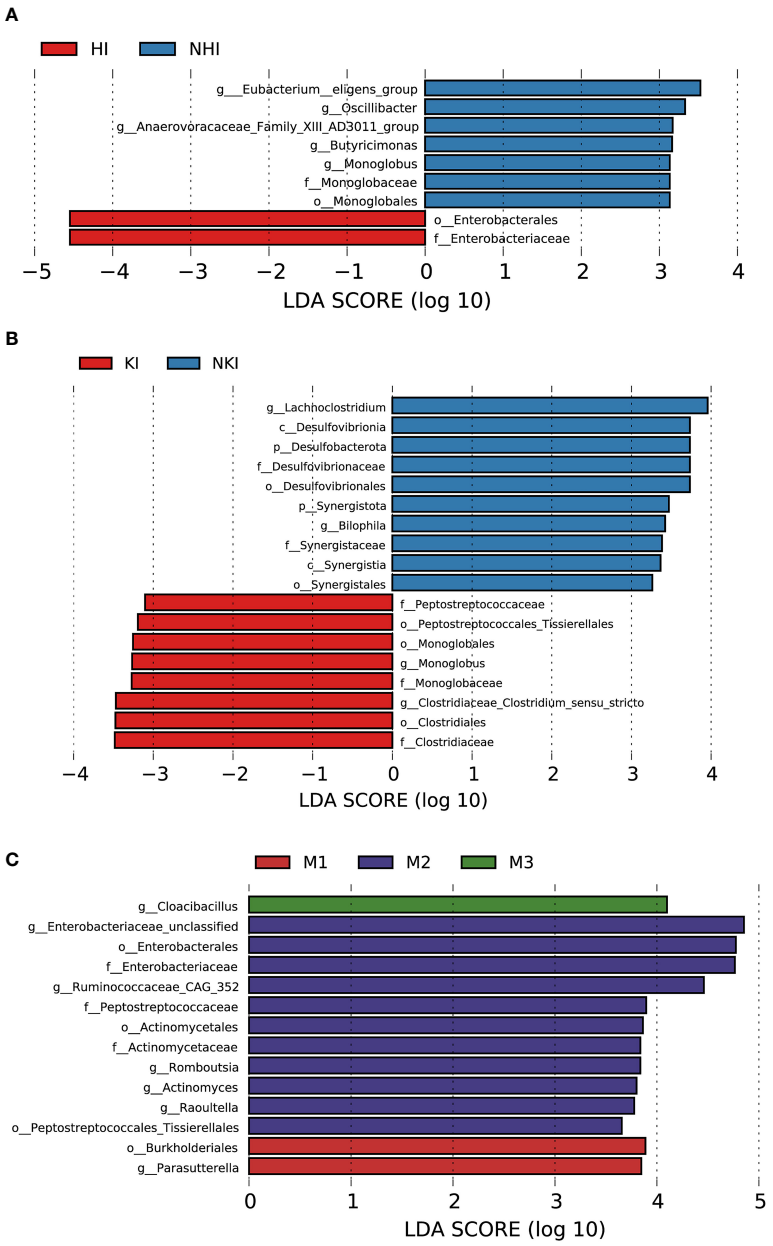

FIGURE 8
Association between baseline gut microbiota and disease severity in AL amyloidosis. Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between **(A)** HI and NHI, **(B)** KI and NKI, **(C)** M1, M2, M3 and M4 (Supplementary Table 14). LDA scores on a log10 scale are indicated at the bottom. The significance of the microbial marker increases with the LDA score. The default criteria was LDA > 2.5 and $p < 0.05$. All the subgroups were matched between age, gender, and BMI (Supplementary Table 13). AMY, patients with AL amyloidosis; LDA, linear discriminant analysis; HI, heart involvement; NHI, non-heart involvement; KI, kidney involvement; NKI, non-kidney involvement; M1, mayo 2012 stage I; M2, mayo 2012 stage II; M3, mayo 2012 stage III; M4, mayo 2012 stage IV.

By random forest, 5 microbial markers were identified for the prediction of hematological complete response (Supplementary Figure 4A, B), including ASV97 (*Roseburia*), ASV183 (*Lachnospiraceae unclassified*), ASV352 (*Monoglobus*), ASV421 (*Lachnospira*), and ASV457 (*Clostridium innocuum group*). The AUC for the training set reached 0.9615(95% CI 0.8952-1, $p = 0.0002$, Supplementary Figure 5A).

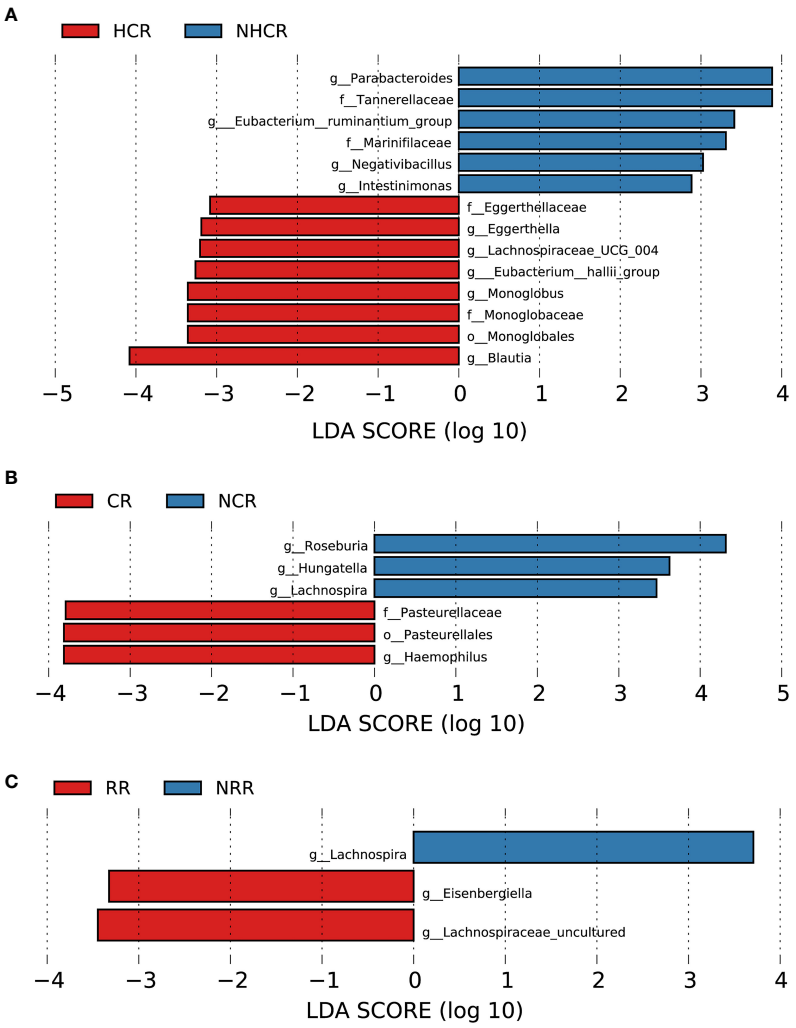

FIGURE 9
Association between baseline gut microbiota and hematological and organ response in AL amyloidosis. Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between **(A)** HCR and NHCR. **(B)** CR and NCR. **(C)** RR and NRR (Supplementary Table 14). LDA scores on a log10 scale are indicated at the bottom. The significance of the microbial marker increases with the LDA score. The default criteria was LDA > 2.5 and $p < 0.05$. All the subgroups were matched between age, gender, and BMI (Supplementary Table 13). AMY, patients with AL amyloidosis; LDA, linear discriminant analysis; HCR, hematological complete response; NHCR, no hematological complete response; CR, cardiac response; NCR, Non-cardiac response; RR, renal response; NRR, Non-renal response.

3.8.5 Association between baseline gut microbiota and cardiac and renal response in AL amyloidosis

In terms of organ response, a total of 13 and 18 patients could be evaluated for cardiac response and renal response, respectively. Patients in the cardiac response group (5 patients) had a higher abundance of one taxonomic chain, *o-Pasteurellales.f-Pasteurellaceae.g-Haemophilus* in phylum *Proteobacteria*, compared with patients with the cardiac response (8 patients). While three genera *Hungatella*, *Lachnospira*, and *Roseburia* of phylum *Firmicutes* were found enriched in the non-cardiac response group (Figure 9B, all $p < 0.05$, Supplementary Table 14). By random forest, 6 microbial markers were identified for the prediction of cardiac response (Supplementary Figures 4C, D), including ASV147 (*Roseburia*), ASV412 (*Escherichia-Shigella*), ASV655 (*Bacteroides*), ASV752 (*Enterobacteriaceae unclassified*), ASV777 (*Odoribacter*), and ASV782 (*Roseburia*). The AUC for the training set reached 0.875(95% CI 0.6197-1, $p = 0.0295$, Supplementary Figure 5B).

Compared with patients without renal response, genera *Eisenbergiella* and *Lachnospiraceae uncultured* of phylum *Firmicutes* had a higher abundance in patients with renal response. While genus *Lachnospira* of phylum *Firmicutes* was

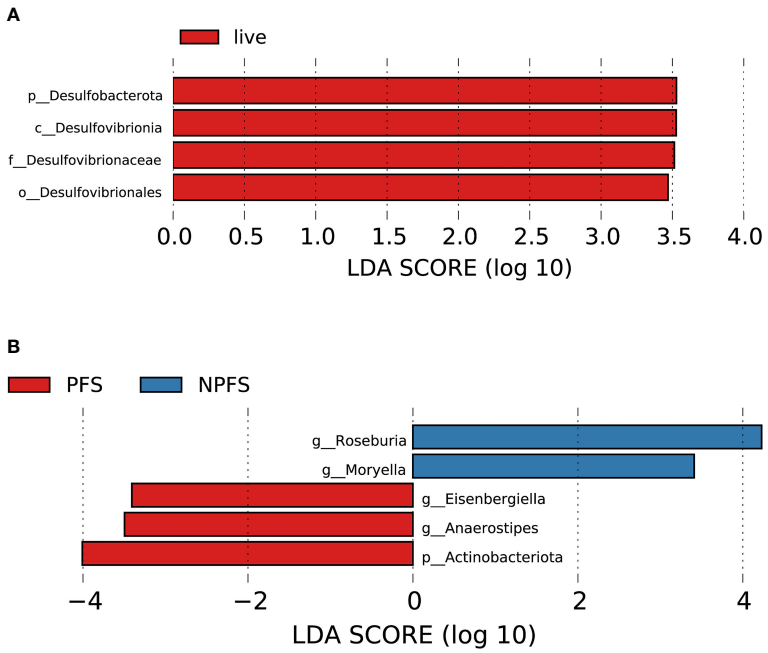

FIGURE 10
Association between baseline gut microbiota and prognosis in AL amyloidosis. Histogram of LDA scores calculated for selected taxa showed significant differences in microbe type and abundance between **(A)** die and live group; **(B)** PFS and NPFS group (Supplementary Table 14). LDA scores on a log10 scale are indicated at the bottom. The significance of the microbial marker increases with the LDA score. The default criteria was LDA > 2.5 and $p < 0.05$. All the subgroups were matched between age, gender, and BMI (Supplementary Table 13). AMY, patients with AL amyloidosis; LDA, linear discriminant analysis; PFS, progression-free survival; NPFS, patients without progression-free survival.

found abundant in patients without renal response (Figure 9C, all $p < 0.05$, Supplementary Table 14). By random forest, 2 microbial markers were identified for the prediction of renal response (Supplementary Figures 4E, F), including ASV71 (*Lachnospira*) and ASV504 (*Faecalibacterium*). The AUC for the training set reached 0.9351(95% CI 0.8036-1, $p = 0.0012$, Supplementary Figure 5C).

3.8.6 Association between baseline gut microbiota and the prognosis of patients with AL amyloidosis

During the follow-up time, four patients died, who had a lower abundance of one bacterial taxon chain, *p-Desulfobacterota.c-Desulfovibrionia.o-Desulfovibrionales.f-Desulfovibrionaceae* (Figure 10A). Patients without progression-free survival had a higher abundance of genus *Roseburia* and *Moryella* in phylum *Firmicutes*, but a decreased abundance of genus *Anaerostipes* and *Eisenbergiella* of phylum *Firmicutes* and phylum *Actinobacteriota* (Figure 10B, Supplementary Table 14). By random forest, 2 microbial markers were identified for the prediction of progression-free survival (Supplementary Figures 4G, H), including ASV584 (*Anaerostipes*) and ASV823 (*Eisenbergiella*). The AUC for the training set reached 0.9412 (95% CI 0.8259-1, $p < 0.0001$, Supplementary Figure 5D).

4 Discussion

In this study, we used 16S rRNA sequencing to analyze the gut microbiota of newly diagnosed patients with AL amyloidosis. Although no significant differences in community richness and diversity were observed between AL amyloidosis and HCs, significant differences were found in bacterial composition between the two groups. Notably, this study was also the first to successfully establish and validate a diagnosis model based on microbial ASV markers for AL amyloidosis. Further subgroup analysis revealed the association between baseline gut microbiota and disease severity, treatment response, and prognosis of patients with AL amyloidosis.

Emerging evidence revealed that different diseases have relatively distinct microbial profiles and gut microbial alteration is unique for each disease, such as MM (8), Alzheimer's disease (34), and FMF-related AA amyloidosis (13). In this study, we found treatment naïve AL amyloidosis patients may possess a specific profile of gut microbiota characterized by composition changes at different levels and several enriched taxonomic chains. Notably, phylum *Actinobacteriota* as a whole, as well as several families and genera classified within *Actinobacteriota* were found increased in patients with AL amyloidosis. Specifically, the enrichment of

genera *Bifidobacterium*, *Collinsella* and *Eggerthella* resulted in a subsequent higher abundance of phylum *Actinobacteriota*. Recently, mounting evidence showed *Bifidobacterium* can modulate immune system by increasing immunoglobulins, and inducing or reducing pro- or anti-inflammatory cytokines, respectively (35–37). And it is widely assumed that immunoglobulin light chains are precursors for amyloid deposits in AL amyloidosis. Accordingly, we speculated that a higher abundance of genus *Bifidobacterium* might have a promoting effect on the induction of immunoglobulin production, further facilitating the development of AL amyloidosis.

The increase of phylum *Verrucomicrobia* is most likely attributed to the increase of genus *Akkermansia* in AL amyloidosis. In general, *Akkermansia muciniphila* has been found to be significantly linked to a variety of physiological processes, including glucose and lipid metabolism, as well as immune response (38). A study claimed that the relative abundance of *Akkermansia muciniphila* in mice with colitis was positively correlated with injured histology and colonic inflammation (39) while Kang et al. found extracellular vesicles from *Akkermansia muciniphila* were significantly protective in DSS-treated mice (40). The increase of genus *Akkermansia* in AL amyloidosis was probably one of the mechanisms of self-protection against gut dysfunctions. The relationship between genus *Akkermansia* and the pathogenesis of AL amyloidosis is still unknown and worth further investigation and confirmation.

In addition to the above two increased phyla, we also found a decrease of phylum *Bacteroidota* in AL amyloidosis. *Bacteroidota* is the largest phylum of Gram-negative bacteria inhabiting our gastrointestinal tract and is regarded as the key player in the healthy state and sophisticated homeostasis of gut microbiota (41). Besides, to our knowledge, specific roles have been attributed to some genera of phylum *Bacteroidota* in the development of human diseases such as obesity, diabetes mellitus, rheumatoid arthritis, atherosclerosis, and neurodegenerative diseases (42–46). Further studies are needed to understand the potential relationship between the decrease of phylum *Bacteroidota* and the development of AL amyloidosis.

Interestingly, consistent with the previous study in MM (8), the nitrogen-cycle-bacteria such as the genus *Streptococcus* of phylum *Firmicutes*, was also markedly higher in AL amyloidosis. During the development of MM, increased urea or NH4+ and descending renal function led to the preferred growth of nitrogen-cycle-bacteria. Subsequently, urea is hydrolyzed effectively and utilized to synthesize L-glutamine, which is transferred to the host, hence hastening the progression of MM (8). In our study, ASV605 (*Streptococcus*) was found to be negatively correlated with SBP and positively correlated with the other three ASVs (ASV244, ASV496, and ASV638). We further identified ASV605 (*Streptococcus*) as the most important ASVs of five microbial markers for the diagnosis of AL amyloidosis. Thus, the genus *Streptococcus* may also contribute to the development of AL amyloidosis. Additionally, it is widely known that clonal plasma cells are both presented in AL amyloidosis and MM, and connected these two diseases. Therefore, the impact of gut microbiota on the proliferation of clonal plasma cells may be the focus of future researches. More importantly, the successful validation of their diagnostic efficacy in our cohort further made us believe that gut microbiota-based biomarkers might be valuable non-invasive methods for the diagnosis of AL amyloidosis. Further metagenomic sequencing of targeted species may strengthen the diagnostic efficiency of AL amyloidosis.

Furthermore, we observed significantly different functional abundance of KEGG pathways in patients with AL amyloidosis compared to HCs, which revealed that alterations in the abundance of certain gut microbial species may play a crucial role in regulating metabolic functions and inflammatory response. However, as the first research on gut microbiota in AL amyloidosis, other relevant researches are vacant and their related pathogenesis in this disease is still unclear. Additionally, spearman's analysis revealed the potential correlations between gut microbiota and clinical features. All the above findings need further validation and have laid the foundation for more research on the role of gut microbiota in the development of AL amyloidosis.

With the median follow-up of 19.0 months, some gut microbiota at baseline had been found to be associated with disease severity, treatment response, and prognosis of AL amyloidosis patients. Although ASV-based microbial markers were also identified as potential tools to predict the outcome of AL amyloidosis, further investigation and validation are needed due to the small number of subgroups.

Despite the valuable findings, our study still had certain shortcomings. First, due to the rarity of AL amyloidosis and the strict inclusion criteria, we recruited a relatively small sample size, which resulted in limited number of patients in further subgroup analysis. In order to fully investigate the impact of gut microbiota and further confirm our results, a larger cohort of patients with varying stages of illness is needed. Second, some confounding factors such as dietary habits, should also be taken into account. Due to the average age being too large in these two groups, it is hard to find absolutely healthy people and the mean BMI of the healthy group was slightly high. Moreover, participants in our study were all from China, and a more comprehensive study should be conducted and validated on a larger random sample of individuals from different regions. Third, we only evaluated fecal microbiota, which didn't adequately represent the whole profiles of mucosal microbiota.

In conclusion, for the first time, we highlighted the difference in gut microbiota between AL amyloidosis and HCs based on ASVs. The increase of *Actinobacteriota* and *Verrucomicrobiota* and decrease of *Bacteroidota* may contribute to the development

of AL amyloidosis. Moreover, our study is the first to successfully establish and validate the ASV-based microbial diagnostic model of AL amyloidosis in China. Interestingly, some gut microbiotas at baseline are associated with disease severity, treatment response, and prognosis of AL amyloidosis patients, which needed further investigation and validation. As the first report of the gut microbiota in AL amyloidosis, it injected its own strength into the ocean and opened new avenues for more studies about microbe-based strategies of diagnosis and treatment in patients with AL amyloidosis in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Xijing Hospital of the Fourth military medical university (KY20192070). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JY, SS, and JZ conceived and designed this study. JZ, XN, ZY, W-FG, YQ, YX, QJ, YW, RM, CL, MZ and BH participated in data acquisition, analysis, and interpretation. JY and JZ drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.973760/full#supplementary-material

References

1. Merlini G, Bellotti V. Molecular mechanisms of amyloidosis. *N Engl J Med* (2003) 349(6):583–96. doi: 10.1056/NEJMra023144

2. Barlogie B, Alexanian R, Jagannath S. Plasma cell dyscrasias. *Jama* (1992) 268 (20):2946–51. doi: 10.1001/jama.1992.03490200198025

3. Kyle RA, Larson DR, Kurtin PJ, Kumar S, Cerhan JR, Therneau TM, et al. Incidence of Al amyloidosis in Olmsted county, Minnesota, 1990 through 2015. *Mayo Clinic Proc* (2019) 94(3):465–71. doi: 10.1016/j.mayocp.2018.08.041

4. Gertz MA. Immunoglobulin light chain amyloidosis: 2020 update on diagnosis, prognosis, and treatment. *Am J Hematol* (2020) 95(7):848–60. doi: 10.1002/ajh.25819

5. Kastritis E, Leleu X, Arnulf B, Zamagni E, Cibeira MT, Kwok F, et al. Bortezomib, melphalan, and dexamethasone for light-chain amyloidosis. *J Clin Oncol: Off J Am Soc Clin Oncol* (2020) 38(28):3252–60. doi: 10.1200/jco.20.01285

6. Kastritis E, Palladini G, Minnema MC, Wechalekar AD, Jaccard A, Lee HC, et al. Daratumumab-based treatment for immunoglobulin light-chain amyloidosis. *N Engl J Med* (2021) 385(1):46–58. doi: 10.1056/NEJMoa2028631

7. Hasib Sidiqi M, Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2021. *Blood Cancer J* (2021) 11(5):90. doi: 10.1038/s41408-021-00483-7

8. Jian X, Zhu Y, Ouyang J, Wang Y, Lei Q, Xia J, et al. Alterations of gut microbiome accelerate multiple myeloma progression by increasing the relative abundances of nitrogen-recycling bacteria. *Microbiome* (2020) 8(1):74. doi: 10.1186/s40168-020-00854-5

9. Zhang B, Gu J, Liu J, Huang B, Li J. Fecal microbiota taxonomic shifts in Chinese multiple myeloma patients analyzed by quantitative polimerase chain reaction (Qpcr) and 16s rrna high-throughput sequencing. *Med Sci Monit* (2019) 25:8269–80. doi: 10.12659/MSM.919988

10. Calcinotto A, Brevi A, Chesi M, Ferrarese R, Garcia Perez L, Grioni M, et al. Microbiota-driven interleukin-17-Producing cells and eosinophils synergize to accelerate multiple myeloma progression. *Nat Commun* (2018) 9(1):4832. doi: 10.1038/s41467-018-07305-8

11. Khan N, Lindner S, Gomes ALC, Devlin SM, Shah GL, Sung AD, et al. Fecal microbiota diversity disruption and clinical outcomes after auto-hct: A multicenter observational study. *Blood* (2021) 137(11):1527–37. doi: 10.1182/blood.2020006923

12. Pianko MJ, Devlin SM, Littmann ER, Chansakul A, Mastey D, Salcedo M, et al. Minimal residual disease negativity in multiple myeloma is associated with intestinal microbiota composition. *Blood Adv* (2019) 3(13):2040–4. doi: 10.1182/bloodadvances.2019032276

13. Deshayes S, Fellahi S, Bastard JP, Launay JM, Callebert J, Fraisse T, et al. Specific changes in faecal microbiota are associated with familial Mediterranean fever. *Ann Rheumatic Dis* (2019) 78(10):1398–404. doi: 10.1136/annrheumdis-2019-215258

14. Eckhardt ER, Witta J, Zhong J, Arsenescu R, Arsenescu V, Wang Y, et al. Intestinal epithelial serum amyloid a modulates bacterial growth in vitro and pro-inflammatory responses in mouse experimental colitis. *BMC Gastroenterol* (2010) 10:133. doi: 10.1186/1471-230x-10-133

15. Reigstad CS, Lundén GO, Felin J, Bäckhed F. Regulation of serum amyloid A3 (Saa3) in mouse colonic epithelium and adipose tissue by the intestinal microbiota. *PloS One* (2009) 4(6):e5842. doi: 10.1371/journal.pone.0005842

16. Jang WY, Jeong J, Kim S, Kang MC, Sung YH, Choi M, et al. Serum amyloid A1 levels and amyloid deposition following a high-fat diet challenge in transgenic mice overexpressing hepatic serum amyloid A1. *Appl Physiol Nutrition Metab = Physiologie Appliquee Nutr Metabolisme* (2016) 41(6):640–8. doi: 10.1139/apnm-2015-0369

17. Larsen P, Nielsen JL, Dueholm MS, Wetzel R, Otzen D, Nielsen PH. Amyloid adhesins are abundant in natural biofilms. *Environ Microbiol* (2007) 9 (12):3077–90. doi: 10.1111/j.1462-2920.2007.01418.x

18. Asti A, Gioglio L. Can a bacterial endotoxin be a key factor in the kinetics of amyloid fibril formation? *J Alzheimer's Disease: JAD* (2014) 39(1):169–79. doi: 10.3233/jad-131394

19. Lundmark K, Westermark GT, Olsén A, Westermark P. Protein fibrils in nature can enhance amyloid protein a amyloidosis in mice: Cross-seeding as a disease mechanism. *Proc Natl Acad Sci USA* (2005) 102(17):6098–102. doi: 10.1073/pnas.0501814102

20. Westermark GT, Fändrich M, Westermark P. Aa amyloidosis: Pathogenesis and targeted therapy. *Annu Rev Pathol* (2015) 10:321–44. doi: 10.1146/annurev-pathol-020712-163913

21. Friedman S, Janowitz HD. Systemic amyloidosis and the gastrointestinal tract. *Gastroenterol Clinics North America* (1998) 27(3):595–614. doi: 10.1016/s0889-8553(05)70022-4

22. Kahn MS, Kranjac D, Alonzo CA, Haase JH, Cedillos RO, McLinden KA, et al. Prolonged elevation in hippocampal aβ and cognitive deficits following repeated endotoxin exposure in the mouse. *Behav Brain Res* (2012) 229(1):176–84. doi: 10.1016/j.bbr.2012.01.010

23. Harach T, Marungruang N, Duthilleul N, Cheatham V, Mc Coy KD, Frisoni G, et al. Reduction of abeta amyloid pathology in Apppss1 transgenic mice in the absence of gut microbiota. *Sci Rep* (2017) 7:41802. doi: 10.1038/srep41802

24. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol Aging* (2017) 49:60–8. doi: 10.1016/j.neurobiolaging.2016.08.019

25. Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR, et al. Effect of probiotic supplementation on cognitive function and metabolic status in alzheimer's disease: A randomized, double-blind and controlled trial. *Front Aging Neurosci* (2016) 8:256. doi: 10.3389/fnagi.2016.00256

26. Pepe MS, Feng Z, Janes H, Bossuyt PM, Potter JD. Pivotal evaluation of the accuracy of a biomarker used for classification or prediction: Standards for study design. *J Natl Cancer Institute* (2008) 100(20):1432–8. doi: 10.1093/jnci/djn326

27. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (Al): A consensus opinion from the 10th international symposium on amyloid and amyloidosis, tours, France, 18-22 April 2004. *Am J Hematol* (2005) 79(4):319–28. doi: 10.1002/ajh.20381

28. Kumar S, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Colby C, et al. Revised prognostic staging system for light chain amyloidosis incorporating cardiac biomarkers and serum free light chain measurements. *J Clin Oncol: Off J Am Soc Clin Oncol* (2012) 30(9):989–95. doi: 10.1200/jco.2011.38.5724

29. Palladini G, Dispenzieri A, Gertz MA, Kumar S, Wechalekar A, Hawkins PN, et al. New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: Impact on survival outcomes. *J Clin Oncol: Off J Am Soc Clin Oncol* (2012) 30(36):4541–9. doi: 10.1200/jco.2011.37.7614

30. Palladini G, Schönland SO, Sanchorawala V, Kumar S, Wechalekar A, Hegenbart U, et al. Clarification on the definition of complete haematologic response in light-chain (Al) amyloidosis. *Amyloid: Int J Exp Clin Investigation: Off J Int Soc Amyloidosis* (2021) 28(1):1–2. doi: 10.1080/13506129.2020.1868810

31. Dong R, Bai M, Zhao J, Wang D, Ning X, Sun S. A comparative study of the gut microbiota associated with immunoglobulin a nephropathy and membranous nephropathy. *Front Cell Infect Microbiol* (2020) 10:557368. doi: 10.3389/fcimb.2020.557368

32. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* (2011) 12(6): R60. doi: 10.1186/gb-2011-12-6-r60

33. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. Picrust2 for prediction of metagenome functions. *Nat Biotechnol* (2020) 38 (6):685–8. doi: 10.1038/s41587-020-0548-6

34. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, Johnson SC, et al. Gut microbiome alterations in alzheimer's disease. *Sci Rep* (2017) 7 (1):13537. doi: 10.1038/s41598-017-13601-y

35. Muñoz JA, Chenoll E, Casinos B, Bataller E, Ramón D, Genovés S, et al. Novel probiotic bifidobacterium longum subsp. infantis cect 7210 strain active against rotavirus infections. *Appl Environ Microbiol* (2011) 77(24):8775–83. doi: 10.1128/aem.05548-11

36. Holscher HD, Czerkies LA, Cekola P, Litov R, Benbow M, Santema S, et al. Bifidobacterium lactis Bb12 enhances intestinal antibody response in formula-fed infants: A randomized, double-blind, controlled trial. *JPEN J Parenteral Enteral Nutr* (2012) 36(1 Suppl):106s–17s. doi: 10.1177/0148607111430817

37. Lim HJ, Shin HS. Antimicrobial and immunomodulatory effects of bifidobacterium strains: A review. *J Microbiol Biotechnol* (2020) 30(12):1793–800. doi: 10.4014/jmb.2007.07046

38. Zhai Q, Feng S, Arjan N, Chen W. A next generation probiotic, akkermansia muciniphila. *Crit Rev Food Sci Nutr* (2019) 59(19):3227–36. doi: 10.1080/10408398.2018.1517725

39. Castro-Mejía J, Jakesevic M, Krych Ł, Nielsen DS, Hansen LH, Sondergaard BC, et al. Treatment with a monoclonal anti-Il-12p40 antibody induces substantial gut microbiota changes in an experimental colitis model. *Gastroenterol Res Pract* (2016) 2016:4953120. doi: 10.1155/2016/4953120

40. Kang CS, Ban M, Choi EJ, Moon HG, Jeon JS, Kim DK, et al. Extracellular vesicles derived from gut microbiota, especially akkermansia muciniphila, protect the progression of dextran sulfate sodium-induced colitis. *PloS One* (2013) 8(10): e76520. doi: 10.1371/journal.pone.0076520

41. Gibiino G, Lopetuso LR, Scaldaferri F, Rizzatti G, Binda C, Gasbarrini A. Exploring bacteroidetes: Metabolic key points and immunological tricks of our gut commensals. *Digestive Liver disease: Off J Ital Soc Gastroenterol Ital Assoc Stud Liver* (2018) 50(7):635–9. doi: 10.1016/j.dld.2018.03.016

42. Cui L, Zhao T, Hu H, Zhang W, Hua X. Association study of gut flora in coronary heart disease through high-throughput sequencing. *BioMed Res Int* (2017) 2017:3796359. doi: 10.1155/2017/3796359

43. Perry RJ, Peng L, Barry NA, Cline GW, Zhang D, Cardone RL, et al. Acetate mediates a microbiome-Brain-β-Cell axis to promote metabolic syndrome. *Nature* (2016) 534(7606):213–7. doi: 10.1038/nature18309

44. Most J, Goossens GH, Reijnders D, Canfora EE, Blaak EE. Gut microbiota composition strongly correlates to peripheral insulin sensitivity in obese men but not in women. *Benef Microbes* (2017) 8(4):1–6. doi: 10.3920/BM2016.0189

45. Quigley EMM. Microbiota-Brain-Gut axis and neurodegenerative diseases. *Curr Neurol Neurosci Rep* (2017) 17(12):94. doi: 10.1007/s11910-017-0802-6

46. Marietta EV, Murray JA, Luckey DH, Jeraldo PR, Lamba A, Patel R, et al. Suppression of inflammatory arthritis by human gut-derived prevotella histicola in humanized mice. *Arthritis Rheumatol (Hoboken NJ)* (2016) 68(12):2878–88. doi: 10.1002/art.39785

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Role of gut microbiota in the pathogenesis and treatment of diabetes mullites: Advanced research-based review

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Gut microbiota plays an important role in the proper functioning of human organisms, while its dysbiosis is associated with disease in various body organs. Diabetes mellitus (DM) is a set of heterogeneous metabolic diseases characterized by hyperglycemia caused by direct or indirect insulin deficiency. There is growing evidence that gut microbiota dysbiosis is closely linked to the development of DM. Gut microbiota composition changes in type 1 diabetes mullites (T1DM) and type 2 diabetes mullites (T2DM) patients, which may cause gut leakiness and uncontrolled entry of antigens into the circulation system, triggering an immune response that damages the isle β cells or metabolic disorders. This review summarizes gut microbiota composition in healthy individuals and compares it to diabetes mullites patients. The possible pathogenesis by which gut microbiota dysbiosis causes DM, particularly gut leakiness and changes in gut microbiota metabolites is also discussed. It also presents the process of microbial-based therapies of DM.

KEYWORDS

gut microbiota, type 1 diabetes mullites, type 2 diabetes mullites, gut leakiness, gut microbiota metabolites, microbiological therapy

Introduction

The gut microbiota plays an essential role in the proper functioning of human organisms (Vrancken et al., 2019). It co-evolves and symbioses with humans by combating pathogenic bacteria, assisting nutrient digestion, maintaining the integrity of the intestinal epithelia, and promoting immunological development (Stecher and Hardt, 2011; Dave et al., 2012; Natividad et al., 2012; Shreiner et al., 2015). However, when the balance of the microbiota community is affected, known as gut microbiota dysbiosis, it may lead to various diseases, as summarized in Figure 1 (Hou et al., 2022a). Diabetes mullites (DM) is a set of heterogeneous metabolic disorders characterized by hyperglycemia and glucose

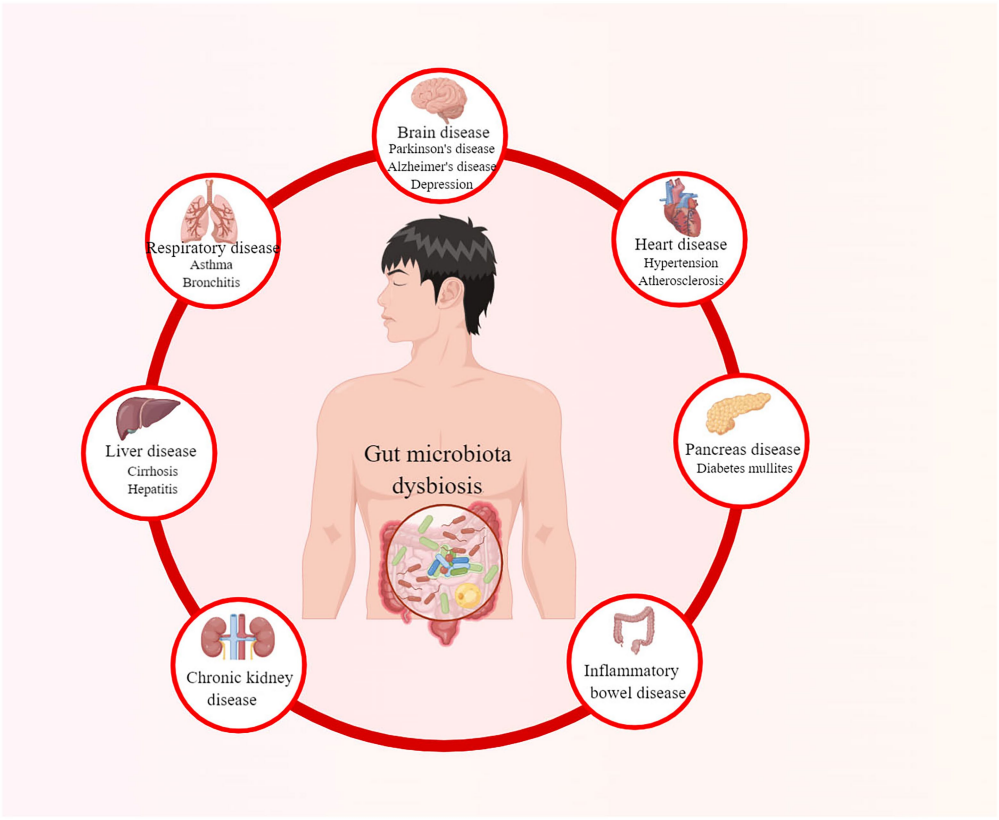

FIGURE 1
Diseases caused by gut microbiota dysbiosis.

intolerance, with high and increasing prevalence and multiple complications (Danaei et al., 2011; Saeedi et al., 2019; Classification and Diagnosis of Diabetes, 2022). The classical opinion is that type 1 diabetes mullites (T1DM) results from autoreactive T-cell-mediated partially or absolute destruction of pancreatic β cells in patients (Atkinson et al., 2014). In contrast, type 2 diabetes mullites (T2DM) is the outcome of a progressive loss of sufficient pancreatic β-cell insulin secretion in the context of insulin resistance (IR) (Kahn et al., 2014). According to International Diabetes Federation, there were more than 463 million DM patients in 2019 over the world and this number is estimated to rise to 578 million by 2030 and even 700 million by 2045 (Saeedi et al., 2019).

In the past decade, studies on the gut microbiome have developed rapidly due to the advancements in sequencing technologies and data analysis. It has been observed that gut microbiota dysbiosis is presented in both T1DM and T2DM patients (Bibbò et al., 2017; Vallianou et al., 2018). Gut microbiota dysbiosis may cause gut leakiness, which leads to external antigens uncontrollably entering the circulatory system (Di Tommaso et al., 2021). These antigens could activate islet autoimmunity and directly damage pancreatic β cells, and gut microbiota metabolites may also cause hormonal effects leading to metabolic disorders (Sun et al., 2015; Zhu and Goodarzi, 2020). Immune and metabolic disorders play an important role in the pathogenesis of DM (Bachem et al., 2019; Hou et al., 2022a). In addition, many diabetic complications are proven to be linked to the gut microbiota, including diabetic retinopathy, diabetes-induced cognitive impairment, diabetic peripheral neuropathy, and diabetic nephropathy (Zhou et al., 2022). Although the role of gut microbiota in the pathogenesis of DM is not yet understood, more and more researchers are pinning new hopes for treating DM in microbiological therapy.

Diabetes mellitus and its complications cause physical and mental injury to patients and a great economic burden to the medical system. However, there is no single treatment that can sustainably and consistently prevent the progression of β-cell failure after the onset of DM. Elucidating the role of gut microbiota in the onset and progression of DM will contribute to a better understanding of DM and the development of new treatments for it. This review summarizes the gut microbiota composition in healthy individuals and compares it with T1DM and T2DM patients. Furthermore, we review the role of gut microbiota dysbiosis in the pathogenesis of DM, particularly gut leakiness, immune disorders, and metabolite disorders. This paper will also present the process of microbial-based therapies of DM in the final.

Gut microbiota in healthy, T1DM, and T2DM individuals

Gut microbiota has 10 times the number of human cells and 150 times larger gene sets than humans, known as the "human second Genene" (Qin et al., 2010). In healthy individuals, the gut microbiota exhibits high taxonomic diversity, abundant microbial genes, and stable core microbiota (Fan and Pedersen, 2021). Herein, we reviewed the composition of gut microbes in healthy individuals at phylum and genus levels.

At the phyla level, approximately 80–90% of the gut microbiota belongs to *Firmicutes* and *Bacteroidetes*. *Firmicutes* dominate the gut microbiota composition of healthy individuals, while *Bacteroidetes* can favor inflammation by distributing the gut epithelial cells' barrier function (Tlaskalová-Hogenová et al., 2011). Therefore, *Firmicutes* to *Bacteroidetes* ratio (F/B ratio) is suggested to be a criterion of the health of gut microbiomes (Li and Ma, 2020). In addition, in the human gut, *Actinobacteria*, *Verrucomicrobia* and *Proteobacteria* are the major microbial phyla (Sommer and Bäckhed, 2013; Jandhyala et al., 2015; Landman and Quévrain, 2016). *Actinobacteria*, represented by the *Bifidobacterium* genus, contribute to producing butyrate and inhibiting bacterial translocation (Arboleya et al., 2016). Maintaining diversity in the gut microbiota is essential to keeping healthy, and its dysbiosis is linked to the development of metabolic diseases, including DM (Vallianou et al., 2018). At the genus level, *Bifidobacterium*, *Akkermansia*, *Lachnospira*, *Prevotela* and the butyrate-producing genera, including *Roseburia*, *Faecalibacterium*, *Anaerostipes*, *Subdoligranulum*, and *Eubacterium*, are abundant gut microbiota in healthy individuals (Mokhtari et al., 2021).

Notably, the human gut microbiota composition is not immutable but varies due to individual differences, age, and environmental factors. *Akkermansia muciniphila*, *Veillonella*, *Bacteroides*, *Clostridium botulinum* spp. and *Clostridium coccoides spp*. dominate the diversity of children's microbiota until a stable gut microbiota is formed (Amabebe et al., 2020). The gut microbiota alters rapidly in the first 2 years of life, matures around age three, and remains relatively stable (Isolauri, 2012; Yatsunenko et al., 2012; Durazzo et al., 2019). At maturity, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* become the dominant gut microbiota in healthy individuals (Yatsunenko et al., 2012). *Bifidobacterium* tends to decrease in older adults, while *Clostridium* and *Proteobacteria* tend to increase (Guigoz et al., 2008).

Gut microbiota dysbiosis in T1DM

Previous studies have identified differences in the gut microbiota between healthy individuals and T1DM patients (Bibbò et al., 2017). The stability, connectivity, abundance, and composition of the gut microbiota are probably linked to the development of T1DM (Han et al., 2018). Decreased microbiota diversity is a common gut microbiota shift associated with T1DM development (Leiva-Gea et al., 2018).

At the phyla level, it was well documented that proportions of *Firmicutes* phyla decreased in T1DM patients compared to the healthy individual group (Murri et al., 2013; Kostic et al., 2015; Leiva-Gea et al., 2018), while *Bacteroidetes* abundance increased successively (Alkanani et al., 2015; Pellegrini et al., 2017). Furthermore, previous research indicated that the F/B ratio significantly increased over time in children who eventually progressed to clinical T1DM and T1DM (Murri et al., 2013; Pellegrini et al., 2017; Leiva-Gea et al., 2018). However, research also showed no difference in the F/B ratio between T1DM patients and healthy individuals (Qi et al., 2016). Studies assessing the relationship between *Proteobacteria* abundance and T1DM have reported conflicting results, with some reporting a positive association (Brown et al., 2011; Cinek et al., 2018), some reporting a negative association (Leiva-Gea et al., 2018), and some reporting no difference (Murri et al., 2013).

At the genus level, T1DM patients present with a higher abundance of 12 different genera, including *Bifidobacterium*, *Bacteroides*, *Escherichia*, *Veillonella*, *Clostridium*, *Enterobacter*, *Lactobacillus*, *Ruminococcus*, *Streptococcus*, *Sutterella*, *Lactococcus*, and *Blautia*, among which *Bacteroides* is reported to be the dominant genus in the most research literature (Mokhtari et al., 2021). A more recent two-sample Mendelian randomization analysis revealed a close link between a higher relative abundance of the *Bifidobacterium* and a higher risk of T1DM (Xu et al., 2021).

Gut microbiota dysbiosis in T2DM

Environmental factors play an essential role in the onset and development of T2DM and recent evidence indicates that gut microbiota dysbiosis is one of them (Gurung et al., 2020; Hou et al., 2022b). Like T1DM, the gut microbiota in T2DM differs from those in healthy individuals (Vallianou et al., 2018; Que et al., 2021). The Integrative Human Microbiome Project found that insulin-resistant (IR) individuals had distinguishable molecular and microbial patterns at baseline from the healthy controls group (The Integrative Human Microbiome Project, 2019).

At the phyla level, *Firmicutes* level was reported to be lower in T2DM patients than in healthy individuals, while *Bacteroidetes* level was increased (Larsen et al., 2010). However, some recent research reported the opposite result; *Firmicutes* increased but *Bacteroidetes* decreased in T2DM patients (Sedighi et al., 2017; Zhao et al., 2019). Uniformly, the F/B ratio was reported to increase in some studies, but decreased in some studies (Sedighi et al., 2017; Zhao et al., 2019). In addition, the recent research also indicated an increased level of *Proteobacteria* in T2DM individuals (Sedighi et al., 2017; Zhao et al., 2019).

At the genus level, a large-scale metagenomic analysis in China compared the structural characteristics of the gut microbiota in healthy control and T2DM patients (Qin et al., 2012). It was recognized that conditioned pathogens (*Escherichia*

coli, *Bacteroides caccae*, some *Clostridium* species, and *Eggerthella lenta* mainly) were abundant in T2DM patients. On the contrary, the abundance of butyrate-producing gut microbiota (*Roseburia intestinalis*, *Roseburia inulinivorans*, *Eubacterium rectale*, *Faecalibacterium prausnitzii* and Clostridiales sp. *SS3/4*) was decreased. Another large-scale metagenome analysis in Europe demonstrated an increase in the abundance of *Lactobacillus gasseri*, *Streptococcus mutans*, some *Clostridiales* species, and *Lactobacillus* in T2DM patients, while a reduction in the abundance of butyrate-producing microbiota (*Roseburia*, *Eubacterium eligens*, *Bacteroides intestinalis*) (Karlsson et al., 2013). Notably, the above two studies both reported a decrease in butyrate-producing microbiota in T2DM patients, particularly *Roseburia* (Qin et al., 2012; Karlsson et al., 2013). In addition, recent research confirmed the decrease of the *Clostridium* genus in T2DM patients (Allin et al., 2018). Similarly, a lower level of *Akkermansia mucinphila*, which is responsible for degenerating mucin in the gut, was also considered a risk factor for T2DM (Allin et al., 2018; Hasani et al., 2021).

Role of the gut microbiota in the development of DM

From the above discussion, we can conclude that DM patients' gut microbiota is characterized by an increased level of opportunistic pathogens and decreased level of probiotics. Furthermore, *Firmicutes* are negatively associated with T1DM, while *Bacteroidetes* is a positive factor at the phyla level. However, conflicting results have been reported on the difference between healthy individuals and T2DM patients. At the genus level, *Bacteroides* showed a promotive association with both T1DM and T2DM. In addition, both T1DM and T2DM patients presented a lower level of butyrate-producing microbiota. Figure 2 depicts the role of gut microbiota dysbiosis in the development of DM.

Gut leakiness: A possible origin of DM

An epithelial layer-based physical barrier and a mucosal immune cell-based functional barrier make up the majority of the intestinal barrier and separate the host from the external environment (Mu et al., 2017). Tight junctions (TJ) and mucins are key to maintaining the integrity of the intestinal barrier (Durazzo et al., 2019). Butyrate-producing gut microbiota, such as *Firmicutes*, has been shown to promote TJ assembly, mucin synthesis, and anti-inflammatory properties (Hague et al., 1996; Peng et al., 2007, 2009). On the contrary, *Bacteroides* can inhibit the production of TJ protein and thus promote gut leakiness (Brown et al., 2011; Tlaskalová-Hogenová et al., 2011). Besides, *Bifidobacterium*, *Bacteroides*, and *Ruminococcus* can degrade mucin and thus induce gut leakiness (Hooper et al., 2002). Some bacterial metabolites, such as butyrate, have also been identified to play an important role in maintaining intestinal epithelial integrity (Wang et al., 2012; Kayama et al., 2020). Correspondingly, a low abundance of the butyrate-producing gut microbiota and increased abundance of the *Bacteroides* are presented in DM patients. Of note, hyperglycemia can induce gut leakiness by changing the TJ and adherence junctions (Thaiss et al., 2018). Immune and metabolic disorders caused by uncontrolled exogenous substances entering the circulatory system as a result of gut leakiness may be the origin of DM.

Immune disorder: The key to developing T1DM caused by gut microbiota dysbiosis and gut leakiness

Gut microbiota significantly influences the development of the immune system. Human and animal studies support a causal relationship between early microbial exposure and immune function development (Zhou et al., 2021). Defective immune response maturation is associated with T1DM progression later in life (Zhou et al., 2021). Moreover, the hygiene hypothesis posits that environmental improvements and antibiotic use lead to a reduction in microbiota diversity, possibly associated with a significant increase in allergic diseases and certain autoimmune diseases (Bach, 2018). Notably, the host's genetic set-up of can also interact with the gut microbiota, leading to alterations in microbial composition, immune system activation, and T1DM susceptibility (Alkanani et al., 2015; Bonder et al., 2016).

It is suggested that gut leakiness possibly leads to uncontrol entry of external antigens (Di Tommaso et al., 2021), which could activate islet autoimmunity and directly damage pancreatic β cells (Sun et al., 2015; Bachem et al., 2019). Lipopolysaccharides (LPS) are a possible molecular link between the gut microbiota, inflammation, and T1DM (Bachem et al., 2019), an integral part of the outer membrane of Gram-negative bacterial species. The leakage of fatty acids and LPS can activate the toll-like receptor 4 (TLR4) (Velloso et al., 2015). Activation of TLR contributes to the maturation of dendritic cells and the recognition of pathogen-associated molecular patterns (Li et al., 2013). TLR4 is a member of patten-recognition receptors, and its activation helps activate pro-inflammatory signaling pathways, expressing and secreting cytokine while bacterial pathogens present (Shi et al., 2006; Cani et al., 2007). A case–control study proved that T1DM patients have higher circulating LPS levels than healthy individuals (Devaraj et al., 2009). In addition, these antigens may be absorbed by antigen-presenting cells (APCs) in the gut mucosa, which then activates the islet-reactive T cells that would be subsequently transported to pancreatic lymph nodes and islets to induce the damage of β cells in genetically predisposed individuals (Sorini et al., 2019). Some antigens may significantly be homologous to islet autoantigen, known as molecular mimicry, so they can directly induce the activation of pathogenic CD8+ T cells to promote DM development (Tai et al., 2016).

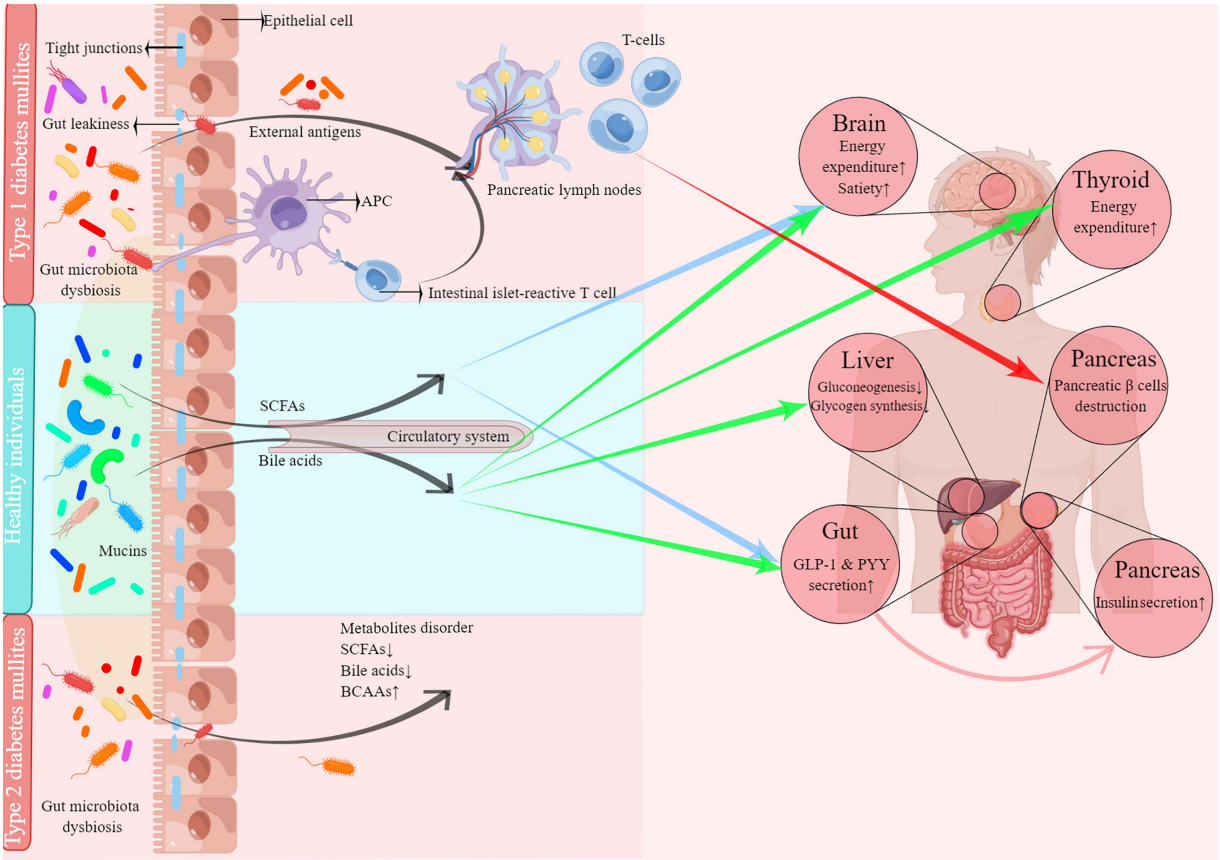

FIGURE 2
The role of gut microbiota dysbiosis in the development of diabetes mullites.

Metabolites disorder: The key to developing T2DM induced by gut microbiota dysbiosis and gut leakiness

Like T1DM, gut microbiota dysbiosis in T2DM can increase serum LPS concentration to injure the intestinal barrier and change in mucosal immune response (Allcock et al., 2001). LPS and other antigens can activate TLR4 on immune cells and induce pro-inflammatory response and IR (Medzhitov, 2001; Medzhitov and Horng, 2009; Janssen and Kersten, 2017). Besides, gut leakiness leads to macrophage infiltration, which causes local inflammation by producing and activating serum IL-6, TNF-α, and other inflammatory cytokines (Ghosh et al., 2020). Gut leakiness may also introduce the gut microbiota and its metabolite into the blood systemic circulation and promote local and systemic immune responses (Sudo et al., 2002; Sumida et al., 2022). However, T2DM is traditionally characterized as a metabolic disease, so the role of gut microbiota metabolite is more remarkable in the development of T2DM. Gut microbiota produces bioactive metabolites, including short-chain fatty acids (SCFAs), ammonia, phenols, endotoxins, etc., through dietary macronutrients (Schroeder and Bäckhed, 2016). SCFAs, bile acids, indole derivatives, sulfur-containing amino acids, and vitamins are gut microbiota metabolisms that prevent DM, whereas Branched-chain amino acids (BCAAs), phenol, p-cresol, methane, amines and ammonia are gut microbiota metabolic that promote DM progression (Khan et al., 2014). Herein, we review the effect of several important metabolites in T2DM.

The short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are products of the metabolism of soluble fiber and amino acids by gut microbiota. Widely reported studies have demonstrated that SCFAs contribute to improved glucose homeostasis and metabolism in tissues such as the liver, adipose, and muscle (Canfora et al., 2015). Acetate can induce the browning of adipose tissue, and propionate stimulates the release of peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) to reduce energy intake, whereas butyrate can reduce inflammation and reverse the decline in GLP-1 receptor expression in the liver (Sahuri-Arisoylu et al., 2005; Chambers et al., 2015; Jin et al., 2015; Zhou et al., 2017, 2018). In addition, two receptors for SCFAs, free fatty acid receptor 3 (GPR41) and free fatty acid receptor 2 (GPR43), have been identified to be directly implicated in T2DM development, which are expressed in enteroendocrine cells, intestinal epithelial cells, pancreatic islet cells, and other cells (Stoddart and Smith, 2008; Priyadarshini et al., 2018). Activation of

GPR41 was reported to stimulate leptin secretion to regulate energy expenditure and long-term food intake (Xiong et al., 2004) and peptide YY (PYY) to increase satiety (Larraufie et al., 2018). In addition, activating the GPR41 in the sympathetic nervous system can stimulate energy expenditure and reduce the risk of T2DM (Kimura et al., 2011). GLP-1 will be increasingly released when GPR43 is activated, which enhances insulin secretion, suppresses glucagon production, and increases satiety (Tolhurst et al., 2012). Of note, GLP-1 might also improve endothelial cell function by changing the composition of gut microbiota (Chen et al., 2022). The GPR43 transgenic mouse showed improved metabolic parameters, such as reduced obesity, improved homeostasis, improved lean meat quality, and higher GLP-1 secretion (Bjursell et al., 2011; Tolhurst et al., 2012; Kimura et al., 2013). Moreover, it has been demonstrated that SCFAs can decrease inflammation in mucosal and chronic systemic, possibly because of their ability to inhibit pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) (Roelofsen et al., 2010), induct anti-inflammatory cytokines (Säemann et al., 2000) and decrease the infiltration of inflammatory cells into adipose tissue (Meijer et al., 2010).

Primary bile acids are generated by the liver from cholesterol and released into the intestine by the gallbladder. The majority of these bile acids are reabsorbed in the intestinal tract, while a small amount reaches the lower intestine tract and is converted by the gut microbiota into secondary bile acid. Glucose homeostasis and intestinal fat absorption are both impacted by primary and secondary bile acids, which may significantly impact the pathophysiology of T2DM (Ahmad and Haeusler, 2019). Bile acids can lessen the amount of gluconeogenesis in the liver, enhance the production of glycogen, boost energy expenditure, stimulate the release of insulin, and reduce inflammation (Shapiro et al., 2018). These bile acids attach to and trigger nuclear hormone receptors like G-protein-coupled bile acid receptor-1 (TGR5) and nuclear hormone receptors, including the Pregnane X receptor (PXR) and Farnesoid X receptor (FXR). TGR5 is presented in digestive, immune, and adipose tissues. Intestinal TGR5 activation promotes insulin secretion and satiety by inducing the release of GLP-1 and PYY (Katsuma et al., 2005; Thomas et al., 2009; Kuhre et al., 2018). On the contrary, the knockout of TRG5 in myeloid-lineage cells and macrophages promoted IR and inflammation in adipose tissue (Perino et al., 2014). Moreover, the action of thyroid hormones may be promoted by bile acid-driven activation of TGR5 in adipose tissue, leading to an increase in energy expenditure (Watanabe et al., 2006). FXP is widely expressed in the liver, gut, kidney, and other tissues. The application of a synthetic FXR agonist was shown to significantly reduce plasma glucose, free fatty acids, triglycerides, cholesterol, and hepatic steatosis in DM mice (Zhang et al., 2006; Fang et al., 2015). Activation of PXR contributed to reducing bile acid production and increasing bile acid clearance to promote the absorption of lipid and fat-soluble vitamins (Staudinger et al., 2001). In addition, bile acids can stimulate GLP-1 secretion to regulate glucose metabolism and improve insulin sensitivity (Pathak et al., 2018; Shapiro et al., 2018).

Branched-chain amino acids (BCAAs) are essential amino acids produced by gut microbiota, including isoleucine, leucine, and valine. A case-cohort study supported that higher baseline BCAAs and their increase over 1 year were associated with a risk of T2DM (Ruiz-Canela et al., 2018). The obese mice model restored metabolic health while feeding them a reduced BCAAs diet, including improved insulin sensitivity and glucose tolerance, despite continuing to consume a high-sugar and high-fat diet (Cummings et al., 2018). However, a mendelian randomization analysis study suggested that the genetic risk score (GRS) for circulating BCAAs level was not linked to the homeostasis model assessment of insulin resistance (HOMA-IR), while the GRS for IR traits was significantly linked to increased circulating BCAAs level (Mahendran et al., 2017). This result indicates that it is not the increase of BCAAs that leads to IR, but IR that leads to the increase of BCAAs. The altered BCAAs levels are possibly due to reduced suppression of proteolysis caused by IR and reductions in BCAA catabolism in peripheral tissues caused by adiponectin signaling in T2DM (Lian et al., 2015; Giesbertz and Daniel, 2016).

In addition to the metabolites mentioned above, Trimethylamine (TMA), Indole derivatives, Imidazole propionate, etc., are associated with T2DM. TMA will be oxidized in the liver into Trimethylamine oxide (TMAO), and a case–control study reported that TMAO is positively associated with newly diagnosed T2DM (Shan et al., 2017). Indolepropionic acid is the product of the microbial metabolism of tryptophan, which is negatively linked to the risk of developing IR, low-grade inflammation, and T2DM (de Mello et al., 2017). Imidazole propionate is a microbial metabolite from histidine, which was demonstrated to be higher in T2DM patients compared with healthy individuals (Koh et al., 2018).

Progress in microbial-based therapies in DM

With the growing understanding of the role of gut microbiota in DM, more and more researchers are trying to use microbial-based therapies to treat DM. These therapies directly or indirectly alter the composition of gut microbiota to function. Herein, we review the progress in microbial-based therapies to treat DM.

Probiotics and prebiotics

Probiotics are referred to living microorganisms that are beneficial to the host's health when administered in adequate amounts (Hill et al., 2014). Research on probiotics has pointed

out their ability to enhance gut barrier function, regulate immunity, and competitively adhere to mucus and epithelial cells to benefit DM (Lomax and Calder, 2009; Ohland and Macnaughton, 2010). In animal models, oral administration of the probiotics increased the anti-inflammatory cytokines, such as TGF-β and IL-10 (Calcinaro et al., 2005; Sturm et al., 2005), decreased the pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β (Mariño et al., 2017), and regulated the immune balance between Th1/Th2/ Th17/Treg cells (Lau et al., 2011; Jia et al., 2017). In addition, bone mineral density was positively correlated with the number of lactic acid bacteria and *Bifidobacterium* in DM patients (Hou et al., 2017).

Human and animal trials have demonstrated the potential of probiotics in preventing and treating DM (Que et al., 2021). It has been reported that the onset of DM in non-obese diabetics (NOD) was reduced by the early supplement of complex-probiotic-preparation VSL#3 (Calcinaro et al., 2005). Another research identified that early supplemental probiotic use reduced the risk of islet autoimmunity in HLA genotype T1DM high-risk children (Uusitalo et al., 2016), while another demonstrated a negative result (Savilahti et al., 2018). Early oral probiotic medication *Clostridium butyricum* was also observed to prevent NOD mice from developing DM (Jia et al., 2017). Combination of galactooligosaccharides, *Bifidobacterium breve strain Yakult*, and *Lacticaseibacillus paracasei strain Shirota* improved the amounts of total *lactobacilli* and *Bifidobacterium*, the proportions of *Bifidobacterium*, and the concentrations of acetate and butyrate in excrement in T2DM patients (Kanazawa et al., 2021).

Colonizing the host gut is an important process by which probiotics function. However, according to a systematic review, probiotics had no impact on the fecal microbiome composition in six of the seven studies that have been examined (Kristensen et al., 2016). Therefore, using prebiotics to promote the propagation of probiotics is another idea to regulate gut microbiota composition. Prebiotics are non-digestible dietary components that promote the host's health by regulating the gut microbiota composition, especially by augmenting the abundance of *Bifidobacteria* and/or *Lactobacillus* (Jakaitis and Denning, 2014). Prebiotics improved glycemic control and decreased intestinal permeability in T1DM patients in a randomized placebo-controlled study, which enhanced insulin sensitivity (Ho et al., 2019). Human milk oligosaccharides (HMOs), the most common prebiotics that comprises multiple prebiotic oligosaccharides, are one of the most prevalent constituents of human milk (Knip and Honkanen, 2017). HMOs are believed to protect against autoimmune DM, possibly through selectively stimulating the growth of *Bifidobacteria* (Brown et al., 2011; de Goffau et al., 2013). In addition, HMOs also have microbially independent immunomodulatory properties, such as the induction of Treg cells (Lehmann et al., 2015) and the ability to maintain intestinal integrity (Aakko et al., 2017). Other prebiotics such as long-chain inulin-type fructosan and β-glucan-rich products have also been demonstrated to directly inhibit the progression of insulitis in NOD mice and reduce the incidence of DM (Chen et al., 2017; Gudi et al., 2019).

Fecal microbiota transplantation

FMT refers to the transfer of healthy microbiomes to dysregulated receptors in the gut microbiota to restore the normal bacterial community (Vindigni and Surawicz, 2017). Notably, these healthy microbiomes can be not only allogeneic (allo-FMT) but also autologous (auto-FMT). Auto-FMT is often implemented by collecting someone's microbiota when they are healthy and transplanting it back when their gut microbiota is out of whack. Participants with abdominal obesity or dyslipidemia can maintain losing weight and controlling blood glucose after accepting auto-FMT whose gut microbiota was collected during the weight-loss intervention phase (Rinott et al., 2021). Another recent randomized controlled trial discovered that auto-FMT prevented the decline in endogenous insulin secretion for 12 months after onset in patients with a recent diagnosis of T1DM, suggesting FMT can possibly prevent the ongoing β cells damage in T1DM patients (de Groot et al., 2021). Compared to auto-FMT, allo-FMT transplants healthy donors' gut microbiota to receivers, which has been an important therapy in treating chronic diarrhea caused by *Clostridium difficile* infections (van Nood et al., 2013; Saha et al., 2021). Early-life FMT from MyD88-deficient mice has been shown to significantly delay the onset of T1DM in NOD mice (Peng et al., 2014). In addition, the development of T1DM was also significantly delayed by the non-selective transplantation of human gut microbiota into GF NOD mice (Neuman et al., 2019). On the contrary, the incidence of T1DM significantly increased when mice received antibiotics (Livanos et al., 2016). Of note, in newly diagnosed T1DM patients, auto-FMT delivered through duodenal tubes is more effective than allo-FMT in protecting β-cell function (de Groot et al., 2021).

In recent years, the use of FMT in the treatment of T2DM has also made great progress. A study with T2DM mice discovered that FMT can reduce hyperglycemia, improve IR, inhibit the level of chronic inflammation in pancreatic, and reduce β-cell apoptosis (Wang et al., 2019). Similarly, it has been demonstrated that the insulin sensitivity of patients with metabolic syndrome increased after receiving the transfer of gut microbiota from lean donors (Vrieze et al., 2012; Kootte et al., 2017). In addition, a randomized clinical trial indicated that butyrate-producing microbiota increased while transferring the microbiota from healthy lean donors to T2DM patients (Ng et al., 2022). Moreover, recent research began to try to combine FMT with dietary or lifestyle intervention to treat T2DM and got better results than single FMT (Ng et al., 2022; Su et al., 2022). It has been observed that FMT can increase the

abundance of beneficial microbiota such as *Bifidobacterium* and decrease the level of *Sulfate-reducing bacteria*, *Desulfovibrio*, and *Bilophila* (Ng et al., 2022; Su et al., 2022). Similar to probiotics, FMT also has difficulty in colonizing the gut, but repeated FMTs can significantly increase the engraftment of lean-associated microbiota (Ng et al., 2022). Notably, FMT transfers not only the healthy microbiota to recipients but also compounds of potentially dangerous microbes (Walker, 2017; Hanssen et al., 2021).

Dietary intervention

Diet and nutrition play the most important influence on how the gut microbiota and the host interact over a lifetime. The ingestion of nutrients influences the composition of microbial metabolism and serves as a substrate for it (Albenberg and Wu, 2014). Therefore, diet interventions are effective ways to alter the gut microbiota composition and influence the host's health. The earliest human diet in life is breastmilk, and a meta-analysis elucidate that breastfed infant presents a stable and *Bifidobacteria*-dominating gut microbiota community, which is conducive to immune maturation (Uusitalo et al., 2016). A case–control study that found a lengthy breastfeeding time to be linked to a lower risk of T1DM suggests that exclusive and long-term breastfeeding is an independent protective factor for T1DM (Rosenbauer et al., 2008).

It has been reported that an animal-based diet reduced the abundance of metabolized plant polysaccharides *Firmicutes*, leading to a reduction in beneficial SCFAs (David et al., 2014), while high dietary fiber intake selectively promoted the growth of a group of SCFA-producing gut microbiota and increased HbA1c levels possibly by increasing the production of GLP-1 (Zhao et al., 2018). After 1 month of a vegan diet, obese patients with T2DM and/or hypertension presented a reduced F/B ratio and increased *Clostridium* and *Bacteroidetes fragile*, and they experienced significant reductions in HbA1c and triglyceride levels, weight loss, and improved fasting and postprandial glucose levels (Kim et al., 2013).In addition, high-fat diets regulate the composition of gut microbiota, mainly reducing the number of *Bifidobacterium* (Marietta et al., 2013). F/B ratio renewed while obese patients accepted carbohydrate-restricted or fat-restricted low-calorie diets (Cotillard et al., 2013; Bouter et al., 2017). Fiber-rich diet contributes to increasing *Prevotella*, whereas a protein-rich diet is associated with an increased abundance of *Bacteroides*. (Hartstra et al., 2015).

Concretely, green tea, caffeine, and omega-3 polyunsaturated fatty acids are beneficial for restoring the changed gut microbiota composition (Lau and Wong, 2018; Pascale et al., 2018). Consuming guar gum promoted T2DM patients to experience lipid-lowering effects (Li et al., 2021). Gluten intake promotes T1DM development by altering gut microbiota composition and immune response resulting in β -cell damage (Marietta et al., 2013). Zinc deficiency influences the inflammatory response and metabolic control (Xia et al., 2017), whereas Vitamin A deficiency increases the F/B ratio and decreases butyrate-producing gut microbiota level (Tian et al., 2018).

Antidiabetic drugs

The gut microbiota interacts with various popular hypoglycemic drugs, such as metformin, liraglutide, acarbose, and thiazolidinedione (Gurung et al., 2020; Lee et al., 2021; Smits et al., 2021; Takewaki et al., 2021). The anti-hyperglycemic effect of metformin has been traditionally attributed to its direct action on the signaling process in liver cells, leading to lower hepatic gluconeogenesis. However, it has been reported that metformin can alleviate the reduction of butyrate-producing microbiota, and gut microbiota supports the therapeutic effects of metformin through SCFAs production (Forslund et al., 2015). Treating the newly diagnosed T2DM naively with metformin can increase the amount of the bile acid GUDCA, thus improving IR (Sun et al., 2018). A randomized controlled trial showed that metformin strongly affected the gut microbiota and germ-free mice that received a transfer of fecal samples from metformin-treated donors presented improved glucose tolerance (Wu et al., 2017). In a rodent model, the level of *Lactobacillus* in the upper small intestine was increased, and sodium-glucose cotransporter-1 (SGLT1) expression was restored, thus increasing glucose sensitivity after using metformin (Bauer et al., 2018).

Conclusion and perspectives

In conclusion, the gut microbiota plays an important role in the occurrence and development of DM, and its composition in both T1DM and T2DM patients differs from that of healthy individuals. In general, opportunistic pathogens are usually reported increasing in DM patients, while probiotics are decreased. This review discusses the role of immune responses, inflammatory responses, metabolic disorders, and other aspects caused by gut microbiota dysbiosis in the pathogenesis of DM, enriching the content of intervention of DM through gut microbiota. This article also summarizes the progress of microbiological therapy in the prevention and treatment of DM, especially the current situation and application prospect of FMT in DM. Nowadays, there are a variety of drugs available to intervene in DM, but these drugs cannot sustainably and consistently prevent the progression of β-cell failure after the onset of DM. Therefore, it is of profound significance to intervene in DM through gut microbiota. In the future, we will verify the effectiveness of gut microbiota intervention in DM through clinical trials, and further, explore the advantages of gut microbiota intervention in DM.

Author contributions

JY and ZW were responsible for the design and manuscript writing. JY, ZW, and YZ were responsible for editing the structure of the article, obtaining the documents, and further arranging the manuscripts. YS and WL were responsible for the supervision, review, and final editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aakko, J., Kumar, H., Rautava, S., Wise, A., Autran, C., Bode, L., et al. (2017). Human milk oligosaccharide categories define the microbiota composition in human colostrum. *Benefic. Microbes* 8, 563–567. doi: 10.3920/BM2016.0185

Ahmad, T. R., and Haeusler, R. A. (2019). Bile acids in glucose metabolism and insulin signalling-mechanisms and research needs. *Nat. Rev. Endocrinol.* 15, 701–712. doi: 10.1038/s41574-019-0266-7

Albenberg, L. G., and Wu, G. D. (2014). Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology* 146, 1564–1572. doi: 10.1053/j.gastro.2014.01.058

Alkanani, A. K., Hara, N., Gottlieb, P. A., Ir, D., Robertson, C. E., Wagner, B. D., et al. (2015). Alterations in intestinal microbiota correlate with susceptibility to type 1 diabetes. *Diabetes* 64, 3510–3520. doi: 10.2337/db14-1847

Allcock, G. H., Allegra, M., Flower, R. J., and Perretti, M. (2001). Neutrophil accumulation induced by bacterial lipopolysaccharide: effects of dexamethasone and annexin 1. *Clin. Exp. Immunol.* 123, 62–67. doi: 10.1046/j.1365-2249.2001.01370.x

Allin, K. H., Tremaroli, V., Caesar, R., Jensen, B. A. H., Damgaard, M. T. F., Bahl, M. I., et al. (2018). Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* 61, 810–820. doi: 10.1007/s00125-018-4550-1

Amabebe, E., Robert, F. O., Agbalalah, T., and Orubu, E. S. F. (2020). Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. *Br. J. Nutr.* 123, 1127–1137. doi: 10.1017/S0007114520000380

Arboleya, S., Watkins, C., Stanton, C., and Ross, R. P. (2016). Gut Bifidobacteria populations in human health and aging. *Front. Microbiol.* 7:1204. doi: 10.3389/fmicb.2016.01204

Atkinson, M. A., Eisenbarth, G. S., and Michels, A. W. (2014). Type 1 diabetes. *Lancet* 383, 69–82. doi: 10.1016/S0140-6736(13)60591-7

Bach, J. F. (2018). The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat. Rev. Immunol.* 18, 105–120. doi: 10.1038/nri.2017.111

Bachem, A., Makhlouf, C., Binger, K. J., de Souza, D. P., Tull, D., Hochheiser, K., et al. (2019). Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated CD8(+) T cells. *Immunity* 51, 285–97.e5. doi: 10.1016/j.immuni.2019.06.002

Bauer, P. V., Duca, F. A., Waise, T. M. Z., Rasmussen, B. A., Abraham, M. A., Dranse, H. J., et al. (2018). Metformin alters upper small intestinal microbiota that impact a glucose-SGLT1-sensing Glucoregulatory pathway. *Cell Metab.* 27:e5, 101–117.e5. doi: 10.1016/j.cmet.2017.09.019

Bibbò, S., Dore, M. P., Pes, G. M., Delitala, G., and Delitala, A. P. (2017). Is there a role for gut microbiota in type 1 diabetes pathogenesis? *Ann. Med.* 49, 11–22. doi: 10.1080/07853890.2016.1222449

Bjursell, M., Admyre, T., Göransson, M., Marley, A. E., Smith, D. M., Oscarsson, J., et al. (2011). Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am. J. Physiol. Endocrinol. Metab.* 300, E211–E220. doi: 10.1152/ajpendo.00229.2010

Bonder, M. J., Kurilshikov, A., Tigchelaar, E. F., Mujagic, Z., Imhann, F., Vila, A. V., et al. (2016). The effect of host genetics on the gut microbiome. *Nat. Genet.* 48, 1407–1412. doi: 10.1038/ng.3663

Bouter, K. E., van Raalte, D. H., Groen, A. K., and Nieuwdorp, M. (2017). Role of the gut microbiome in the pathogenesis of obesity and obesity-related metabolic dysfunction. *Gastroenterology* 152, 1671–1678. doi: 10.1053/j.gastro.2016.12.048

Brown, C. T., Davis-Richardson, A. G., Giongo, A., Gano, K. A., Crabb, D. B., Mukherjee, N., et al. (2011). Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. *PLoS One* 6:e25792. doi: 10.1371/journal.pone.0025792

Calcinaro, F., Dionisi, S., Marinaro, M., Candeloro, P., Bonato, V., Marzotti, S., et al. (2005). Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. *Diabetologia* 48, 1565–1575. doi: 10.1007/s00125-005-1831-2

Canfora, E. E., Jocken, J. W., and Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* 11, 577–591. doi: 10.1038/nrendo.2015.128

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56, 1761–1772. doi: 10.2337/db06-1491

Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zac-Varghese, S. E., et al. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 64, 1744–1754. doi: 10.1136/gutjnl-2014-307913

Chen, K., Chen, H., Faas, M. M., de Haan, B. J., Li, J., Xiao, P., et al. (2017). Specific inulin-type fructan fibers protect against autoimmune diabetes by modulating gut immunity, barrier function, and microbiota homeostasis. *Mol. Nutr. Food Res.* 61. doi: 10.1002/mnfr.201601006

Chen, F., He, L., Li, J., Yang, S., Zhang, B., Zhu, D., et al. (2022). Polyethylene glycol Loxenatide injection (GLP-1) protects vascular endothelial cell function in middle-aged and elderly patients with type 2 diabetes by regulating gut microbiota. *Front. Mol. Biosci.* 9:879294. doi: 10.3389/fmolb.2022.879294

Cinek, O., Kramna, L., Mazankova, K., Odeh, R., Alassaf, A., Ibekwe, M. U., et al. (2018). The bacteriome at the onset of type 1 diabetes: a study from four geographically distant African and Asian countries. *Diabetes Res. Clin. Pract.* 144, 51–62. doi: 10.1016/j.diabres.2018.08.010

Classification and Diagnosis of Diabetes (2022). Standards of medical Care in Diabetes-2022. *Diabetes Care* 45, S17–s38. doi: 10.2337/dc22-S002

Cotillard, A., Kennedy, S. P., Kong, L. C., Prifti, E., Pons, N., Le Chatelier, E., et al. (2013). Dietary intervention impact on gut microbial gene richness. *Nature* 500, 585–588. doi: 10.1038/nature12480

Cummings, N. E., Williams, E. M., Kasza, I., Konon, E. N., Schaid, M. D., Schmidt, B. A., et al. (2018). Restoration of metabolic health by decreased consumption of branched-chain amino acids. *J. Physiol.* 596, 623–645. doi: 10.1113/JP275075

Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., et al. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. *Lancet* 378, 31–40. doi: 10.1016/S0140-6736(11)60679-X

Dave, M., Higgins, P. D., Middha, S., and Rioux, K. P. (2012). The human gut microbiome: current knowledge, challenges, and future directions. *Transl. Res.* 160, 246–257. doi: 10.1016/j.trsl.2012.05.003

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563. doi: 10.1038/nature12820

de Goffau, M. C., Luopajärvi, K., Knip, M., Ilonen, J., Ruohtula, T., Härkönen, T., et al. (2013). Fecal microbiota composition differs between children with β-cell autoimmunity and those without. *Diabetes* 62, 1238–1244. doi: 10.2337/db12-0526

de Groot, P., Nikolic, T., Pellegrini, S., Sordi, V., Imangaliyev, S., Rampanelli, E., et al. (2021). Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut* 70, 92–105. doi: 10.1136/gutjnl-2020-322630

de Mello, V. D., Paananen, J., Lindström, J., Lankinen, M. A., Shi, L., Kuusisto, J., et al. (2017). Indolepropionic acid and novel lipid metabolites are associated with a lower risk of type 2 diabetes in the Finnish diabetes prevention study. *Sci. Rep.* 7:46337. doi: 10.1038/srep46337

Devaraj, S., Dasu, M. R., Park, S. H., and Jialal, I. (2009). Increased levels of ligands of toll-like receptors 2 and 4 in type 1 diabetes. *Diabetologia* 52, 1665–1668. doi: 10.1007/s00125-009-1394-8

Di Tommaso, N., Gasbarrini, A., and Ponziani, F. R. (2021). Intestinal barrier in human health and disease. *Int. J. Environ. Res. Public Health* 18. doi: 10.3390/ijerph182312836

Durazzo, M., Ferro, A., and Gruden, G. (2019). Gastrointestinal microbiota and type 1 diabetes mellitus: the state of art. *J. Clin. Med.* 8. doi: 10.3390/jcm8111843

Fan, Y., and Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* 19, 55–71. doi: 10.1038/s41579-020-0433-9

Fang, S., Suh, J. M., Reilly, S. M., Yu, E., Osborn, O., Lackey, D., et al. (2015). Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat. Med.* 21, 159–165. doi: 10.1038/nm.3760

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 528, 262–266. doi: 10.1038/nature15766

Ghosh, S. S., Wang, J., Yannie, P. J., and Ghosh, S. (2020). Intestinal barrier dysfunction, LPS translocation, and disease development. Journal of the endocrine. *Society* 4:bvz039. doi: 10.1210/jendso/bvz039

Giesbertz, P., and Daniel, H. (2016). Branched-chain amino acids as biomarkers in diabetes. *Curr. Opin. Clin. Nutr. Metab. Care* 19, 48–54. doi: 10.1097/MCO.0000000000000235

Gudi, R., Perez, N., Johnson, B. M., Sofi, M. H., Brown, R., Quan, S., et al. (2019). Complex dietary polysaccharide modulates gut immune function and microbiota, and promotes protection from autoimmune diabetes. *Immunology* 157, 70–85. doi: 10.1111/imm.13048

Guigoz, Y., Doré, J., and Schiffrin, E. J. (2008). The inflammatory status of old age can be nurtured from the intestinal environment. *Curr. Opin. Clin. Nutr. Metab. Care* 11, 13–20. doi: 10.1097/MCO.0b013e3282f2bfdf

Gurung, M., Li, Z., You, H., Rodrigues, R., Jump, D. B., Morgun, A., et al. (2020). Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* 51:102590. doi: 10.1016/j.ebiom.2019.11.051

Hague, A., Butt, A. J., and Paraskeva, C. (1996). The role of butyrate in human colonic epithelial cells: an energy source or inducer of differentiation and apoptosis? *Proc. Nutr. Soc.* 55, 937–943. doi: 10.1079/PNS19960090

Han, H., Li, Y., Fang, J., Liu, G., Yin, J., Li, T., et al. (2018). Gut microbiota and type 1 diabetes. *Int. J. Mol. Sci.* 19. doi: 10.3390/ijms19040995

Hanssen, N. M. J., de Vos, W. M., and Nieuwdorp, M. (2021). Fecal microbiota transplantation in human metabolic diseases: from a murky past to a bright future? *Cell Metab.* 33, 1098–1110. doi: 10.1016/j.cmet.2021.05.005

Hartstra, A. V., Bouter, K. E., Bäckhed, F., and Nieuwdorp, M. (2015). Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 38, 159–165. doi: 10.2337/dc14-0769

Hasani, A., Ebrahimzadeh, S., Hemmati, F., Khabbaz, A., Hasani, A., and Gholizadeh, P. (2021). The role of Akkermansia muciniphila in obesity, diabetes, and atherosclerosis. *J. Med. Microbiol.* 70. doi: 10.1099/jmm.0.001435

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document: The international scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11, 506–514. doi: 10.1038/nrgastro.2014.66

Ho, J., Nicolucci, A. C., Virtanen, H., Schick, A., Meddings, J., Reimer, R. A., et al. (2019). Effect of prebiotic on microbiota, intestinal permeability, and glycemic control in children with type 1 diabetes. *J. Clin. Endocrinol. Metab.* 104, 4427–4440. doi: 10.1210/jc.2019-00481

Hooper, L. V., Midtvedt, T., and Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu. Rev. Nutr.* 22, 283–307. doi: 10.1146/annurev.nutr.22.011602.092259

Hou, K. J., Lin, C. J., Chen, C., Wu, B. T., Zhu, D., Zhong, W. J., et al. (2017). Association of probiotics and bone mineral density in Chinese patients with type 2 diabetes. *Biomed. Res.* 28, 129–133.

Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., et al. (2022a). Microbiota in health and diseases. *Signal Transduct. Target. Ther.* 7:135. doi: 10.1038/s41392-022-00974-4

Hou, K., Zhang, S., Wu, Z., Zhu, D., Chen, F., Lei, Z. N., et al. (2022b). Reconstruction of intestinal microecology of type 2 diabetes by fecal microbiota transplantation: why and how. *Bosn. J. Basic Med. Sci.* 22, 315–325. doi: 10.17305/bjbms.2021.6323

Isolauri, E. (2012). Development of healthy gut microbiota early in life. *J. Paediatr. Child Health* 48, 1–6. doi: 10.1111/j.1440-1754.2012.02489.x

Jakaitis, B. M., and Denning, P. W. (2014). Human breast milk and the gastrointestinal innate immune system. *Clin. Perinatol.* 41, 423–435. doi: 10.1016/j.clp.2014.02.011

Jandhyala, S. M., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., and Nageshwar, R. D. (2015). Role of the normal gut microbiota. *World J. Gastroenterol.* 21, 8787–8803. doi: 10.3748/wjg.v21.i29.8787

Janssen, A. W., and Kersten, S. (2017). Potential mediators linking gut bacteria to metabolic health: a critical view. *J. Physiol.* 595, 477–487. doi: 10.1113/JP272476

Jia, L., Shan, K., Pan, L. L., Feng, N., Lv, Z., Sun, Y., et al. (2017). Clostridium butyricum CGMCC0313.1 protects against autoimmune diabetes by modulating intestinal immune homeostasis and inducing pancreatic regulatory T cells. *Front. Immunol.* 8:1345. doi: 10.3389/fimmu.2017.01345

Jin, C. J., Sellmann, C., Engstler, A. J., Ziegenhardt, D., and Bergheim, I. (2015). Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH). *Br. J. Nutr.* 114, 1745–1755. doi: 10.1017/S0007114515003621

Kahn, S. E., Cooper, M. E., and Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet* 383, 1068–1083. doi: 10.1016/S0140-6736(13)62154-6

Kanazawa, A., Aida, M., Yoshida, Y., Kaga, H., Katahira, T., Suzuki, L., et al. (2021). Effects of synbiotic supplementation on chronic inflammation and the gut microbiota in obese patients with type 2 diabetes mellitus: a randomized controlled study. *Nutrients* 13. doi: 10.3390/nu13020558

Karlsson, F. H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C. J., Fagerberg, B., et al. (2013). Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 498, 99–103. doi: 10.1038/nature12198

Katsuma, S., Hirasawa, A., and Tsujimoto, G. (2005). Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem. Biophys. Res. Commun.* 329, 386–390. doi: 10.1016/j.bbrc.2005.01.139

Kayama, H., Okumura, R., and Takeda, K. (2020). Interaction between the microbiota, epithelia, and immune cells in the intestine. *Annu. Rev. Immunol.* 38, 23–48. doi: 10.1146/annurev-immunol-070119-115104

Khan, M. T., Nieuwdorp, M., and Bäckhed, F. (2014). Microbial modulation of insulin sensitivity. *Cell Metab.* 20, 753–760. doi: 10.1016/j.cmet.2014.07.006

Kim, M. S., Hwang, S. S., Park, E. J., and Bae, J. W. (2013). Strict vegetarian diet improves the risk factors associated with metabolic diseases by modulating gut microbiota and reducing intestinal inflammation. *Environ. Microbiol. Rep.* 5, 765–775. doi: 10.1111/1758-2229.12079

Kimura, I., Inoue, D., Maeda, T., Hara, T., Ichimura, A., Miyauchi, S., et al. (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc. Natl. Acad. Sci. U. S. A.* 108, 8030–8035. doi: 10.1073/pnas.1016088108

Kimura, I., Ozawa, K., Inoue, D., Imamura, T., Kimura, K., Maeda, T., et al. (2013). The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4:1829. doi: 10.1038/ncomms2852

Knip, M., and Honkanen, J. (2017). Modulation of type 1 diabetes risk by the intestinal microbiome. *Curr. Diab. Rep.* 17:105. doi: 10.1007/s11892-017-0933-9

Koh, A., Molinaro, A., Ståhlman, M., Khan, M. T., Schmidt, C., Mannerås-Holm, L., et al. (2018). Microbially produced imidazole propionate impairs insulin signaling through mTORC1. *Cells* 175, 947–61.e17. doi: 10.1016/j.cell.2018.09.055

Kootte, R. S., Levin, E., Salojärvi, J., Smits, L. P., Hartstra, A. V., Udayappan, S. D., et al. (2017). Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab.* 26, 611–9.e6. doi: 10.1016/j.cmet.2017.09.008

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A. M., et al. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 17, 260–273. doi: 10.1016/j.chom.2015.01.001

Kristensen, N. B., Bryrup, T., Allin, K. H., Nielsen, T., Hansen, T. H., and Pedersen, O. (2016). Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Med.* 8:52. doi: 10.1186/s13073-016-0300-5

Kuhre, R. E., Wewer Albrechtsen, N. J., Larsen, O., Jepsen, S. L., Balk-Møller, E., Andersen, D. B., et al. (2018). Bile acids are important direct and indirect regulators of the secretion of appetite and metabolism-regulating hormones from the gut and pancreas. *Mol. Metab.* 11, 84–95. doi: 10.1016/j.molmet.2018.03.007

Landman, C., and Quévrain, E. (2016). Gut microbiota: description, role and pathophysiologic implications. *La Revue de Med. Int.* 37, 418–423. doi: 10.1016/j.revmed.2015.12.012

Larraufie, P., Martin-Gallausiaux, C., Lapaque, N., Dore, J., Gribble, F. M., Reimann, F., et al. (2018). SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Sci. Rep.* 8:74. doi: 10.1038/s41598-017-18259-0

Larsen, N., Vogensen, F. K., van den Berg, F. W., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., et al. (2010). Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 5:e9085. doi: 10.1371/journal.pone.0009085

Lau, K., Benitez, P., Ardissone, A., Wilson, T. D., Collins, E. L., Lorca, G., et al. (2011). Inhibition of type 1 diabetes correlated to a lactobacillus johnsonii N6.2-mediated Th17 bias. *J. Immunol.* 186, 3538–3546. doi: 10.4049/jimmunol.1001864

Lau, L. H. S., and Wong, S. H. (2018). Microbiota, obesity and NAFLD. *Adv. Exp. Med. Biol.* 1061, 111–125. doi: 10.1007/978-981-10-8684-7_9

Lee, Y., Kim, A. H., Kim, E., Lee, S., Yu, K. S., Jang, I. J., et al. (2021). Changes in the gut microbiome influence the hypoglycemic effect of metformin through the altered metabolism of branched-chain and nonessential amino acids. *Diabetes Res. Clin. Pract.* 178:108985. doi: 10.1016/j.diabres.2021.108985

Lehmann, S., Hiller, J., van Bergenhenegouwen, J., Knippels, L. M., Garssen, J., and Traidl-Hoffmann, C. (2015). *In vitro* evidence for immune-modulatory properties of non-digestible oligosaccharides: direct effect on human monocyte derived dendritic cells. *PLoS One* 10:e0132304. doi: 10.1371/journal.pone.0132304

Leiva-Gea, I., Sánchez-Alcoholado, L., Martín-Tejedor, B., Castellano-Castillo, D., Moreno-Indias, I., Urda-Cardona, A., et al. (2018). Gut microbiota differs in composition and functionality between children with type 1 diabetes and MODY2 and healthy control subjects: a case-control study. *Diabetes Care* 41, 2385–2395. doi: 10.2337/dc18-0253

Li, J., Chen, R., Chen, Y., Zhu, D., Wu, Z., Chen, F., et al. (2021). The effect of guar gum consumption on the lipid profile in type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *Crit. Rev. Food Sci. Nutr.* 1–10. doi: 10.1080/10408398.2021.1981228

Li, W., and Ma, Z. S. (2020). FBA ecological guild: trio of firmicutes-bacteroidetes alliance against actinobacteria in human oral microbiome. *Sci. Rep.* 10:287. doi: 10.1038/s41598-019-56561-1

Li, J., Wang, X., Zhang, F., and Yin, H. (2013). Toll-like receptors as therapeutic targets for autoimmune connective tissue diseases. *Pharmacol. Ther.* 138, 441–451. doi: 10.1016/j.pharmthera.2013.03.003

Lian, K., Du, C., Liu, Y., Zhu, D., Yan, W., Zhang, H., et al. (2015). Impaired adiponectin signaling contributes to disturbed catabolism of branched-chain amino acids in diabetic mice. *Diabetes* 64, 49–59. doi: 10.2337/db14-0312

Livanos, A. E., Greiner, T. U., Vangay, P., Pathmasiri, W., Stewart, D., McRitchie, S., et al. (2016). Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nat. Microbiol.* 1:16140. doi: 10.1038/nmicrobiol.2016.140

Lomax, A. R., and Calder, P. C. (2009). Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. *Curr. Pharm. Des.* 15, 1428–1518. doi: 10.2174/138161209788168155

Mahendran, Y., Jonsson, A., Have, C. T., Allin, K. H., Witte, D. R., Jørgensen, M. E., et al. (2017). Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. *Diabetologia* 60, 873–878. doi: 10.1007/s00125-017-4222-6

Marietta, E. V., Gomez, A. M., Yeoman, C., Tilahun, A. Y., Clark, C. R., Luckey, D. H., et al. (2013). Low incidence of spontaneous type 1 diabetes in non-obese diabetic mice raised on gluten-free diets is associated with changes in the intestinal microbiome. *PLoS One* 8:e78687. doi: 10.1371/journal.pone.0078687

Mariño, E., Richards, J. L., McLeod, K. H., Stanley, D., Yap, Y. A., Knight, J., et al. (2017). Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat. Immunol.* 18, 552–562. doi: 10.1038/ni.3713

Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1, 135–145. doi: 10.1038/35100529

Medzhitov, R., and Horng, T. (2009). Transcriptional control of the inflammatory response. *Nat. Rev. Immunol.* 9, 692–703. doi: 10.1038/nri2634

Meijer, K., de Vos, P., and Priebe, M. G. (2010). Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health? *Curr. Opin. Clin. Nutr. Metab. Care* 13, 715–721. doi: 10.1097/MCO.0b013e32833eebe5

Mokhtari, P., Metos, J., and Anandh Babu, P. V. (2021). Impact of type 1 diabetes on the composition and functional potential of gut microbiome in children and adolescents: possible mechanisms, current knowledge, and challenges. *Gut Microbes* 13, 1–18. doi: 10.1080/19490976.2021.1926841

Mu, Q., Kirby, J., Reilly, C. M., and Luo, X. M. (2017). Leaky gut as a danger signal for autoimmune diseases. *Front. Immunol.* 8:598. doi: 10.3389/fimmu.2017.00598

Murri, M., Leiva, I., Gomez-Zumaquero, J. M., Tinahones, F. J., Cardona, F., Soriguer, F., et al. (2013). Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med.* 11:46. doi: 10.1186/1741-7015-11-46

Natividad, J. M., Petit, V., Huang, X., de Palma, G., Jury, J., Sanz, Y., et al. (2012). Commensal and probiotic bacteria influence intestinal barrier function and susceptibility to colitis in nod 1−/−; nod 2−/− mice. *Inflamm. Bowel Dis.* 18, 1434–1446. doi: 10.1002/ibd.22848

Neuman, V., Cinek, O., Funda, D. P., Hudcovic, T., Golias, J., Kramna, L., et al. (2019). Human gut microbiota transferred to germ-free NOD mice modulate the progression towards type 1 diabetes regardless of the pace of beta cell function loss in the donor. *Diabetologia* 62, 1291–1296. doi: 10.1007/s00125-019-4869-2

Ng, S. C., Xu, Z., Mak, J. W. Y., Yang, K., Liu, Q., Zuo, T., et al. (2022). Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial. *Gut* 71, 716–723. doi: 10.1136/gutjnl-2020-323617

Ohland, C. L., and Macnaughton, W. K. (2010). Probiotic bacteria and intestinal epithelial barrier function. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298, G807–G819. doi: 10.1152/ajpgi.00243.2009

Pascale, A., Marchesi, N., Marelli, C., Coppola, A., Luzi, L., Govoni, S., et al. (2018). Microbiota and metabolic diseases. *Endocrine* 61, 357–371. doi: 10.1007/s12020-018-1605-5

Pathak, P., Xie, C., Nichols, R. G., Ferrell, J. M., Boehme, S., Krausz, K. W., et al. (2018). Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. *Hepatology* 68, 1574–1588. doi: 10.1002/hep.29857

Pellegrini, S., Sordi, V., Bolla, A. M., Saita, D., Ferrarese, R., Canducci, F., et al. (2017). Duodenal mucosa of patients with type 1 diabetes shows distinctive inflammatory profile and microbiota. *J. Clin. Endocrinol. Metab.* 102, 1468–1477. doi: 10.1210/jc.2016-3222

Peng, L., He, Z., Chen, W., Holzman, I. R., and Lin, J. (2007). Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr. Res.* 61, 37–41. doi: 10.1203/01.pdr.0000250014.92242.f3

Peng, L., Li, Z. R., Green, R. S., Holzman, I. R., and Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr.* 139, 1619–1625. doi: 10.3945/jn.109.104638

Peng, J., Narasimhan, S., Marchesi, J. R., Benson, A., Wong, F. S., and Wen, L. (2014). Long term effect of gut microbiota transfer on diabetes development. *J. Autoimmun.* 53, 85–94. doi: 10.1016/j.jaut.2014.03.005

Perino, A., Pols, T. W., Nomura, M., Stein, S., Pellicciari, R., and Schoonjans, K. (2014). TGR5 reduces macrophage migration through mTOR-induced C/EBPβ differential translation. *J. Clin. Invest.* 124, 5424–5436. doi: 10.1172/JCI76289

Priyadarshini, M., Navarro, G., and Layden, B. T. (2018). Gut microbiota: FFAR reaching effects on islets. *Endocrinology* 159, 2495–2505. doi: 10.1210/en.2018-00296

Qi, C. J., Zhang, Q., Yu, M., Xu, J. P., Zheng, J., Wang, T., et al. (2016). Imbalance of fecal microbiota at newly diagnosed type 1 diabetes in Chinese children. *Chin. Med. J.* 129, 1298–1304. doi: 10.4103/0366-6999.182841

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., et al. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490, 55–60. doi: 10.1038/nature11450

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65. doi: 10.1038/nature08821

Que, Y., Cao, M., He, J., Zhang, Q., Chen, Q., Yan, C., et al. (2021). Gut bacterial characteristics of patients with type 2 diabetes mellitus and the application potential. *Front. Immunol.* 12:722206. doi: 10.3389/fimmu.2021.722206

Rinott, E., Youngster, I., Yaskolka Meir, A., Tsaban, G., Zelicha, H., Kaplan, A., et al. (2021). Effects of diet-modulated autologous fecal microbiota transplantation on weight regain. *Gastroenterology* 160, 158–73.e10. doi: 10.1053/j.gastro.2020.08.041

Roelofsen, H., Priebe, M. G., and Vonk, R. J. (2010). The interaction of short-chain fatty acids with adipose tissue: relevance for prevention of type 2 diabetes. *Benefic. Microbes* 1, 433–437. doi: 10.3920/BM2010.0028

Rosenbauer, J., Herzig, P., and Giani, G. (2008). Early infant feeding and risk of type 1 diabetes mellitus-a nationwide population-based case-control study in pre-school children. *Diabetes Metab. Res. Rev.* 24, 211–222. doi: 10.1002/dmrr.791

Ruiz-Canela, M., Guasch-Ferré, M., Toledo, E., Clish, C. B., Razquin, C., Liang, L., et al. (2018). Plasma branched chain/aromatic amino acids, enriched Mediterranean diet and risk of type 2 diabetes: case-cohort study within the PREDIMED trial. *Diabetologia* 61, 1560–1571. doi: 10.1007/s00125-018-4611-5

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., et al. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the international diabetes federation diabetes atlas, 9(th) edition. *Diabetes Res. Clin. Pract.* 157:107843. doi: 10.1016/j.diabres.2019.107843

Säemann, M. D., Böhmig, G. A., Osterreicher, C. H., Burtscher, H., Parolini, O., Diakos, C., et al. (2000). Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB J.* 14, 2380–2382. doi: 10.1096/fj.00-0359fje

Saha, S., Mara, K., Pardi, D. S., and Khanna, S. (2021). Long-term safety of fecal microbiota transplantation for recurrent Clostridioides difficile infection. *Gastroenterology* 160, 1961–9.e3. doi: 10.1053/j.gastro.2021.01.010

Sahuri-Arisoylu, M., Brody, L. P., Parkinson, J. R., Parkes, H., Navaratnam, N., Miller, A. D., et al. (2005). Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int. J. Obes.* 40, 955–963.

Savilahti, E., Härkönen, T., Savilahti, E. M., Kukkonen, K., Kuitunen, M., and Knip, M. (2018). Probiotic intervention in infancy is not associated with development of beta cell autoimmunity and type 1 diabetes. *Diabetologia* 61, 2668–2670. doi: 10.1007/s00125-018-4738-4

Schroeder, B. O., and Bäckhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nat. Med.* 22, 1079–1089. doi: 10.1038/nm.4185

Sedighi, M., Razavi, S., Navab-Moghadam, F., Khamseh, M. E., Alaei-Shahmiri, F., Mehrtash, A., et al. (2017). Comparison of gut microbiota in adult patients with type 2 diabetes and healthy individuals. *Microb. Pathog.* 111, 362–369. doi: 10.1016/j.micpath.2017.08.038

Shan, Z., Sun, T., Huang, H., Chen, S., Chen, L., Luo, C., et al. (2017). Association between microbiota-dependent metabolite trimethylamine-N-oxide and type 2 diabetes. *Am. J. Clin. Nutr.* 106, 888–894. doi: 10.3945/ajcn.117.157107

Shapiro, H., Kolodziejczyk, A. A., Halstuch, D., and Elinav, E. (2018). Bile acids in glucose metabolism in health and disease. *J. Exp. Med.* 215, 383–396. doi: 10.1084/jem.20171965

Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., and Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *J. Clin. Invest.* 116, 3015–3025. doi: 10.1172/JCI28898

Shreiner, A. B., Kao, J. Y., and Young, V. B. (2015). The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* 31, 69–75. doi: 10.1097/MOG.0000000000000139

Smits, M. M., Fluitman, K. S., Herrema, H., Davids, M., Kramer, M. H. H., Groen, A. K., et al. (2021). Liraglutide and sitagliptin have no effect on intestinal microbiota composition: a 12-week randomized placebo-controlled trial in adults with type 2 diabetes. *Diabetes Metab.* 47:101223. doi: 10.1016/j.diabet.2021.101223

Sommer, F., and Bäckhed, F. (2013). The gut microbiota--masters of host development and physiology. *Nat. Rev. Microbiol.* 11, 227–238. doi: 10.1038/nrmicro2974

Sorini, C., Cosorich, I., Lo Conte, M., De Giorgi, L., Facciotti, F., Lucianò, R., et al. (2019). Loss of gut barrier integrity triggers activation of islet-reactive T cells and autoimmune diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 116, 15140–15149. doi: 10.1073/pnas.1814558116

Staudinger, J. L., Goodwin, B., Jones, S. A., Hawkins-Brown, D., MacKenzie, K. I., LaTour, A., et al. (2001). The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3369–3374. doi: 10.1073/pnas.051551698

Stecher, B., and Hardt, W. D. (2011). Mechanisms controlling pathogen colonization of the gut. *Curr. Opin. Microbiol.* 14, 82–91. doi: 10.1016/j.mib.2010.10.003

Stoddart, L. A., and Smith, N. J. (2008). Milligan G. International Union of Pharmacology. LXXI. Free fatty acid receptors FFA1, −2, and −3: pharmacology and pathophysiological functions. *Pharmacol. Rev.* 60, 405–417. doi: 10.1124/pr.108.00802

Sturm, A., Rilling, K., Baumgart, D. C., Gargas, K., Abou-Ghazalé, T., Raupach, B., et al. (2005). Escherichia coli Nissle 1917 distinctively modulates T-cell cycling and expansion via toll-like receptor 2 signaling. *Infect. Immun.* 73, 1452–1465. doi: 10.1128/IAI.73.3.1452-1465.2005

Su, L., Hong, Z., Zhou, T., Jian, Y., Xu, M., Zhang, X., et al. (2022). Health improvements of type 2 diabetic patients through diet and diet plus fecal microbiota transplantation. *Sci. Rep.* 12:1152. doi: 10.1038/s41598-022-05127-9

Sudo, N., Yu, X. N., Aiba, Y., Oyama, N., Sonoda, J., Koga, Y., et al. (2002). An oral introduction of intestinal bacteria prevents the development of a long-term Th2-skewed immunological memory induced by neonatal antibiotic treatment in mice. *Clin. Exp. Allergy* 32, 1112–1116. doi: 10.1046/j.1365-2222.2002.01430.x

Sumida, K., Han, Z., Chiu, C. Y., Mims, T. S., Bajwa, A., Demmer, R. T., et al. (2022). Circulating microbiota in Cardiometabolic disease. *Front. Cell. Infect. Microbiol.* 12:892232. doi: 10.3389/fcimb.2022.892232

Sun, J., Furio, L., Mecheri, R., van der Does, A. M., Lundeberg, E., Saveanu, L., et al. (2015). Pancreatic β-cells limit autoimmune diabetes via an immunoregulatory antimicrobial peptide expressed under the influence of the gut microbiota. *Immunity* 43, 304–317. doi: 10.1016/j.immuni.2015.07.013

Sun, L., Xie, C., Wang, G., Wu, Y., Wu, Q., Wang, X., et al. (2018). Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat. Med.* 24, 1919–1929. doi: 10.1038/s41591-018-0222-4

Tai, N., Peng, J., Liu, F., Gulden, E., Hu, Y., Zhang, X., et al. (2016). Microbial antigen mimics activate diabetogenic CD8 T cells in NOD mice. *J. Exp. Med.* 213, 2129–2146. doi: 10.1084/jem.20160526

Takewaki, F., Nakajima, H., Takewaki, D., Hashimoto, Y., Majima, S., Okada, H., et al. (2021). Habitual dietary intake affects the altered pattern of gut microbiome by Acarbose in patients with type 2 diabetes. *Nutrients* 13. doi: 10.3390/nu13062107

Thaiss, C. A., Levy, M., Grosheva, I., Zheng, D., Soffer, E., Blacher, E., et al. (2018). Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science* 359, 1376–1383. doi: 10.1126/science.aar3318

The Integrative Human Microbiome Project (2019). The Integrative Human Microbiome Project. *Nature* 569, 641–648. doi: 10.1038/s41586-019-1238-8

Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., et al. (2009). TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* 10, 167–177. doi: 10.1016/j.cmet.2009.08.001

Tian, Y., Nichols, R. G., Cai, J., Patterson, A. D., and Cantorna, M. T. (2018). Vitamin a deficiency in mice alters host and gut microbial metabolism leading to altered energy homeostasis. *J. Nutr. Biochem.* 54, 28–34. doi: 10.1016/j.jnutbio.2017.10.011

Tlaskalová-Hogenová, H., Stěpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tučková, L., et al. (2011). The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cell. Mol. Immunol.* 8, 110–120. doi: 10.1038/cmi.2010.67

Tolhurst, G., Heffron, H., Lam, Y. S., Parker, H. E., Habib, A. M., Diakogiannaki, E., et al. (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* 61, 364–371. doi: 10.2337/db11-1019

Uusitalo, U., Liu, X., Yang, J., Aronsson, C. A., Hummel, S., Butterworth, M., et al. (2016). Association of Early Exposure of probiotics and islet autoimmunity in the TEDDY study. *JAMA Pediatr.* 170, 20–28. doi: 10.1001/jamapediatrics.2015.2757

Vallianou, N. G., Stratigou, T., and Tsagarakis, S. (2018). Microbiome and diabetes: where are we now? *Diabetes Res. Clin. Pract.* 146, 111–118. doi: 10.1016/j.diabres.2018.10.008

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., de Vos, W. M., et al. (2013). Duodenal infusion of donor feces for recurrent Clostridium difficile. *N. Engl. J. Med.* 368, 407–415. doi: 10.1056/NEJMoa1205037

Velloso, L. A., Folli, F., and Saad, M. J. (2015). TLR4 at the crossroads of nutrients, gut microbiota, and metabolic inflammation. *Endocr. Rev.* 36, 245–271. doi: 10.1210/er.2014-1100

Vindigni, S. M., and Surawicz, C. M. (2017). Fecal microbiota transplantation. *Gastroenterol. Clin. N. Am.* 46, 171–185. doi: 10.1016/j.gtc.2016.09.012

Vrancken, G., Gregory, A. C., Huys, G. R. B., Faust, K., and Raes, J. (2019). Synthetic ecology of the human gut microbiota. *Nat. Rev. Microbiol.* 17, 754–763. doi: 10.1038/s41579-019-0264-8

Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R. S., Bartelsman, J. F., et al. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 143, 913–6.e7. doi: 10.1053/j.gastro.2012.06.031

Walker, W. A. (2017). The importance of appropriate initial bacterial colonization of the intestine in newborn, child, and adult health. *Pediatr. Res.* 82, 387–395. doi: 10.1038/pr.2017.111

Wang, H., Lu, Y., Yan, Y., Tian, S., Zheng, D., Leng, D., et al. (2019). Promising treatment for type 2 diabetes: fecal microbiota transplantation reverses insulin resistance and impaired islets. *Front. Cell. Infect. Microbiol.* 9:455.

Wang, H. B., Wang, P. Y., Wang, X., Wan, Y. L., and Liu, Y. C. (2012). Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig. Dis. Sci.* 57, 3126–3135. doi: 10.1007/s10620-012-2259-4

Watanabe, M., Houten, S. M., Mataki, C., Christoffolete, M. A., Kim, B. W., Sato, H., et al. (2006). Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439, 484–489. doi: 10.1038/nature04330

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Mannerås-Holm, L., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.* 23, 850–858. doi: 10.1038/nm.4345

Xia, T., Lai, W., Han, M., Han, M., Ma, X., and Zhang, L. (2017). Dietary ZnO nanoparticles alters intestinal microbiota and inflammation response in weaned piglets. *Oncotarget* 8, 64878–64891. doi: 10.18632/oncotarget.17612

Xiong, Y., Miyamoto, N., Shibata, K., Valasek, M. A., Motoike, T., Kedzierski, R. M., et al. (2004). Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc. Natl. Acad. Sci. U. S. A.* 101, 1045–1050. doi: 10.1073/pnas.2637002100

Xu, Q., Ni, J. J., Han, B. X., Yan, S. S., Wei, X. T., Feng, G. J., et al. (2021). Causal relationship between gut microbiota and autoimmune diseases: a two-sample Mendelian randomization study. *Front. Immunol.* 12:746998. doi: 10.3389/fimmu.2021.746998

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., et al. (2012). Human gut microbiome viewed across age and geography. *Nature* 486, 222–227. doi: 10.1038/nature11053

Zhang, Y., Lee, F. Y., Barrera, G., Lee, H., Vales, C., Gonzalez, F. J., et al. (2006). Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1006–1011. doi: 10.1073/pnas.0506982103

Zhao, L., Lou, H., Peng, Y., Chen, S., Zhang, Y., and Li, X. (2019). Comprehensive relationships between gut microbiome and faecal metabolome in individuals with type 2 diabetes and its complications. *Endocrine* 66, 526–537. doi: 10.1007/s12020-019-02103-8

Zhao, L., Zhang, F., Ding, X., Wu, G., Lam, Y. Y., Wang, X., et al. (2018). Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. *Science* 359, 1151–1156. doi: 10.1126/science.aao5774

Zhou, D., Chen, Y. W., Zhao, Z. H., Yang, R. X., Xin, F. Z., Liu, X. L., et al. (2018). Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Exp. Mol. Med.* 50, 1–12. doi: 10.1038/s12276-018-0183-1

Zhou, D., Pan, Q., Xin, F. Z., Zhang, R. N., He, C. X., Chen, G. Y., et al. (2017). Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J. Gastroenterol.* 23, 60–75. doi: 10.3748/wjg.v23.i1.60

Zhou, Z., Sun, B., Yu, D., and Zhu, C. (2022). Gut microbiota: an important player in type 2 diabetes mellitus. *Front. Cell. Infect. Microbiol.* 12:834485. doi: 10.3389/fcimb.2022.834485

Zhou, H., Sun, L., Zhang, S., Zhao, X., Gang, X., and Wang, G. (2021). The crucial role of early-life gut microbiota in the development of type 1 diabetes. *Acta Diabetol.* 58, 249–265. doi: 10.1007/s00592-020-01563-z

Zhu, T., and Goodarzi, M. O. (2020). Metabolites linking the gut microbiome with risk for type 2 diabetes. *Curr. Nutr. Rep.* 9, 83–93. doi: 10.1007/s13668-020-00307-3

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The role and mechanism of gut microbiota-derived short-chain fatty in the prevention and treatment of diabetic kidney disease

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Diabetic kidney disease (DKD), an emerging global health issue, is one of the most severe microvascular complications derived from diabetes and a primary pathology contributing to end-stage renal disease. The currently available treatment provides only symptomatic relief and has failed to delay the progression of DKD into chronic kidney disease. Recently, multiple studies have proposed a strong link between intestinal dysbiosis and the occurrence of DKD. The gut microbiota-derived short-chain fatty acids (SCFAs) capable of regulating inflammation, oxidative stress, fibrosis, and energy metabolism have been considered versatile players in the prevention and treatment of DKD. However, the underlying molecular mechanism of the intervention of the gut microbiota–kidney axis in the development of DKD still remains to be explored. This review provides insight into the contributory role of gut microbiota-derived SCFAs in DKD.

KEYWORDS

diabetes, gut microbiota, diabetic kidney disease, pathogenesis, short chain fatty acids

1 Introduction

Diabetic kidney disease (DKD) is the leading cause of death among patients diagnosed with diabetes mellitus (DM) and is also a contributor to end-stage renal disease (ESRD) (1). According to the latest data from the International Diabetes Federation, the global population diagnosed with diabetes will reach 537 million and is expected to increase to 693 million by 2045. More than 147 million people affected by DKD are from China; the disease lays a heavy social burden on the medical care system, accompanied by increased family financial costs, low quality of life, and psychological problems (2).

DKD is a complex disease often related to four main characterized pathological changes, including mesangial proliferation, glomerular basement membrane thickening, podocyte loss, and glomerular sclerosis (3). The factors contributing to these pathological changes in patients with diabetes are mainly poor control of hyperglycemia, hypertension, and micro-inflammation (4). To counteract these apparently adverse factors, the recommended first-line therapies include strict blood glucose control with a dipeptidyl peptidase-4 inhibitor or metformin, hypertension control with a renin–angiotensin–aldosterone system inhibitor, and dietary control by reduced carbohydrate intake (5, 6). However, even after following these tight controls, some patients with diabetes still develop DKD, which eventually progresses to ESRD. Thus, it is crucial to fully elucidate the mechanism for the prevention and treatment of DKD (7).

Over recent years, the research community has attached great importance to the idea of the "gut–kidney" axis, first introduced by Meijiers in 2011 (8). The increasing understanding of the "gut–kidney" axis has provided deep insight into the symbiotic relationship between the gut microbiota and the kidney (9). The human gut microbiota harbors a diverse and complex microbial community with more than 100 trillion microorganisms and serves a key role in maintaining the integrity and function of the intestinal tract, regulating immune-inflammatory responses, the absorption and metabolism of nutrients, and removing toxins (10); these functions are beneficial in maintaining a dynamic balance between the gut microbiota and the host's health (11, 12).

Short-chain fatty acids (SCFAs), a type of saturated fatty acid containing less than six carbon atoms produced by the fermentation of the gut microbiome, are the most studied metabolite (13). The low concentration of SCFAs was observed in both patients with diabetes and diabetic mice but could be improved by supplementation (14, 15), implicating low levels of SCFAs in the pathogenesis of DKD and the positive impact of SCFA supplementation (16, 17). In this review, we summarize the functional role of SCFAs in the prevention and treatment of DKD and discuss the potential association between DKD and the intestinal microbiota microenvironment.

2 The pathogenesis regulating the development of diabetic kidney disease

2.1 The role of inflammation in diabetic kidney disease

A persistent inflammation induced by high-glucose is a leading cause of DKD *via* the activation of the generated advanced glycation end product (AGE) pathway, protein kinase C pathway, and polyol pathway, which promotes the expression of cytokines (such as monocyte-chemotactic protein-1, interleukin-1β, and toll-like receptors (TLRs) related to inflammatory pathways and macrophage infiltration leading to insulin resistance, proteinuria, and renal interstitial fibrosis (18–20). Podocytes are a type of terminally differentiated epithelial cell that play an important role in inhibiting the leakage of protein in a positive manner (21); Pyroptosis mediated by the nucleotide oligomerization domain-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome is a new type of cell death that serves as a key pathological factor contributing to accelerated DKD injury (22, 23). The absence of NLRP3 in animal disease models contributes to lowered expression of pro-inflammatory factors and improved renal fibrosis (24, 25). Studies on NLRP3 revealed suppressed activation of inflammasome and infiltration of macrophage in NLRP3 knock-out db/db mice and attenuated renal fibrosis through the blockage of the expression of profibrotic factors, such as transforming growth factor-β (TGF-β), mothers against decapentaplegic homolog 2 (Smad2), and Smad3 (26). Notably, AGEs are strongly toxic to cell survival by inducing the activation of pyroptosis (27). AGEs bind to their receptor expressed in podocytes and sharply reduce the survival rate of podocytes, leading to severe renal injury and the generation of proteinuria through the activation of NLRP3-mediated inflammation manifested by the increased expression of interleukin (IL)-1 β and IL-18 (28, 29). Other inflammatory cytokines like IL-1, IL-6, IL-18, and IL-17 with a potent pro-inflammatory impact were found to be upregulated in urine from patients with DKD, which could be employed as a potential inflammation biomarker in the diagnosis of DKD (30). In conclusion, the hyperglycemia-induced release of inflammatory cytokines is a vital indicator of DKD (31). Earlier detection and inhibition of these inflammatory cytokines may slow the development of DKD and its associated complications.

2.2 The role of oxidative stress in diabetic kidney disease

Oxidative stress is another key risk factor involved in the development of DKD (32). A certain amount of reactive oxygen species (ROS) is produced by metabolism in the human body and is capable of degrading bacteria (33). A normal antioxidant system

can eliminate the overgeneration of ROS (34). Altogether, the balance between ROS generation and degradation by the antioxidant system plays a large part in maintaining host health (35). However, in patients with DM and poor diabetic control, hyperglycemia induces ROS overproduction that exceeds the eliminating capacity of the antioxidant system, resulting in oxidative stress (22). ROS influences the progress of DKD mainly through the dysregulation of energy metabolism, inhibition of cell growth contributing to cell cycle arrest, alteration in the synergistic or antagonistic effects of a related protein, and activation of inflammation and the immune response mediated by various signaling pathways (23, 36). In one study, tubular epithelial cells treated with high-glucose medium induced ROS over-production leading to the deposition of mesangial cells and thickening of glomerular basement membrane *via* increased expression of TGF-β and collagen-I (37); ROS also facilitated the expression of pro-inflammatory factors that cause kidney injury. The ROS-induced NLRP3 inflammasome-mediated pyroptosis is a novel pathway involved in the development of DKD (38). Under the diabetic condition, the antioxidant enzymes lose their capacity against oxidative stress by glycation, which leads to the accumulation of ROS, consequently causing severe damage to podocytes resulting in accelerated tubular injury (39). Nicotinamide adenine dinucleotide phosphate oxidase (NADPH) is widely expressed in renal tissue and is also a major source of ROS (40). The mechanism of the role of ROS in the formation of renal fibrosis can be interpreted as follows: High glucose induces the increased expression of NADPH oxidase 4 (NOX4), contributing to the overproduction of ROS, which activates the TGF-β/Smads pathway and other pro-fibrotic factors (36, 41). Moreover, ROS-induced mitochondrial damage aggravates DKD (42). The electrons leak from the respiratory chain and combine with oxygen to form superoxide, which promotes ROS production. Subsequently, ROS disturb the mitochondrion antioxidant capacity, followed by the overexpression of Bcl-2-associated X-protein and caspase-3, leading to the apoptosis of podocytes (43).

2.3 The role of autophagy in diabetic kidney disease

Autophagy, an adaptive system reacting to various stimuli, plays a significant role in maintaining the homeostasis of the intracellular environment *via* impaired protein degradation and recycling of these degraded materials as an energy source for cellular activity (44). Energy metabolism is believed to greatly impact the activation of autophagy. The mammalian targets of rapamycin (mTOR), adenosine monophosphate-activated protein kinase (AMPK), and sirtuins (SIRT) are the three best-known targets that regulate nutrient sensing pathways (45). In DKD, mTOR is a vital autophagy regulator and includes two complexes, the mTOR complex 1 (mTORC1) and complex 2 (mTORC2) (46). Under normal conditions, autophagy is negatively regulated by mTORC1 *via* phosphorylated unc-51-like kinase 1 (ULK1). However, the higher expression of mTORC1 is often detected in rodents with DKD, which leads to the inhibition of autophagy (47). In streptozotocin (STZ)-induced diabetic mice with hyperactivation of mTORC1, the inhibition of autophagy contributes to renal injury and proteinuria, and the pathological change is manifested by extracellular matrix accumulation and cell death that promote DKD progression to renal failure (48). Notably, the inhibition of autophagy was reversed by rapamycin (a mTORC1 inhibitor) in high-glucose-treated podocytes (49). On the other hand, AMPk promotes the activation of autophagy by sensing the AMP/ATP ratio (50). Excessive energy production reduces AMPk expression in animals with diabetes, which was rescued by treatment with an AMPk activator that alleviated kidney injury (51). Autophagy was also boosted in high-glucose cultured podocytes by the administration of an AMPk activator for apoptosis induction. Mechanistically, AMPk-mediated regulation of autophagy is related to the phosphorylation of ULK1, TSC1/2, and raptor and the inhibition of mTORC1 (52). The effect of SRIT1 is similar to that of APMk in inducing autophagy. Under the diabetic condition, SIRT1 expression is largely suppressed, inhibiting autophagy and contributing to accelerated renal damage (53). The treatment of the animal model with resveratrol (a SIRT1 activator) led to the elevated expression of SIRT1, which could restore autophagy activity and thus protect cells from hyperglycemia. Particularly, overexpression of SIRT1 in STZ-induced diabetic mice had a beneficial effect on the inhibition of podocyte damage and renal fibrosis (54). The mechanism of SIRT1-mediated protection of autophagy relies on its role in deacetylating essential autophagy proteins, such as autophagy-related (ATG)5, ATG7, and light chain3. In conclusion, the restoration of autophagy exhibited a great renal protective effect in preventing DKD (53).

2.4 The role of fibrotic mediators in DKD

TGF-β is key factor leading to end-stage renal disease with the features of glomerulosclerosis and renal fibrosis. Its mechanism is associated with activating intracellular signal pathways such as protein kinase and enhanced expression of cytokines to promote fibrosis, which leads to end-stage renal disease (55). Almost all types of kidney cells are capable of secreting TGF-β, and highly expressed TGF-β receptors were detected in those cell membranes. They exerted pro-fibrotic function *via* autocrine and paracrine pathways that contributed to the occurrence and development of renal fibrosis in diabetes (56). The TGF-β is an upstream regulator of TGF-β/Smads signaling pathway, which is central to the fibrotic component of diabetic kidney damage (57). Multiple exogenous stimulators such as angiotensin-II, protein kinase C, and 38 mitogen-activated protein kinase-dependent pathways

could trigger the expression of TGF-β. Under diabetic conditions, hyperglycemia induced massive amounts of ROS production and attacked cell membranes, which lead to upregulation of TGF-β (58). TGF-β mediated renal fibrosis *via a* complex mechanism, including over-generation of the extracellular matrix, and dedifferentiation of tubular epithelial and glomerular endothelial cells. Smad signaling is key downstream regulator of the TGF-β pathway. In the process of renal fibrosis in an animal model with DKD, the increased level of TGF-β interacts with its receptors and triggers activation of Smad-dependent pathways, Smad2 and Smad3 presented higher expression in kidney tissues, and Smad7 was inhibited (57). Altogether, the TGF-β/Smads pathway was activated to accelerate the formation of renal fibrosis.

2.5 The role of abnormal metabolism regulation in DKD

The abnormal glucose metabolism in patients with DKD is characterized by the increased production of advanced glycation end products (AGEs), activation of the protein kinase C (PKC) pathway, and enhanced polyol pathways, which play a significant role in promoting the development of DKD (59). Long-term chronic hyperglycemia stimulates the overgeneration of AGEs by interacting with its receptor, which upregulated the levels of nuclear factor κB (NF-κB), vascular endothelial growth factor (VEGF), transforming growth factor-β 1(TGF-β 1), and monocyte chemoattractant protein-1 (MCP-1) (60). It was believed that progressive glomerulosclerosis in DKD is strongly associated with the increased expression of these protein levels, acting as pro-inflammatory and pro-fibrotic factors that contribute to podocyte injury and extra mesangial matrix (ECM) accumulation. Endothelial nitric oxide synthase (eNOS) is an important enzyme capable of protecting the integrity of vascular endothelial cells (61). The upregulation of PKC induced by high glucose resulted in the decreased production of eNOS and an increase in VEGF levels, which accelerated the development of DKD (62).

3 The role of gut microbiota dysbiosis implicated in the pathogenesis of diabetic kidney disease

3.1 The reduction in short-chain fatty acids generation aggravates diabetic kidney disease

Intestinal microbiota dysbiosis was observed in patients with DKD, which was attributed to the limited uptake of foods containing high fiber and fruits, resulting in the insufficient production of SCFAs (63). SCFAs, including acetate, propionate, and butyrate, are the end products of polysaccharide fermentation in the distal gut microbiome (64). The absorption of butyrate by intestinal epithelium is a major source of energy for the phosphorylation of AMPK and for promoting the release of glucagon-like peptide-1 (GLP1) (17). The acetate and propionate must bind to G-protein-coupled receptors (GPCRs) 41 or 43 expressed on intestinal epithelium to perform their basic functions (17). The activation of GPR41 promotes the secretion of peptide YY (PYY) and controls satiety and intestinal transit. On the other hand, GPR43 inhibits the production of pro-inflammatory factors and enhances GLP1 secretion, which contributes to the production and induces the proliferation of pancreatic β cells and thus exerts renal protection against DKD by reducing blood glucose levels (65). Altered gut microbiota composition was detected in STZ-induced diabetic mice and led to a decrease in the concentration of SCFAs, causing a decline in the secretion of PYY and GLP1, and hence accelerating the development of DKD manifested by proteinuria, loss of renal structure integrity, and renal fibrosis (66). The administration of SCFAs or GPR41 agonists can both hamper the development of DKD by inhibiting the expansion of the high-glucose-induced mesangial cell line, the generation of ROS, and suppression of the expression of pro-inflammatory cytokines, such as monocyte-chemotactic protein-1 (MCP-1) and IL-1β (67). These aspects altogether prove that the low level of SCFAs in the intestinal tract due to intestinal dysbiosis in DM patients has a strong link to the development of DKD.

3.2 The role of LPS in diabetic kidney disease

Although a tight control of hyperglycemia is effective against DM and is beneficial to the delay in the progression of DKD, in one study, some patients with diabetes did not reach the stage of DKD even after poor control over hyperglycemia (68). Multiple studies have proposed the idea of alteration in the gut microbiome composition, which serves as a novel indicator of host health. A large number of toxic metabolites are generated and accumulated in patients with DKD due to the dysfunction of gut microbiota dysbiosis in degrading these toxic metabolites that consequently lead to oxidative stress and inflammation (69). In addition, the integrity of the intestinal barrier is impaired, resulting in increased permeability and thus favoring the invasion of pathogenic bacteria and their toxic metabolites (69). The circulating pathogenic bacteria or toxic metabolites in the bloodstream attack the cells and favor the expression of an inflammatory response mediated by endogenous danger signal transduction, which affects the development of DKD (70). Lipopolysaccharides (LPSs), also called endotoxin, is one of the most potent toxic metabolites and is an antigen present on

the surface of Gram-negative bacteria; LPSs regulate the activation of inflammation and immune reaction, which thus leads to numerous metabolic diseases (71). The intestinal barrier loses its integrity under dysbiosis conditions, facilitating LPS influx into the systemic circulation to distal organs such as the kidney, which leads to accelerated renal impairment. Prolonged inflammation is a major contributor to DKD. TLRs, such as TLR2 and TLR4, are implicated in the pathogenesis of DKD through the stimulation of an inflammatory response. Growing evidence supports LPS-mediated inflammation in renal tissue by the activation of TLR2 and TLR4-related pathways (72). The mechanism of accelerated DKD damage is associated with the activation of the MyD88/NF-κB pathway by the binding of LPS to TLRs that contributes to increased production of pro-inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-1(IL-1), and IL-6 (73). In TLR2 knock-out mice, low MyD88 expression is observed in renal tissue. Meanwhile, TLR2 deficiency has a positive impact on the attenuation of podocyte damage, the reduction of proteinuria, and inhibiting macrophage infiltration. The LPS–TLR4 axis is responsible for the regulation of inflammatory reaction that results in the upregulation of TGF-β, contributing to accelerated renal fibrosis (74). It is becoming increasingly apparent that targeting the LPS–TLRs–MyD88-mediated inflammatory pathway by restoring dysbiotic gut microbiome activation may be a novel therapy against DKD.

3.3 The role of abnormal gut microbiota in activating the immune response

The synergistic or antagonistic correlation between the gut microbiome and immune axis is necessary for maintaining host health. The immune system eliminates pathobionts and thus plays an important role in keeping the host healthy (75). A study on DKD indicated that toxic metabolites produced by an abnormal microbial community contribute to accelerated DKD damage by obstructing the capacity of the immune system to degrade toxin materials. Enhanced activation of complement 5 (C5), a vital regulator in the initial stage of the immune system, was observed in db/db mice, which promoted the overexpression of inflammatory factors and activation of the TGF-β/Smads-mediated fibrotic pathway in the kidney, causing both chronic inflammation and kidney injury (76). Under the pathological condition of DKD, the immune complexes formed by pathogens and antibodies depose in the glomeruli to stimulate complement activation, which is responsible for accelerated kidney injury *via* the recruitment of immune cells (77). A large amount of C5a resides in the intestinal tract, suggesting that the altered intestinal tract microenvironment negatively affects the abundance of C5 by modifying the composition of the intestinal microbiome. The gut tract of db/db mice is characterized by a low abundance of *Proteobacteria* and *Epsilonbacteraeota*, which were restored at the phylum level after administration of the C5aR antagonist (C5aRA) (78). Meanwhile, treatment with C5aRA also rescued the decreased production of SCFAs. Over-activation of C5-induced abnormal gut microbiota was found to be responsible for reduced production of SCFAs (79). The C5-mediated STAT3 pathway participates in the development of DKD, leading to the inflammatory response. However, SCFA supplementation exerts a positive effect on the remission of DKD by inhibiting C5 expression. Also, other toxic metabolites, such as trimethylamine N-oxide derived from permeate into the circulatory system through the intestinal epithelium and accumulate in the kidney, which can be recognized by the immune system and forms an immune complex, which subsequently initiates a series of immune-mediated reactions, such as inflammation that affect the progression of DKD (80). In conclusion, an immune reaction mediated by gut microbiota dysbiosis damages the structure and function of the kidney in the DM condition.

3.4 Gut microbiota dysbiosis increased intestinal permeability in DKD

Under normal circumstances, the tight junctions, intestinal epithelial cell membranes, mucus secretion, and gut innate immune defensive mechanisms are the four main parts constituting a complete gut barricade defense system, which is of great importance to preclude the entry of hazardous substances or microorganisms from the intracavity to the circulation system (81); It is becoming increasingly apparent that the occurrence of DKD has a strong link to the disrupted gut barrier defense system. Amounting studies indicated the fact that under the influence of gut microbiota dysbiosis, the permeability of the gut barricade was increased in both patients with DKD and animal models due to structural and functional abnormalities of the gut tract. The intestinal dysbiosis-derived bacteria may take advantage of the leaky gut barrier to enter the bloodstream and act as pathogens or antigens, which trigger an immune reaction. ZO-1 and occluding are two important epithelial tight junction proteins keeping a tight state of the gut tract (82). It was reported that the altered gut microbiota composition in high-fat diet-fed db/db mice caused the downregulation of ZO-1 and occluding levels, which led to increased intestinal permeability and enhanced gut microbiota dysbiosis-derived pathogen absorption, thereby speeding up the deterioration of DKD through triggering tissue inflammatory responses, ROS generation and inflammatory

cell infiltration (83). Notably, these unfavorable changes could be attenuated by employing antibiotic therapy. The underlying mechanism of these beneficial effects may be associated with some agents' capacity for improving the gut barricade and reducing intestinal permeability by regulating gut microbiota and alleviating DKD-related complications (84).

3.5 Other toxic metabolites derived from gut microbiota aggravated the development of DKD

The human body in a healthy state can tolerate a small number of toxic products derived from gut microbiota. However, the increased production of toxic metabolites damaged the gut barricade due to the disorders of the gut environment and alteration in the composition of the gut microbiota, which led to insulin resistance, energy metabolism disorder and immune-inflammatory response in those DKD diagnosed. Trimethylamine N-oxide (TMAO) is a waste product derived from the digestion of red meat by gut microbiota, which has recently emerged as a gut microbiota-dependent metabolite linked to DKD. TMAO participates in the regulation of lipid and glucose metabolism and influences the gut, liver, kidney, and heart through a physiological connection. Multiple studies indicated that the increased concentration of TMAO in the bloodstream is positively correlated with the occurrence of atherosclerosis, thrombosis, and diabetes (85). The elevated TMAO plasma level was observed in C57BL/6J mice after chronic exposure to the provision of supplementary choline or the TMAO diet, with overexpression of collagen and tubulointerstitium ECM accumulation (86). These pathological changes contribute to progressive renal functional loss and renal fibrosis. Moreover, clinical studies have demonstrated that, compared to non-diabetes, the TMAO plasma level is higher in patients with diabetes (87). The unhealthy lifestyle and lack of fiber in the diet have become a major part of modern life and contribute to disorders of the gut microbiota, which are widely found. The uremic toxins, a waste product generated by the imbalance of gut microbiota, play a regulatory role in the activation of various cellular signaling pathways that mediate inflammation, oxidative stress, and apoptosis and influence the development of DKD (88). Under the influence of uremic conditions, the persistent activation of aryl hydrocarbon receptors (AhRs) induced by overgeneration of indoxyl sulfate (IS) results in the apoptosis of podocytes, and a decline in the glomerular filtration rate, and upregulation of pro-inflammatory cytokines (89). In addition, phenyl sulfate (PS), a metabolite derived from intestinal microflora, is reported to be associated with the occurrence of proteinuria in diabetes patients. Taken together, these systemic circulating toxic metabolite levels could be employed as a hallmark of gut microbiota dysfunction.

The above-discussed mechanism of intestinal microbiota dysregulation in the pathogenesis of DKD is summarized in Figure 1.

4 The role of short-chain fatty acids in the prevention and treatment of the diabetic kidney disease

Sufficient production of SCFAs ensures the host's health by exerting multiple protective effects against various diseases, including diabetes, obesity, and cardiovascular disease. This beneficial outcome is contributed by SCFAs in regulating energy metabolism, inhibiting inflammation and oxidative stress, and regulating the immune response. Studies on the role and related mechanisms of SCFAs in the prevention and treatment of DKD are summarized in Table 1 and Figure 2.

4.1 The role of short chain fatty acids in regulating energy metabolism

The SCFAs provide energy for the maintenance of normal physiological activity of the gut tract (92). The disturbance in energy supply caused by the SCFAs is attributed to the altered composition of intestinal microbiota that causes unwanted effects such as obesity, poor diabetic control, and severe DKD-related vascular diseases. To date, studies have focused on the protective effect of SCFAs as energy metabolism regulators in treating DKD (13). Low production of SCFAs was found in patients with DKD. The administration of SCFAs or transplantation of normal fecal samples from individuals with normal blood glucose could have a positive impact on reducing blood glucose and proteinuria and improving life quality (13). The dietary supplementation of SCFAs or a high fiber diet (a major source of SCFAs) could attenuate albuminuria excretion, lower blood lipids, and prevent renal glomerulus injury in STZ-induced diabetic mice. These positive results were not only observed in animal experiments but also in clinical studies on human beings. In mice with an obesity or DKD diagnosis, less progress was made in controlling the fasting blood glucose level or the body weight after strict control of food intake and drugs for therapy. However, after long-term treatment with acetate or butyrate, DKD or obesity were relieved (65). The high affinity of SCFAs for binding GPCRs is necessary for multiple functions of SCFAs. The protective mechanism of SCFAs in regulating a stable blood glucose level may be related to their involvement in glucose synthesis. GLP1 and PYY are two important hormones responsible for energy metabolism *via* SCFA-mediated GPCR activation (15). The binding of SCFAs to GPR43 expressed on the cell membrane directly stimulates the goblet cells of the colon to secrete GLP1; the generation of GLP-1 is necessary for the induction and proliferation of pancreatic β cells to perform their role in controlling body weight, reducing blood glucose level, increasing insulin sensitivity, and inhibiting risk factors associated with diabetes (67). The SCFA-mediated GPR41 pathway stimulates entero-endocrine cells to secrete more PYY, which suppresses the increase in blood glucose levels,

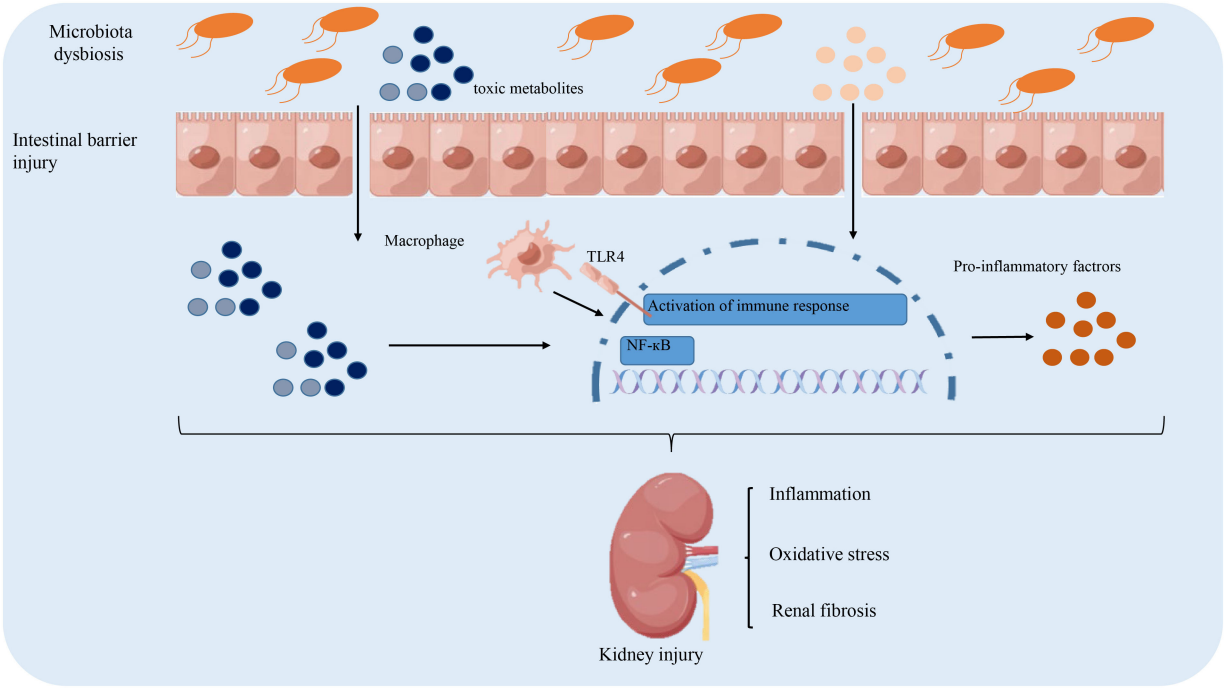

FIGURE 1
The role of gut microbiota dysbiosis implicated in the pathogenesis of diabetic kidney disease. Intestinal dysbiosis results in the accumulation of toxic metabolites, which damage the integrity of the intestinal barrier to facilitate the influx of toxic metabolites into the circulation. This triggers an immune response to secrete pro-inflammatory cytokines that contribute to renal fibrosis and oxidative stress. Altogether, these pathological factors lead to the accelerated progression of DKD.

prohibits gastric emptying, and reduces food intake. In conclusion, the SCFA-mediated GPRs pathway modulates energy metabolism by regulating the secretion of different hormones.

4.2 The role of short-chain fatty acids in inhibiting inflammation and oxidative stress

Prolonged inflammation is a characteristic of DKD. Although the mechanisms by which gut microbiome influences host health and renal therapy have not yet been fully explained, the role of SCFAs has been partly implicated in modulating inflammation (20). Several in-depth studies provide evidence of SCFAs serving as a protective regulator in the reduction of inflammation *via* inhibition of monocyte recruitment and chemokine production. An investigation into the pharmacological effect of SCFAs indicated a significant attenuation of renal function after sodium propionate treatment in DM patients diagnosed with DKD (93). Propionate exerts its anti-inflammatory effect by curbing the expression of inflammatory indicators, including IL-2, IL-17, IL-6, and TNF-α, and for promoting the expression of

TABLE 1 Short-chain fatty acids exert multiple functions against diabetic kidney disease.

Effects of SCFAs	Protective mechanism-related index	References
Anti-inflammation	Reducing pro-inflammatory factors: IL-1, IL-6, TNF-α. Blocking the activation of the NF-κB pathway.	Wu et al. (90)
Anti-oxidative stress	Inhibiting HDAC-mediated NOX2/ROS signaling pathway to reduce ROS generation.	Al-Harbi et al. (91)
Improvement in energy metabolism	Promoting the secretion of GLP1and PYY to increase insulin sensitivity, inhibit gastric emptying, and reduce body weight.	Everard et al. (65)
Improvement in renal function	Protecting the structural integrity of podocytes to reduce proteinuria.	Li et al. (15)

TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; NF-κB, nuclear factor kappa beta; HDAC, Histone deacetylase; NOX, NADHP oxidase; ROS, reactive oxygen species; GLP1, Glucagon-like peptide-1; PYY, peptide YY.

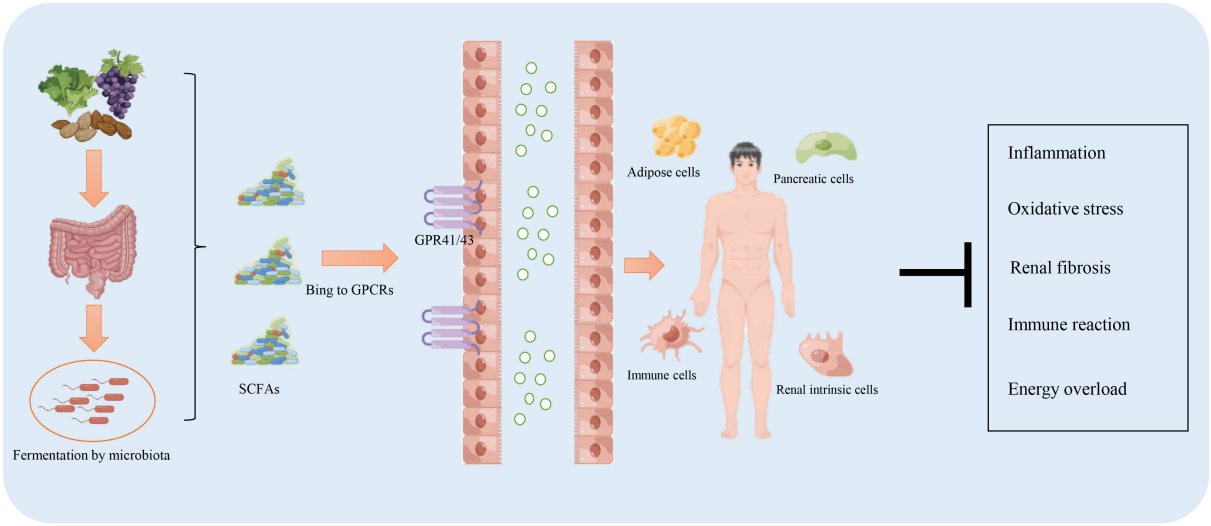

FIGURE 2
The function of short-chain fatty acids (SCFAs) in the prevention and treatment of diabetic kidney disease. The intake of a high-fiber diet promotes the production of SCFAs through fermentation by the microbiota residing in the digestive system. SCFAs facilitate resistance to inflammation, the immune response, energy overload, and renal fibrosis mainly *via* binding to G protein-coupled receptors (GPR)41/43.

anti-inflammatory factor (IL-10) (14). Other studies also suggest the benefits of daily consumption of a certain amount of high-fiber diet in producing more SCFAs in patients with DKD. These beneficial results were also observed in other kidney diseases, such as acute kidney injury (AKI) (94). AKI in animal models is characterized by a fast decline in renal function and a high death rate. The NF-kB signaling pathway plays an irrefutable role in promoting inflammation and contributing to AKI, which could be blocked by the SCFAs (95). The anti-inflammatory effect of SCFAs is also related to their inhibitory effect on HDAC activity. Under diabetic conditions, oxidative stress and inflammation coexist and interact with each other to enhance the condition. Toll-like receptor 4 (TLR4) is a pro-inflammatory factor that is highly expressed in diabetic mice. The oxidative stress condition results in the increased expression of NOX2, which further deteriorates the antioxidant system (94). The generation of ROS and inflammation, enhanced by the TLR4/NOX2 pathway, was reversed by acetate-mediated inhibition of HDAC activity (73). Thus, a better understanding of the benefits derived from SCFAs involved in DKD will have important clinical value.

4.3 The role of short-chain fatty acids in regulating the immune response

Apart from the beneficial results mentioned above, SCFAs regulate immune system homeostasis and the integrity of the intestinal epithelial barrier. The close interaction between epithelial cells and immune cells establishes a solid defense system against the invasion and accumulation of lethal microorganisms (96). Intensive studies have been conducted to understand the relationship between the SCFA-mediated immune response axis and DKD. Most patients with diabetes are troubled by various infectious diseases, such as respiratory tract and urinary tract infections, caused by the decline in immunity induced by intestinal dysbiosis (97). The STZ-induced DKD mice treated with SCFAs had a renal protective effect in terms of reduced proteinuria, a decrease in blood glucose level, and attenuated renal fibrosis. These beneficial outcomes are associated with the inhibitory effect of SCFAs on the expression of TLR4 as well as blocking NF-κB pathway activation (98). Moreover, less macrophage infiltration was observed in renal tissues. The large scale of Th17 cell infiltration was observed in the animal model of DKD, which is generally recognized as a key to initiating an immune response through the secretion of IL-17A and IL17F to produce chemokines, which in turn recruit more Th17 cells, finally leading to inflammation (99). The mechanism of SCFA-mediated renal protection depends on its ability to inhibit the infiltration of Th17 cells, reduce the permeability of the disrupted intestinal barrier, and alleviate oxidative stress conditions.

4.4 The role of short-chain fatty acids in anti-fibrosis

The immune-inflammatory axis plays vital roles in promoting the formation of renal fibrosis, leading to ERSD. SCFAs had

shown great benefits in inhibiting immune response and inflammation. The treatment with acetate and butyrate, the main effective ingredients of SCHFAs, was reported to be capable of alleviating tubule interstitial fibrosis as well as reducing ECM deposition in STZ-induced diabetic mice (100). Increasing the SCFA-producing bacteria by XOS supplementation has a positive impact on inhibiting renal fibrosis as well as the infiltration of M2 macrophages (101). HDAC activity was known to contribute to renal fibrosis. Valproic acid was capable of inhibiting HDAC activity, resulting in the reduced phosphorylation of ERK, which further inhibited the proliferation of pericytes to block Ang II-induced fibrosis (102). Under diabetic conditions, the persistent low-grade inflammation induced higher expression of transforming growth factor beta 1 (TGF-β) presented in stimulated tubular epithelial cells, which was reversed by the administration of butyrate (103). It was worth noticing that inhibiting renal fibrosis is a significant method to delay the progression of DKD to ESRD. The mechanism of SCFAs conferring anti-fibrosis protection on DKD is mainly through inhibiting the activation TGF-β pathway, HDAC activity, and reducing phosphorylation of ERK. It is still necessary to explore other SCFAs' related functions against renal fibrosis.

5 Medical therapies against gut microbiota disorders in DKD

5.1 The role of dietary fiber in regulating gut microbiota

Dietary fiber (DF) provides a large amount of fermentable substrate for intestinal microbiota and regulates the health of the host in an indirect manner, which is recognized as a promising method to delay the progression of DKD (104). Because of the absence of DF-degrading enzymes in the human body, DF cannot be digested and absorbed in the human body. Given its specific chemical composition and structure, DF exerts specific physiological functions, including reducing postprandial blood glucose levels, delaying gastric emptying, and altering gut microbiota composition (105). The undigested DF can be utilized as fuel for the growth of intestinal microorganisms that lead to the proliferation of intestinal flora and production of SCFA (acetate, propionate, and butyrate) through the fermentation process, and increase the abundance of Bifidobacterium and Lactobacillus (106). These beneficial results of DF contribute to anti-inflammation, anti-oxidation, and anti-immune reactions. Non-obese diabetes (NOD) mice were generally used as an animal model to study type I diabetes. After 24 weeks of treatment with long-chain or short-chain inulin fructans, the blood glucose level was significantly improved in NOD mice *via* an increased abundance of rumen Coccidae and lactic acid bacteria and an upregulated ratio of Firmicutes and Bacteroides. The intake of long-chain or short-chain inulin fructans also produced positive results in decreasing the permeability of the gut barricade and restoring the homeostasis state of intestinal microbiota (107).

5.2 The role of exercise in regulating gut microbiota

Lack of exercise, obesity, and stress are the major causes leading to the rising prevalence of DM. It is estimated that about 27% of DM, 30% of ischemic heart disease, and 21%–25% of breast cancer can be attributed to a lack of physical activity (108). Relevant medical guidelines suggest that a healthy diet and regular physical activity are strongly recommended as the most economical means of preventing and treating diabetes (109). Long-term (12 months) moderate intensity aerobic exercise is capable of reducing the expression of inflammatory-related cytokines, such as IL-1 β and TNF-α, with the upregulation of anti-inflammation factors like IL-4 (110). Moderate physical activity also produces a positive outcome in controlling the development of DM by promoting the secretion of GLP-1, which is helpful to improve glucose metabolism (111). After diabetes mice went through six weeks of exercise, favorable changes were observed in the composition of rat intestinal flora, including a reduction in the abundance of Bacteroides and an increase in the abundance of Firmicutes and Proteus, which promoted the generation of SCFAs (112). These SCFAs induced the secretion of peptide (PYY) limiting the intake of food and GLP-1 increasing insulin sensitivity (113). A sedentary lifestyle has a negative influence on host health because of the reduction in abundance of probiotics and the rising ratio of gram-negative bacteria, which resulted in disorders of the gut environment.

Compared with the less active group, the level of bacterial endotoxin was lower in well-trained athletes, accompanied by a higher concentration of heat shock protein, which contributed to reducing intestinal permeability *via* enhancing the upregulated tight junction proteins (114).

5.3 The role of probiotics, synbiotics, and postbiotics in the regulation of the gut microbiota

Probiotics are a series of indispensable living bacteria or beneficial microorganisms for human health that participate in the synthesis of various vitamins, the regulation of food digestion, the inhibition of the proliferation of pathogenic bacteria, and the degradation of toxic substances. A survey of 340 DKD patients receiving probiotic intervention had shown that the administration of probiotics exerted beneficial effects on reducing the expression of genes that are responsible for the generation of inflammation and oxidative stress biomarkers *via*

significantly downregulated levels of CRP and MDA plus enhancing GSH expression (115). It was reported that the benefits of supplemental probiotics may also help to protect the gut barrier. The protective mechanism is associated with enhanced levels of occluding and claudin-1, leading to reduced intestinal permeability (116). Synbiotics, a compound consisting of prebiotics and probiotics, possess good biological functions for the prevention of gut microbiota dysbiosis. A study revealed that the intake of synbiotics containing *Clostridium butyricum* and corn bran played a positive regulatory role in reducing the abundance of pathogens while enhancing the growth of SCFA-producing bacteria, which further led to an increase in acetate and isovalerate (117). Postbiotics is an inanimate microorganism that confers a health benefit on the host. The postbiotic intervention also possesses a regulatory function in inhibiting the differentiation of immature cells into mature adipocytes, reducing body weight gain and lipid accumulation in mice by promoting the activation of the TLR2–AMPK pathway (118). Further studies revealed that the intake of postbiotics could attenuate insulin resistance as well as inhibit inflammation *via* interacting with NOD2 (119). In short, the renal protective mechanism of probiotics, synbiotics, and postbiotics is mainly associated with the promotion of the growth of beneficial bacterial metabolites (such as acetate, propionate, and butyrate) as well as protecting the gut barrier *via* limiting the production of LPS and TMAO. The intake of probiotics, synbiotics, and postbiotics also helped to suppress the activation of signaling pathways that were responsible for oxidative stress, inflammation, and insulin resistance.

6 Conclusion and prospect

DKD is a global health problem with a complex mechanism of pathogenesis. A full understanding of the mechanisms underlying DKD is of great significance for laying out an effective treatment plan for the disease. Inflammation, oxidative stress, and autophagy play negative roles in accelerating the progress of DKD. Treatments involving inhibition of inflammation, reducing oxidative stress, and restoring autophagy activation are shown to protect podocytes and decrease proteinuria. Recent progress in the knowledge of the "gut–kidney" axis is a novel perspective for elucidating the relationship between gut microbiota and DKD. SCFAs are beneficial end products derived from gut bacteria, which regulate a variety of host physiological functions. Nonetheless, the mechanism of attenuation of DKD-related complications by the administration of SCFAs is still in its infancy. The beneficial effects of SCFAs may be associated with their ability to regulate energy metabolism, reduce the inflammatory response, and suppress immune effects. It is apparent that the beneficial properties of SCFAs act as a promising biomarker to diagnose and treat DKD. Still, a few questions need to be addressed before the clinical application of SCFAs.

First, the binding of SCFAs to GPCRs is necessary for their diverse functions. But there are still many unknown receptors with GPCR-like effects yet to be discovered. Discovering these not-yet-known receptors and developing specific targeted drugs will contribute to solving the puzzle of DKD. Second, most of these SCFA-induced beneficial results were observed in animal experiments and have rarely been performed in humans. Further research should be conducted to confirm whether these benefits could be repeated in humans. Lastly, SCFAs undoubtedly have an irrefutable role in attenuating DKD; any possibility that other metabolites would produce the same beneficial results needs to be investigated.

We are confident that these difficulties will and must be overcome to elucidate the detailed mechanism of the function of SCFAs and their promising medical value to mankind.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Raval N, Kumawat A, Kalyane D, Kalia K, Tekade RK. Understanding molecular upsets in diabetic nephropathy to identify novel targets and treatment opportunities. *Drug Discov Today* (2020) 25(5):862–78. doi: 10.1016/j.drudis.2020.01.008

2. Hui WA, Nl A, Tc B, Mw C, Hong SD, Ly E, et al. IDF diabetes atlas: Estimation of global and regional gestational diabetes mellitus prevalence for 2021 by international association of diabetes in pregnancy study group's criteria. *Diabetes Res Clin Pract* (2022) 183:109050. doi: 10.1016/j.diabres.2021.109050

3. Hang WU, Sun Z, Endocrinology DO, Hospital Z, University S, University S. Research progress in the pathogenesis and drug intervention of diabetic nephropathy. *Prog Pharm Sci* (2016) 40(05):337–43.

4. Keating ST, Diepen JV, Riksen NP, El-Osta A. Epigenetics in diabetic nephropathy, immunity and metabolism. *Diabetologia* (2018) 61(1):6–20. doi: 10.1007/s00125-017-4490-1

5. Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat Rev Nephrol* (2019) 15(6):327–45. doi: 10.1038/s41581-019-0135-6

6. Yau K, Dharia MA, Alrowiyti I, David CM. Prescribing SGLT2 inhibitors in patients with chronic kidney disease: Expanding indications and practical considerations. *Kidney Int Rep* (2022) 15(6):327–45. doi: 10.1016/j.ekir.2022.08.016

7. Macisaac RJ, Jerums G, Ekinci EI. Effects of glycaemic management on diabetic kidney disease. *World J Diabetes* (2017) 05):172. doi: 10.4239/wjd.v8.i5.172

8. Maruvada P, Leone V, Kaplan LM, Chang EB. The human microbiome and obesity: Moving beyond associations. *Cell Host Microbe* (2017) 22(5):589–99. doi: 10.1016/j.chom.2017.10.005

9. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med* (2018) 24(4):392–400. doi: 10.1038/nm.4517

10. Kim SK, Guevarra RB, Kim YT, Kwon J, Kim H, Cho JH, et al. Role of probiotics in human gut microbiome-associated diseases. *J Microbiol Biotechnol* (2019) 29(9):1335–40. doi: 10.4014/jmb.1906.06064

11. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol* (2021) 19(1):55–71. doi: 10.1038/s41579-020-0433-9

12. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. *Gut.* (2022) 71(5):1020–32. doi: 10.1136/gutjnl-2021-326789

13. Hu J, Lin S, Zheng B, Cheung PCK. Short-chain fatty acids in control of energy metabolism. *Crit Rev Food Sci Nutr* (2018) 58(8):1243–9. doi: 10.1080/10408398.2016.1245650

14. Huang W, Man Y, Gao C, Zhou L, Gu J, Xu H, 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. doi: 10.1155/2020/4074832

15. Li YJ, Chen X, Kwan TK, Loh YW, Singer J, Liu Y, et al. Dietary fiber protects against diabetic nephropathy through short-chain fatty acid-mediated activation of G protein-coupled receptors GPR43 and GPR109A. *J Am Soc Nephrol.* (2020) 31(6):1267–81. doi: 10.1681/ASN.2019101029

16. Zaky A, Glastras SJ, Wong MYW, Pollock CA, Saad S. The role of the gut microbiome in diabetes and obesity-related kidney disease. *Int J Mol Sci* (2021) 22(17):9641. doi: 10.3390/ijms22179641

17. Portincasa P, Bonfrate L, Vacca M, De Angelis M, Farella I, Lanza E, et al. Gut microbiota and short chain fatty acids: Implications in glucose homeostasis. *Int J Mol Sci* (2022) 23(3):1105. doi: 10.3390/ijms23031105

18. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. *Clin J Am Soc Nephrol.* (2017) 12(12):2032–45. doi: 10.2215/CJN.11491116

19. Sanajou D, Ghorbani Haghjo A, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: Current status and future directions. *Eur J Pharmacol* (2018) 833:158–64. doi: 10.1016/j.ejphar.2018.06.001

20. Pérez-Morales RE, Del Pino MD, Valdivielso JM, Ortiz A, Mora-Fernández C, Navarro-González JF. Inflammation in diabetic kidney disease. *Nephron.* (2019) 143(1):12–6. doi: 10.1159/000493278

21. Xiong Y, Zhou L. The signaling of cellular senescence in diabetic nephropathy. *Oxid Med Cell Longev* (2019) 2019:7495629. doi: 10.1155/2019/7495629

22. Ding T, Wang S, Zhang X, Zai W, Fan J, Chen W, et al. Kidney protection effects of dihydroquercetin on diabetic nephropathy through suppressing ROS and NLRP3 inflammasome. *Phytomedicine.* (2018) 41:45–53. doi: 10.1016/j.phymed.2018.01.026

23. Han Y, Xu X, Tang C, Gao P, Chen X, Xiong X, et al. Reactive oxygen species promote tubular injury in diabetic nephropathy: The role of the mitochondrial ros-txnip-nlrp3 biological axis. *Redox Biol* (2018) 16:32–46. doi: 10.1016/j.redox.2018.02.013

24. An X, Zhang Y, Cao Y, Chen J, Qin H, Yang L. Punicalagin protects diabetic nephropathy by inhibiting pyroptosis based on TXNIP/NLRP3 pathway. *Nutrients.* (2020) 12(5):516. doi: 10.3390/nu12051516

25. Hou Y, Lin S, Qiu J, Sun W, Dong M, Xiang Y, et al. NLRP3 inflammasome negatively regulates podocyte autophagy in diabetic nephropathy. *Biochem Biophys Res Commun* (2020) 521(3):791–8. doi: 10.1016/j.bbrc.2019.10.194

26. Wu M, Yang Z, Zhang C, Shi Y, Han W, Song S, et al. Inhibition of NLRP3 inflammasome ameliorates podocyte damage by suppressing lipid accumulation in diabetic nephropathy. *Metabolism.* (2021) 118:154748. doi: 10.1016/j.metabol.2021.154748

27. Rayego-Mateos S, Morgado-Pascual JL, Opazo-Ríos L, Guerrero-Hue M, García-Caballero C, Vázquez-Carballo C, et al. Pathogenic pathways and therapeutic approaches targeting inflammation in diabetic nephropathy. *Int J Mol Sci* (2020) 21(11):3798. doi: 10.3390/ijms21113798

28. Dai J, Chen H, Chai Y. Advanced glycation end products (AGEs) induce apoptosis of fibroblasts by activation of NLRP3 inflammasome *via* reactive oxygen species (ROS) signaling pathway. *Med Sci Monit* (2019) 25:7499–508. doi: 10.12659/MSM.915806

29. Hong J, Li G, Zhang Q, Ritter J, Li W, Li PL. D-ribose induces podocyte NLRP3 inflammasome activation and glomerular injury *via* AGEs/RAGE pathway. *Front Cell Dev Biol* (2019) 7:259. doi: 10.3389/fcell.2019.00259

30. Yang M, Wang X, Han Y, Li C, Wei L, Yang J, et al. Targeting the NLRP3 inflammasome in diabetic nephropathy. *Curr Med Chem* (2021) 28(42):8810–24. doi: 10.2174/0929867328666210705153109

31. Samsu N. Diabetic nephropathy: Challenges in pathogenesis, diagnosis, and treatment. *BioMed Res Int* (2021) 2021:1497449. doi: 10.1155/2021/1497449

32. Walton LE. Oxidative stress and diabetes: Glucose response in the cROSsfire. *Biomed J* (2017) 40(5):241–4. doi: 10.1016/j.bj.2017.10.001

33. Cui Q, Wang JQ, Assaraf YG, Ren L, Gupta P, Wei L, et al. Modulating ROS to overcome multidrug resistance in cancer. *Drug Resist Updat.* (2018) 41:1–25. doi: 10.1016/j.drup.2018.11.001

34. Madreiter-Sokolowski CT, Thomas C, Ristow M. Interrelation between ROS and Ca(2+) in aging and age-related diseases. *Redox Biol* (2020) 36:101678. doi: 10.1016/j.redox.2020.101678

35. Konno T, Melo EP, Chambers JE, Avezov E. Intracellular sources of ROS/H(2)O(2) in health and neurodegeneration: Spotlight on endoplasmic reticulum. *Cells.* (2021) 10(2):233. doi: 10.3390/cells10020233

36. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis* (2018) 9(2):119. doi: 10.1038/s41419-017-0135-z

37. Lu Q, Wang WW, Zhang MZ, Ma ZX, Qiu XR, Shen M, et al. ROS induces epithelial-mesenchymal transition *via* the TGF-β1/PI3K/Akt/mTOR pathway in diabetic nephropathy. *Exp Ther Med* (2019) 17(1):835–46. doi: 10.3892/etm.2018.7014

38. Hou Y, Wang Q, Han B, Chen Y, Qiao X, Wang L. CD36 promotes NLRP3 inflammasome activation *via* the mtROS pathway in renal tubular epithelial cells of diabetic kidneys. *Cell Death Dis* (2021) 12(6):523. doi: 10.1038/s41419-021-03813-6

39. Liu L, Bai F, Song H, Xiao R, Wang Y, Yang H, et al. Upregulation of TIPE1 in tubular epithelial cell aggravates diabetic nephropathy by disrupting PHB2 mediated mitophagy. *Redox Biol* (2022) 50:102260. doi: 10.1016/j.redox.2022.102260

40. Vermot A, Petit-Härtlein I, Smith SME, Fieschi F. NADPH oxidases (NOX): An overview from discovery, molecular mechanisms to physiology and pathology. *Antioxidants (Basel).* (2021) 10(6):890. doi: 10.3390/antiox10060890

41. Fukai T, Ushio-Fukai M. Cross-talk between NADPH oxidase and mitochondria: Role in ROS signaling and angiogenesis. *Cells.* (2020) 9(8):1849. doi: 10.3390/cells9081849

42. Forbes JM, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol.* (2018) 14(5):291–312. doi: 10.1038/nrneph.2018.9

43. Wei PZ, Szeto CC. Mitochondrial dysfunction in diabetic kidney disease. *Clin Chim Acta* (2019) 496:108–16. doi: 10.1016/j.cca.2019.07.005

44. Yang J, Zhou R, Ma Z. Autophagy and energy metabolism. *Adv Exp Med Biol* (2019) 1206:329–57. doi: 10.1007/978-981-15-0602-4_16

45. King KE, Losier TT, Russell RC. Regulation of autophagy enzymes by nutrient signaling. *Trends Biochem Sci* (2021) 46(8):687–700. doi: 10.1016/j.tibs.2021.01.006

46. Wang Y, Zhang H. Regulation of autophagy by mTOR signaling pathway. *Adv Exp Med Biol* (2019) 1206:67–83. doi: 10.1007/978-981-15-0602-4_3

47. Wang Y, Lu YH, Tang C, Xue M, Li XY, Chang YP, et al. Calcium dobesilate restores autophagy by inhibiting the VEGF/PI3K/AKT/mTOR signaling pathway. *Front Pharmacol* (2019) 10:886. doi: 10.3389/fphar.2019.00886

48. Tao M, Zheng D, Liang X, Wu D, Hu K, Jin J, et al. Tripterygium glycoside suppresses epithelial–to–mesenchymal transition of diabetic kidney disease podocytes by targeting autophagy through the mTOR/Twist1 pathway. *Mol Med Rep* (2021) 24(2):592. doi: 10.3892/mmr.2021.12231

49. Yang S, Lin C, Zhuo X, Wang J, Rao S, Xu W, et al. Glucagon-like peptide-1 alleviates diabetic kidney disease through activation of autophagy by regulating AMP-activated protein kinase-mammalian target of rapamycin pathway. *Am J Physiol Endocrinol Metab* (2020) 319(6):E1019–e30. doi: 10.1152/ajpendo.00195.2019

50. Hu Y, Chen H, Zhang L, Lin X, Li X, Zhuang H, et al. The AMPK-MFN2 axis regulates MAM dynamics and autophagy induced by energy stresses. *Autophagy.* (2021) 17(5):1142–56. doi: 10.1080/15548627.2020.1749490

51. Papandreou I, Lim AL, Laderoute K, Denko NC. Hypoxia signals autophagy in tumor cells *via* AMPK activity, independent of HIF-1, BNIP3, and BNIP3L. *Cell Death Differentiation.* (2008) 15(10):1572–81. doi: 10.1038/cdd.2008.84

52. Madhavi YV, Gaikwad N, Yerra VG, Kalvala AK, Nanduri S, Kumar A. Targeting AMPK in diabetes and diabetic complications: Energy homeostasis, autophagy and mitochondrial health. *Curr Med Chem* (2019) 26(27):5207–29. doi: 10.2174/0929867325666180406120051

53. Ji J, Tao P, Wang Q, Li L, Xu Y. SIRT1: Mechanism and protective in diabetic nephropathy. *Endocrine Metab Immune Disord - Drug Targets(Formerly Curr Drug Targets - Immune Endocrine Metab Disorders).* (2020) 2020:5207–29. doi: 10.2174/1871530320666201029143606

54. Ren H, Shao Y, Wu C, Ma X, Lv C, Wang Q. Metformin alleviates oxidative stress and enhances autophagy in diabetic kidney disease *via* AMPK/SIRT1-FoxO1 pathway. *Mol Cell Endocrinol* (2020) 500:110628. doi: 10.1016/j.mce.2019.110628

55. Nikolic-Paterson DJ, Meng XM, Lan HY. TGF-beta: the master regulator of fibrosis. *Nature Rev Nephrol* (2016) 12(6):325–38. doi: 10.1038/nrneph.2016.48

56. He T, Zhao JH. Resveratrol inhibits renal interstitial fibrosis in diabetic nephropathy by regulating AMPK/NOX4/ROS signaling. *Hong Kong J Nephrol* (2015) 94(12):1359–71. doi: 10.1016/j.hkjn.2015.08.034

57. Lan HY, Chung CK. TGF-β/Smad signaling in kidney disease. *Semin Nephrology.* (2012) 32(3):236–43. doi: 10.1016/j.semnephrol.2012.04.002

58. Ren H, Shao Y, Wu C, Lv C, Wang Q. VASH-1 regulates oxidative stress and fibrosis in diabetic kidney disease *via* SIRT1/HIF1α and TGFβ1/Smad3 signaling pathways. *Front Mol Biosci* (2020) 7. doi: 10.3389/fmolb.2020.00137

59. Akhtar M, Taha NM, Nauman A, Mujeeb IB, Al-Nabet A. Diabetic kidney disease: Past and present. *Adv Anatomic Pathol* (2020) 27:87–97. doi: 10.1097/PAP.0000000000000257

60. Kim JM, Sang SK. Management of hyperglycemia in diabetic kidney disease. *J Korean Diabetes.* (2021) 22(1):21–5. doi: 10.4093/jkd.2021.22.1.21

61. George J, Zhao X. High-fat diet exacerbates diabetic kidney injury in mice lacking eNOS. *FASEB J* (2019) 33(S1):567.8. doi: 10.1096/fasebj.2019.33.1_supplement.567.8

62. Menne J, Meier M, Park JK, Boehne M, Kirsch T, Lindschau C, et al. Nephrin loss in experimental diabetic nephropathy is prevented by deletion of protein kinase c alpha signaling in-vivo. *Kidney Int* (2006) 70(8):1456–62. doi: 10.1038/sj.ki.5001830

63. Khodor SA, Shatat IF. Gut microbiome and kidney disease: a bidirectional relationship. *Pediatr Nephrology.* (2016) 32(6):921–31. doi: 10.1007/s00467-016-3392-7

64. Cong J, Zhou P, Zhang R. Intestinal microbiota-derived short chain fatty acids in host health and disease. *Nutrients.* (2022) 14(9):1977. doi: 10.3390/nu14091977

65. Everard A, Cani PD. Gut microbiota and GLP-1. *Rev Endocr Metab Disord* (2014) 15(3):189–96. doi: 10.1007/s11154-014-9288-6

66. Mandaliya DK, Seshadri S. Short chain fatty acids, pancreatic dysfunction and type 2 diabetes. *Pancreatology.* (2019) 19(2):280–4. doi: 10.1016/j.pan.2019.01.021

67. Kim MH, Kang SG, Park JH, Yanagisawa M, Kim CH. Short-chain fatty acids activate GPR41 and GPR43 on intestinal epithelial cells to promote inflammatory responses in mice. *Gastroenterology.* (2013) 145(2):396–406.e1-10. doi: 10.1053/j.gastro.2013.04.056

68. Yilmaz B, Li H. Gut microbiota and iron: The crucial actors in health and disease. *Pharm (Basel).* (2018) 11(4):98. doi: 10.3390/ph11040098

69. Shimizu Y. Gut microbiota in common elderly diseases affecting activities of daily living. *World J Gastroenterol* (2018) 24(42):4750–8. doi: 10.3748/wjg.v24.i42.4750

70. Fang Q, Liu N, Zheng B, Guo F, Zeng X, Huang X, et al. Roles of gut microbial metabolites in diabetic kidney disease. *Front Endocrinol (Lausanne).* (2021) 12:636175. doi: 10.3389/fendo.2021.636175

71. Bian M, Wang J, Wang Y, Nie A, Zhu C, Sun Z, et al. Chicory ameliorates hyperuricemia *via* modulating gut microbiota and alleviating LPS/TLR4 axis in quail. *BioMed Pharmacother.* (2020) 131:110719. doi: 10.1016/j.biopha.2020.110719

72. Li F, Yang N, Zhang L, Tan H, Huang B, Liang Y, et al. Increased expression of toll-like receptor 2 in rat diabetic nephropathy. *Am J Nephrol.* (2010) 32(2):179–86. doi: 10.1159/000317023

73. Yang M, Gan H, Shen Q. [Effect of LPS on the level of TLR4 and on the expression of NF-κB and Notch1 in monocytes from patients with type 2 diabetic nephropathy]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban.* (2012) 37(6):578–85. doi: 10.3969/j.issn.1672-7347.2012.06.007

74. Salguero MV, Al-Obaide MAI, Singh R, Siepmann T, Vasylyeva TL. Dysbiosis of gram-negative gut microbiota and the associated serum lipopolysaccharide exacerbates inflammation in type 2 diabetic patients with chronic kidney disease. *Exp Ther Med* (2019) 18(5):3461–9. doi: 10.3892/etm.2019.7943

75. Kobayashi N, Takahashi D, Takano S, Kimura S, Hase K. The roles of peyer's patches and microfold cells in the gut immune system: Relevance to autoimmune diseases. *Front Immunol* (2019) 10:2345. doi: 10.3389/fimmu.2019.02345

76. Chi M, Ma K, Wang J, Ding Z, Li Y, Zhu S, et al. The immunomodulatory effect of the gut microbiota in kidney disease. *J Immunol Res* (2021) 2021:5516035. doi: 10.1155/2021/5516035

77. Mattson DL, Dasinger JH, Abais-Battad JM. Gut-Immune-Kidney axis: Influence of dietary protein in salt-sensitive hypertension. *Hypertension* (2022) 79 (11):101161hypertensionaha12218556. doi: 10.1161/HYPERTENSIONAHA.122.18556

78. Tsai YL, Lin TL, Chang CJ, Wu TR, Lai WF, Lu CC, et al. Probiotics, prebiotics and amelioration of diseases. *J BioMed Sci* (2019) 26(1):3. doi: 10.1186/s12929-018-0493-6

79. Zundler S, Günther C, Kremer AE, Zaiss MM, Rothhammer V, Neurath MF. Gut immune cell trafficking: inter-organ communication and immune-mediated inflammation. *Nat Rev Gastroenterol Hepatol* (2022). doi: 10.1038/s41575-022-00663-1

80. Glorieux G, Gryp T, Perna A. Gut-derived metabolites and their role in immune dysfunction in chronic kidney disease. *Toxins (Basel).* (2020) 12(4):245. doi: 10.3390/toxins12040245

81. Courtney CM, Onufer EJ, Mcdonald KG, Steinberger AE, Sescleifer AM, Seiler KM, et al. Small bowel resection increases paracellular gut barrier permeability via alterations of tight junction complexes mediated by intestinal TLR4. *J Surg Research: Clin Lab Invest* (2021) 258-):258. doi: 10.1016/j.jss.2020.08.049

82. Yang Y, Zhao M, He X, Wu Q, Zang WJ. Pyridostigmine protects against diabetic cardiomyopathy by regulating vagal activity, gut microbiota, and branched-chain amino acid catabolism in diabetic mice. *Front Pharmacol* (2021) 12:647481. doi: 10.3389/fphar.2021.647481

83. Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, et al. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. *PLoS One* (2015) 10:e0126931. doi: 10.1371/journal.pone.0126931

84. Joesten WC, Short AH, Kennedy MA. Spatial variations in gut permeability are linked to type 1 diabetes development in non-obese diabetic mice. *BMJ Open Diabetes Res Care* (2019) 7(1):e000793. doi: 10.1136/bmjdrc-2019-000793

85. Anderson KM, Ferranti EP, Alagha EC, Mykityshyn E, French CE, Reilly CM. The heart and gut relationship: a systematic review of the evaluation of the microbiome and trimethylamine-n-oxide (TMAO) in heart failure. *Heart Failure Rev* (2022) 27(6):1–27. doi: 10.1007/s10741-022-10254-6

86. Tang WW, Wang Z, Kennedy DJ, Wu Y, Hazen SL. Gut microbiota-dependent trimethylamine n-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* (2015) 116(3):448–55. doi: 10.1161/CIRCRESAHA.116.305360

87. Winther SA, Llgaard JC, Hansen TW, Scholten BJV, Rossing P. Plasma trimethylamine n-oxide and its metabolic precursors and risk of mortality, cardiovascular and renal disease in individuals with type 2-diabetes and albuminuria. *PLoS One* (2021) 16(3):e0244402. doi: 10.1371/journal.pone.0244402

88. Wang F, Zhang P, Jiang H, Cheng S. Gut bacterial translocation contributes to microinflammation in experimental uremia. *Digestive Dis Sci* (2012) 57 (11):2856–62. doi: 10.1007/s10620-012-2242-0

89. Osamu I, Saori OK, Teppei N, Masaaki U, Yasuhiro K, Chen W, et al. Podocyte injury caused by indoxyl sulfate, a uremic toxin and aryl-hydrocarbon receptor ligand. *PLoS One* (2014) 9(9):e108448. doi: 10.1371/journal.pone.0108448

90. Wu XY, Yu J, Tian HM. Effect of SOCS1 on diabetic renal injury through regulating TLR signaling pathway. *Eur Rev Med Pharmacol Sci* (2019) 23(18):8068–74. doi: 10.26355/eurrev_201909_19023

91. Al-Harbi NO, Nadeem A, Ahmad SF, Alotaibi MR, AlAsmari AF, Alanazi WA, et al. Short chain fatty acid, acetate ameliorates sepsis-induced acute kidney injury by inhibition of NADPH oxidase signaling in T cells. *Int Immunopharmacol.* (2018) 58:24–31. doi: 10.1016/j.intimp.2018.02.023

92. Tang G, Du Y, Guan H, Jia J, Zhu N, Shi Y, et al. Butyrate ameliorates skeletal muscle atrophy in diabetic nephropathy by enhancing gut barrier function and FFA2-mediated PI3K/Akt/mTOR signals. *Br J Pharmacol* (2022) 179(1):159–78. doi: 10.1111/bph.15693

93. Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The gut microbiota and inflammation: An overview. *Int J Environ Res Public Health* (2020) 17(20):7618. doi: 10.3390/ijerph17207618

94. Gharaie S, Noel S, Rabb H. Gut microbiome and AKI: Roles of the immune system and short-chain fatty acids. *Nephron.* (2020) 144(12):662–4. doi: 10.1159/000508984

95. Gong J, Noel S, Pluznick JL, Hamad ARA, Rabb H. Gut microbiota-kidney cross-talk in acute kidney injury. *Semin Nephrol.* (2019) 39(1):107–16. doi: 10.1016/j.semnephrol.2018.10.009

96. Tang S, Yiu WH. Innate immunity in diabetic kidney disease. *Nat Rev Nephrology.* (2020) 16(4):1–17. doi: 10.1038/s41581-019-0234-4

97. Scheithauer TPM, Rampanelli E, Nieuwdorp M, Vallance BA, Verchere CB, van Raalte DH, et al. Gut microbiota as a trigger for metabolic inflammation in obesity and type 2 diabetes. *Front Immunol* (2020) 11:571731. doi: 10.3389/fimmu.2020.571731

98. Kim DH, Park MH, Choi YJ, Chung KW, Park CH, Jang EJ, et al. Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney. *PLoS One* (2013) 8(3):e59316. doi: 10.1371/journal.pone.0059316

99. Yu R, Bo H, Villani V, Spencer PJ, Fu P. The inhibitory effect of rapamycin on toll like receptor 4 and interleukin 17 in the early stage of rat diabetic nephropathy. *Kidney Blood Press Res* (2016) 41(1):55–69. doi: 10.1159/000368547

100. Huang W, Zhou L, Guo H, Xu Y, Xu Y. The role of short-chain fatty acids in kidney injury induced by gut-derived inflammatory response. *Metab Clin Experimental.* (2017) 68:20. doi: 10.1016/j.metabol.2016.11.006

101. Li Q, Li K, Chen Z, Zhou B. Anti-renal fibrosis and anti-inflammation effect of urolithin b, ellagitannin-gut microbial-derived metabolites in unilateral ureteral obstruction rats. *J Funct Foods.* (2019) 65:103748. doi: 10.1016/j.jff.2019.103748

102. Khan S, Jena G. Sodium butyrate, a HDAC inhibitor ameliorates eNOS, iNOS and TGF-β1-induced fibrogenesis, apoptosis and DNA damage in the kidney of juvenile diabetic rats. *Food Chem Toxicol Int J Published Br Ind Biol Res Assoc* (2014) 73:127–39. doi: 10.1016/j.fct.2014.08.010

103. Du Y, Yang Y, Tang G, Jia J, Zhu N, Yuan W. Butyrate alleviates diabetic kidney disease by mediating the miR-7a-5p/P311/TGF-β1 pathway. *FASEB J* (2020) 34(8):10462–75. doi: 10.1096/fj.202000431R

104. Benito-González I, Martínez-Sanz M, Fabra MJ, López-Rubio A. Health effect of dietary fibers. *Dietary Fiber: Properties, Recovery, and Applications* (2019) 125–63. doi: 10.1016/B978-0-12-816495-2.00005-8

105. Weickert MO, Pfeiffer AF. Impact of dietary fiber consumption on insulin resistance and the prevention of type 2 diabetes. *J Nutr* (2018) 148(1):7–12. doi: 10.1093/jn/nxx008

106. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: Short-chain fatty acids as key bacterial metabolites. *Cell.* (2016) 165(6):1332–45. doi: 10.1016/j.cell.2016.05.041

107. Zhang Q, Yu H, Xiao X, Hu L, Yu X. Inulin-type fructan improves diabetic phenotype and gut microbiota profiles inrats. *Peerj.* (2018) 6(3):e4446. doi: 10.7717/peerj.4446

108. Shi Y, Hu FB. The global implications of diabetes and cancer. *Lancet* (2014) 383(9933):1947–8. doi: 10.1016/S0140-6736(14)60886-2

109. Gingras V, Hivert MF, Oken E. Early-life exposures and risk of diabetes mellitus and obesity. *Curr Diabetes Rep* (2018) 18(10):89. doi: 10.1007/s11892-018-1050-0

110. Nicolucci A, Balducci S, Cardelli P, Zanuso S, Pugliese G. Supervised exercise training improves quality of life in subjects with type 2 diabetes. *Arch Internal Med* (2011) 171(21):1951–3. doi: 10.1001/archinternmed.2011.561

111. Janus C, Vistisen D, Amadid H, Witte DR, Torekov SS. Habitual activity associates with lower fasting and greater glucose-induced GLP-1 response in men. *Endocrine Connections.* (2019) 8(12):1607–17. doi: 10.1530/EC-19-0408

112. Codella R, Luzi L, Terruzzi I. Exercise has the guts: How physical activity may positively modulate gut microbiota in chronic and immune-based diseases. *Dig Liver Dis* (2018) 50(4):S1590865817313129. doi: 10.1016/j.dld.2017.11.016

113. Hoffman-Goetz L, Spagnuolo PA, Guan J. Repeated exercise in mice alters expression of IL-10 and TNF-α in intestinal lymphocytes. *Brain Behav Immunity.* (2008) 22(2):195–9. doi: 10.1016/j.bbi.2007.07.002

114. Dokladny K, Moseley PL, Ma TY. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *AJP Gastrointestinal Liver Physiol* (2006) 290(2):G204–12. doi: 10.1152/ajpgi.00401.2005

115. Jb A, In B, Bi B, Mahk C, Zbz D, Arm E. Effect of probiotics on oxidative stress and inflammatory status in diabetic nephropathy: A systematic review and meta-analysis of clinical trials - ScienceDirect. *Heliyon*. (2021) 7(1):e05925. doi: 10.1016/j.heliyon.2021.e05925

116. Vlachou E, Ntikoudi A, Govina O, Lavdaniti M, Kotsalas N, Tsartsalis A, et al. Effects of probiotics on diabetic nephropathy: A systematic review. *Curr Clin Pharmacol* (2020) 15(3):234–42. doi: 10.2174/1574884715666200303112753

117. Zhang J, Sun J, Chen X, Nie C, Zhao J, Guan W, et al. Combination of clostridium butyricum and corn bran optimized intestinal microbial fermentation using a weaned pig model. *Front Microbiol* (2018) 9. doi: 10.3389/fmicb.2018.03091

118. Lee J, Park S, Oh N, Park J, Kwon M, Seo J, et al. Oral intake of lactobacillus plantarum l-14 extract alleviates TLR2- and AMPK-mediated obesity-associated disorders in high-fat-diet-induced obese C57BL/6J mice. *Cell Prolif.* (2021) 54(6):e13039. doi: 10.1111/cpr.13039

119. Cavallari JF, Fullerton M, Duggan BM, Foley KP, Denou E, Smith BK, et al. Muramyl dipeptide-based postbiotics mitigate obesity-induced insulin resistance *via* IRF4. *Cell Metab* (2017) 25(5):1063–74. doi: 10.1016/j.cmet.2017.03.021

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Gut microbiota: The key to the treatment of metabolic syndrome in traditional Chinese medicine – a case study of diabetes and nonalcoholic fatty liver disease

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Metabolic syndrome mainly includes obesity, type 2 diabetes (T2DM), alcoholic fatty liver (NAFLD) and cardiovascular diseases. According to the ancient experience philosophy of Yin-Yang, monarch-minister compatibility of traditional Chinese medicine, prescription is given to treat diseases, which has the advantages of small toxic and side effects and quick effect. However, due to the diversity of traditional Chinese medicine ingredients and doubts about the treatment theory of traditional Chinese medicine, the mechanism of traditional Chinese medicine is still in doubt. Gastrointestinal tract is an important part of human environment, and participates in the occurrence and development of diseases. In recent years, more and more TCM researches have made intestinal microbiome a new frontier for understanding and treating diseases. Clinically, nonalcoholic fatty liver disease (NAFLD) and diabetes mellitus (DM) often co-occur. Our aim is to explain the mechanism of interaction between gastrointestinal microbiome and traditional Chinese medicine (TCM) or traditional Chinese medicine formula to treat DM and NAFLD. Traditional Chinese medicine may treat these two diseases by influencing the composition of intestinal microorganisms, regulating the metabolism of intestinal microorganisms and transforming Chinese medicinal compounds.

KEYWORDS

gastrointestinal flora, diabetes mellitus, non-alcoholic fatty liver disease, traditional Chinese medicine, inflammation, short chain fatty acids

1 Introduction

Intestinal microecology is an important part of human environment. It is composed of intestinal microbiota, intestinal epithelial cells and immune system, forms intestinal mucosal barrier and plays an important role in energy metabolism. Both external and genetic factors affect the composition and function of intestinal microecology. The steady state of microbiota is closely related to human health. There is growing evidence that intestinal microbiota and their metabolites play an important role in the development of obesity, diabetes and nonalcoholic fatty liver disease (1). Diabetes and non-alcoholic fatty liver are two diseases closely related to intestinal microbial homeostasis. T2DM is the main type of diabetes, mainly manifested as metabolic disorders, such as hyperglycemia, hyperlipidemia and insulin resistance. Diabetes can lead to a variety of serious complications, such as retinopathy and diabetic nephropathy, gestational diabetes, atherosclerosis and other cardiovascular diseases, these complications affect the quality of life of a large number of people around the world. The rapid growth of diabetes has brought a great burden on the global society and economic society (2). Nonalcoholic fatty liver disease (NAFLD), as a common chronic liver disease, can be divided into fatty liver, steatohepatitis and liver fibrosis according to the degree of inflammation and fibrosis. The main manifestations of NAFLD are steatosis, lipotoxicity and inflammatory injury, which are associated with glucose homeostasis and persistent low-grade inflammation (1) (3). Studies have found that intestinal flora and metabolites can reverse some metabolic disorders, including high fat, tissue inflammation and low insulin sensitivity and secretion (4). This suggests that intestinal flora can be used in the treatment of diabetes and fatty liver (5).

As an important supplementary means of clinical medicine, traditional Chinese medicine has been widely adopted in some East Asian countries. In some western countries, such as the United States, Britain and Germany, the trend of using traditional Chinese medicine as a treatment for diseases is becoming more and more obvious. Different from chemical drugs and biological agents, traditional Chinese medicine and traditional Chinese medicine formulations under the guidance of traditional Chinese medicine theory are often difficult to determine specific bioactive components. Traditional Chinese medicine prescribes prescriptions to treat diseases under the guidance of ancient empirical philosophy, such as yin-yang, monarch-minister compatibility and so on. Traditional Chinese medicine advocates the concept of wholeness and regards organs such as internal organs as a whole. the destruction of intestinal microbial homeostasis promotes the development of metabolic syndrome such as diabetes, fatty liver and cardiovascular syndrome, which is in line with the "whole" concept of TCM theory. There has been a sharp increase in patients with diabetes and non-alcoholic fatty liver disease worldwide, which studies have shown may be associated with insulin resistance. Patients usually suffer from these two diseases at the same time, which is a difficult problem in clinical treatment (6). As insulin resistance plays an important role in the development of non-alcoholic fatty liver, diabetes drugs are often used as the treatment option for the treatment of non-alcoholic fatty liver (7) (8). On the one hand: there are no approved drugs for nonalcoholic fatty liver disease, and the only approved treatment option is to improve diet and lose weight. On the other hand: the drugs used to treat diabetes are still defective in the treatment of fatty liver. With the discovery of plant-derived natural products quercetin, resveratrol, polysaccharides, berberine and curcumin in the treatment of diseases, researchers have focused on "simple, convenient and low-toxic" herbs. Researchers have found that single herbs such as Coptis chinensis, Radix Astragali, Ginseng and herbal formulations such as SiMiao, Gegen Qinlian decoction, Huanglian jiedu decoction and LLKL have potential therapeutic effects on T2DM and NAFLD. These herbs exert pharmacological effects through intestinal microflora and mainly include two ways: changing the composition of intestinal microorganisms and affecting the metabolism of intestinal microflora. The main purpose of this review is to explain the therapeutic effect of intestinal microbiota on diabetes and non-alcoholic fatty liver.

2 Association between diabetes mellitus and non-alcoholic fatty liver disease

2.1 Diabetes mellitus and non-alcoholic fatty liver disease – two clinically associated diseases

Diabetes is a metabolic disorder characterized by hyperglycemia caused by deficiency of insulin secretion and/or deficiency of insulin action. There are two main types of diabetes, insulin-dependent type 1 diabetes (T1DM) and insulin-independent type 2 diabetes mellitus (T2DM), of which type 2 diabetes accounts for 90% of patients with diabetes (9). Nonalcoholic fatty liver disease (NAFLD) is considered to be the most common form of liver disease in the world, including fatty liver, steatohepatitis (NASH) and liver fibrosis (10). NASH is a progressive form of nonalcoholic fatty liver. NAFLD is a risk factor for metabolic disorders such as obesity, diabetes, especially T2DM and cardiovascular disease. Among obese people undergoing bariatric surgery, the prevalence of NAFLD is as high as 90%, and among diabetics, the prevalence of NAFLD can be as high as 71%. Insulin resistance (IR) exists in almost all patients with NAFLD and T2DM. Through the evaluation of the homeostasis model of

insulin resistance, it was found that there was a significant correlation between IR and the prevalence of steatohepatitis in NAFLD. The relationship between diabetes and nonalcoholic fatty liver gradually evolved into the relationship with simple steatosis (SS), NASH and liver fibrosis (11) (12). Cross-sectional studies have shown that non-alcoholic fatty liver disease usually occurs in patients with type 2 diabetes (13). A systematic review and meta-analysis of 27 clinical trials confirmed the direct relationship between fatty liver and the incidence of diabetes (14). The probability of developing diabetes is also different in different steatosis states. Follow-up results showed that the incidence of diabetes in patients without steatosis, intermittent steatosis and persistent steatosis increased by 5.1%, 14.1% and 27.1%, respectively. It can be speculated that early intervention of steatosis has a resistant effect on the development of diabetes (15) A study based on patients with nonalcoholic fatty liver disease and first-degree relatives in the United States found that familial aggregation of insulin resistance syndrome has a genetic susceptibility to supporting nonalcoholic fatty liver disease (16). Family history of diabetes, especially in non-diabetic patients, is associated with nonalcoholic steatohepatitis (NASH) and fibrosis in NAFLD (17). In addition, the occurrence and development of cardiovascular diseases such as obesity, retinopathy, renal failure, peripheral neuropathy and atherosclerosis are also related to diabetes (9).

2.2 Beneficial effects of various anti-diabetic drugs on non-alcoholic fatty liver disease

In the above part, we have explained the clinical correlation between NAFLD and T2DM. However, NAFLD does not have an explicitly approved drug, and the only approved treatment option is to change diet and exercise. IR plays an important role in the development of NAFLD, and many hypoglycemic drugs have been evaluated for the treatment of NAFLD. These drugs mainly include biguanides, glucagon-like peptide 1 receptor (GLP-1) agonists, peroxisome proliferator-activated receptor (PPAR) agonists and farnesoid X receptor (FXR) agonists. Metformin is known to improve lipid metabolism by activating adenylate-activated protein kinase (AMPK), an important regulator of energy metabolism (18). Metformin exerts the preventive effect of NAFLD by increasing AMPK phosphorylation, inhibiting macrophage polarization, reducing macrophage infiltration and the expression of pro-inflammatory cytokines (TNF- α, IL-1 β and IL-6), relieving liver inflammation and fat accumulation (19, 20). In addition, metformin alleviates fatty liver degeneration in obese mice by affecting the protein levels of CYP7B1 and CH25H, a cholesterol hydroxylase, to regulate cholesterol secretion and metabolism (21). Glucagon-like peptide-1 (GLP-1) is an enterotropic insulin secreted by intestinal endocrine L cells that regulates glucose regulation by slowing gastric emptying and glucose-dependent inhibition of glucagon secretion. GLP-1 can improve liver insulin sensitivity (22, 23)and enhance the direct effect of lipid hydrolysis and oxidation on liver (24–26). Lilarutide is a kind of GLP-1 analogue. Studies have shown that liralutide can reduce liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (27). Lipopeptide is associated with liver lipid metabolism, total cholesterol (TC) and triglyceride (TG) (28). Pioglitazone belongs to PPAR- γ agonist and has insulin sensitizing effect (29). Pioglitazone reduces the accumulation of lipids in the liver (30) by improving fatty acid uptake and transport. Farnesoid X receptor (FXR) is a kind of nuclear receptor activated by bile acid, which is highly expressed in the liver and intestines and is related to bile acid and lipid metabolism (31). FXR agonists can reduce insulin resistance, improve lipid metabolism disorders, and alleviate fatty liver degeneration (32).

2.3 Summary

In conclusion, there is a close relationship between diabetes and nonalcoholic fatty liver disease. Metformin, liralutide and pioglitazone are used in the treatment of diabetes and drugs can be developed for the treatment of non-alcoholic fatty liver.

3 How does the gut microbiota influence T2DM and NAFLD

3.1 Intestinal microbes

Intestinal microecology is an extremely complex ecosystem, which is composed of intestinal microflora, intestinal epithelial cells and intestinal immune system. Intestinal microecology is regarded as an important “organ”, which plays an important role in regulating human metabolism (33). Intestinal microflora, also known as intestinal bacteria, is a complex microbial community living in the gastrointestinal tract of the human body in a symbiotic way, which mainly includes two phyla, thick-walled bacteria and Bacteroides (34). Diabetes and nonalcoholic fatty liver disease are metabolic diseases related to obesity (35). Obesity increases the risk of diabetes and NAFLD in humans (36, 37). Intestinal flora disorders have been repeatedly observed in these metabolic diseases, which seem to be related to changes in the proportion of thick-walled bacteria and actinomycetes in the intestines (38, 39). In patients with T2DM, it was observed that the abundance of Streptococcus faecalis and Rosobacter increased, while the abundance of Shigella and Bifidobacterium decreased (40). The decrease of microbial diversity and the increase of Prevotella abundance were observed in the feces of children with NAFLD. Today, unhealthy Western diets are promoting and aggravating the course of T2DM and NAFLD,

which may be reduced or reversed by intestinal flora treatment (41–43).

3.2 Intestinal microbial metabolites

3.2.1 SCFAs

Intestinal ecological disorders usually lead to changes in intestinal SCFAs levels. Short-chain fatty acids (such as acetate, propionate and butyrate) produced by intestinal microorganisms not only provide nutrition and energy for the host (44), but also participate in lipid metabolism and glucose metabolism through a variety of pathways (45), thus affecting the development of T2DM and NAFLD. It has been found that human cells respond to SCFAs mainly by activating G protein coupled receptor (GPR41,GPR43) and inhibiting histone deacetylase (HDAC) (46, 47). G protein coupled receptors are expressed in adipose tissue (48), liver (49) and pancreatic β cells (50). Acetate is an important substrate for fatty acid synthesis, and the increase of acetate will lead to the accumulation of triglycerides (51, 52). Propionate is an important precursor of gluconeogenesis, and an increase in propionate levels will promote gluconeogenesis in the liver (53). Acetate and propionate activate GPR43 receptors, inhibit insulin signal transduction in adipocytes, inhibit fat accumulation and promote lipid and glucose metabolism in other tissues (54, 55). Butyrate promotes the expression of gluconeogenesis-related genes in a cAMP-dependent manner. In addition, SCFAs stimulates intestinal endocrine cells to secrete glucagon-like peptide 1 (GLP-1) and YY peptide (PYY) through a GPR-dependent mechanism. These two hormones inhibit appetite, promote fat oxidation, promote insulin secretion and reduce glucagon, and inhibit hepatic steatosis and the development of diabetes (47, 56). In addition, propionate and butyrate can also act as HDAC inhibitors to induce increased PYY mRNA levels (57).

3.2.2 TMAO, BAs and BCAAs

Trimethylamine N-oxide (TMAO) is a metabolite associated with diabetes, liver steatosis and other chronic diseases (58). TMAO is derived from intestinal microflora that metabolizes choline. Choline is converted to trimethylamine (TMA) through Flavin-containing monooxygenase, and TMA is converted into TMAO in the liver (59). It was found that TMAO accumulated in the serum of patients with T2DM and NAFLD (60–62). TMAO can play a role in NAFLD by changing bile acid metabolism (63). In addition, TMAO may induce pancreatic β-cell dysfunction and promote the pathogenesis of T2D (64). Bile acids include primary bile acids and secondary bile acids. Primary bile acids are synthesized by cholesterol in the liver. Primary bile acids enter the intestine and are converted into secondary bile acids by intestinal flora. As an important mediator of intestinal-liver crosstalk, bile acid mainly acts on two key receptors, farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), and regulates glucose homeostasis and lipid metabolism (65, 66). Bile acid metabolism is associated with the onset and progression of type 2 diabetes and NAFLD (67). Bile acid chelating agents can inhibit FXR activity in intestinal L cells, promote the production and secretion of GLP-1, and improve blood glucose (68). It can also reverse hepatic steatosis, inflammation and fibrosis by interrupting intestinal bile acid reabsorption (69). Branched chain amino acids (BCAAs) are essential amino acids, including leucine, isoleucine and valine (70). Intestinal microflora can produce and degrade branched chain amino acids. The increase of host branched chain amino acids is related to metabolic fatty liver disease and diabetes (71, 72). Amino acid-induced insulin signal transduction damage and G protein coupled receptor involvement lead to insulin resistance and type 2 diabetes mellitus (73). Leucine affects glucose metabolism by activating rapamycin complex 1mTORC1 (74). Host circulating branched chain amino acids were positively correlated with higher cholesterol level, liver fat content and insulin resistance (IR) (75). However, some studies have found that supplementation of branched chain amino acids can reduce the expression of adipogenesis-related genes FAS and ACC in the liver and reduce fat accumulation in the liver of rats fed with high-fat diet (72, 76).

3.3 Intestinal permeability and inflammation

The intestinal barrier consists of mucin layer and epithelial cells. The destruction of intestinal barrier makes it easier for bacterial metabolites and inflammatory cytokines to enter the circulatory system, which is related to the occurrence of metabolic syndrome (77). It is known that secondary bile acid pass inhibits the expression of intestinal tight junction protein and increases intestinal permeability (78). The production of LPS results from the overgrowth of Gram-negative bacteria in the intestinal tract. LPS circulates through the portal vein to the liver to induce liver injury and inflammation (79, 80). The increase of intestinal permeability and inflammation induced by LPS is mediated by TLR4-dependent activation of ganglion (81). By inducing the activation of TLR4/NF- κ B signal pathway, LPS upregulates the levels of inflammatory factors such as TNF- α, IL-1 and IL-10, and promotes oxidative stress, resulting in insulin resistance and NAFLD (82, 83). Similarly, SCFAs reduces intestinal inflammation by inhibiting the LPS/NF-kappa B/TLR4 pathway (84). SCFAs reduces inflammation by inhibiting the activity of histone deacetylase (HDAC) and promoting the production of regulatory T cells (Treg) (85).

3.4 Summary

From the above introduction, it can be known that intestinal microbiota disorder is the key to the occurrence and development of T2DM and NAFLD. It can induce local organ or systemic inflammation by changing the diversity of intestinal flora, affecting microbial metabolism and destroying intestinal barrier.

4 Intestinal flora– the "target organ" of traditional Chinese medicine in the treatment of diseases

Traditional Chinese medicine has a history of treating diseases in China for more than 2000 years, including single drug treatment and compound drug treatment. T2DM and NAFLD are metabolic diseases characterized by hyperglycemia and fat accumulation. Intestinal flora mediates the occurrence and development of metabolic diseases and is used as an important organ to participate in metabolic regulation. A series of experimental results also show that the hypoglycemic and lipid-lowering effect of traditional Chinese medicine is related to intestinal flora. Below, we will introduce the molecular mechanism of traditional Chinese medicine in the treatment of diabetes and fatty liver from the point of view of intestinal flora structure, intestinal barrier and intestinal metabolites. The mechanism of the therapeutic effect of traditional Chinese medicine (TCM) is shown in Figures 1, 2.

4.1 Individual herbs or herbal extracts

It is well known that traditional Chinese medicine extracts resveratrol, berberine, ginsenosides and curcumin play a beneficial regulatory role in lipid and glucose metabolism. Resveratrol is a natural polyphenol compound found in most herbs and has the potential to relieve diabetes and liver steatosis (86). It has been proved that the therapeutic effect of resveratrol is mediated by intestinal flora. For example, resveratrol alleviates the progression of diabetic nephropathy by reversing the low levels of Bacteroides, Alistipes, Rikenella, Odoribacter, Bacteroides and Alloprevotella in db/db mouse model. The therapeutic effect of resveratrol on db/db mice is related to resveratrol reversing the imbalance of intestinal flora, improving intestinal barrier, reducing intestinal permeability and inflammation (87). In addition, resveratrol can act as a potential NAFLD replacement therapy, and its therapeutic effect has been evaluated and confirmed in a number of trials. Previous experimental results have shown that resveratrol can improve lipid metabolism and reduce lipogenesis and inflammation in high-fat-fed mice, thus reducing hepatic steatosis (88). A new study found that high-fat diet (HFD)-

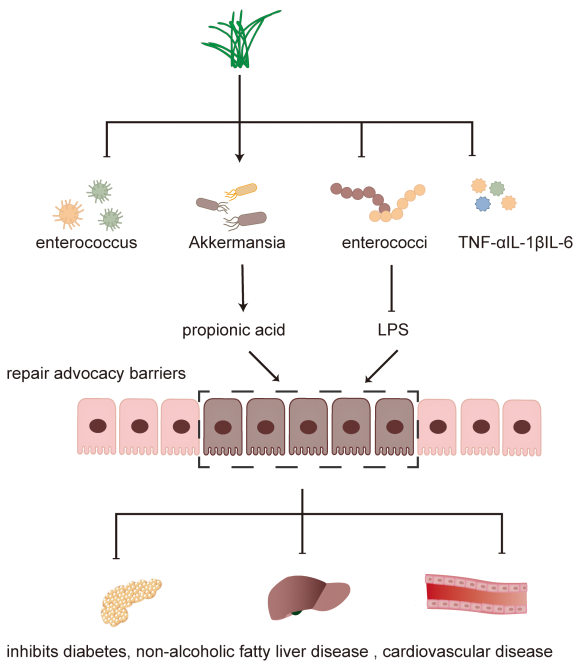

FIGURE 1
Mechanism of TCM in treating diseases.

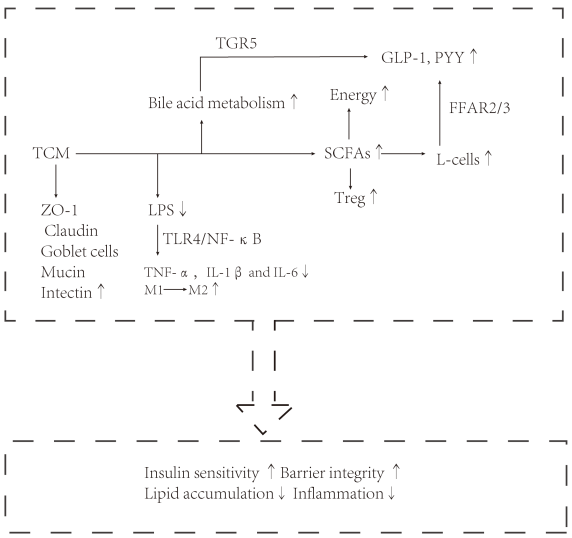

FIGURE 2
Traditional Chinese medicine exerts a therapeutic effect through intestinal flora, such as enterococcus, Akkermansia, and Vibrio desulfurization.

induced NAFLD mice treated with resveratrol reduced the enrichment of lipid and glucose metabolism-related pathways, and this change was closely related to changes in intestinal flora. Resveratrol can reshape the diversity and composition of intestinal flora at different levels of the family. At the phylum level, the number of thick-walled bacteria increased significantly, while that of Bacteroides decreased significantly; at the family level, the erysipelaceae increased; at the genus level, the Olsenella content increased. Resveratrol reduces the invasion of harmful substances by up-regulating tight junction protein zo-1 and ameliorates liver inflammation by down-regulating inflammatory factors (IL-1, TNF- α, MyD88 and TLR-4) (89).

Berberine is a natural isoquinoline alkaloid extracted from herbal plants, which is the main activity of Coptis chinensis and Berberis (90, 91). The interaction between berberine and intestinal flora can alleviate metabolic disorders such as T2DM and NAFLD. Intestinal flora affects the absorption and transformation of berberine in gastrointestinal tract, and berberine also interferes with the structure and function of intestinal flora (92). In a study of Sprague-Dawley (SD) rats, the intestinal microflora diversity and richness of rats treated with berberine changed. At the gate level, there are higher abundance of Bacteroides and lower abundance of Proteus and verrucous microorganisms. At the family level, the family of Lactobacillus was significantly up-regulated. The concentrations of tyrosine, tryptophan and phenylalanine, the metabolites of intestinal flora, decreased in intestine and serum. Some studies have shown that high concentrations of aromatic amino acids are positively correlated with the risk of diabetes (93). Therefore, berberine treatment reduced the risk of diabetes in SD rats (94). Berberine is metabolized to berberine in the liver (95). It has been proved that berberine can regulate bile acid metabolism, activate intestinal farnesoid X receptor (FXR) and inhibit hepatic gluconeogenesis, and has significant lipid-lowering and hypoglycemic effects (95). Interestingly, by analyzing the composition of intestinal flora in high-fat (HFD)-fed mice, the researchers found that berberine increased intestinal beneficial bacteria, such as ileobacteria and myxobacteria. In addition, berberine reduced fat accumulation in the liver of HFD mice and decreased the levels of ALT and AST, which were beneficial to the treatment of NAFLD. Berberine can improve the imbalance of glucose homeostasis in HFD mice by affecting the expression of proteins related to glucose metabolism (PPAR γ, G6Pase, GLUT2,p-GSK) (96).

Ginsenosides are a kind of bioactive components extracted from plant medicine Ginseng. Ginsenosides can fight a variety of diseases through intestinal flora (97). Ginsenoside Rg1 can relieve T2D symptoms induced by HFD and streptozotocin (STZ) in rats, which may be related to the increase of the proportion of lactic acid bacteria and Lachnospiraceae and the decrease of the proportion of Lactobacillus by Rg1. Spearman correlation analysis showed that Lactobacillus was positively correlated with IL-1 β, IL-6, TNF- α and ROS levels (98). Lachnospiraceae is the main source of intestinal SCFAs, especially butyric acid (99, 100). Rg5 relieves inflammation by reducing plasma LPS levels and inhibiting the activation of TLR4-related signaling pathways in db/db mice. The hypoglycemic effect of Rg5 is related to its reducing the abundance of thick-walled bacteria and verrucous microorganisms and increasing the abundance of Bacteroides and Proteus in db/db mice (101). 25-hydroxy-protopanaxatriol (T19) is a new type of ginsenoside. Lachnospiraceae is a

beneficial bacteria that regulates glucose and lipid metabolism. It was found that T19 significantly improved the abnormal glycolipid levels induced by HFD and STZ by significantly increasing the relative abundance of Lachnospiraceae family (102).

Curcumin is a polyphenol compound, mainly found in turmeric root (103). Curcumin attenuates dextran sulfate-induced T2MD symptoms in mice by reshaping the balance of Th17 and Treg in lymphoid cells. Th17 and Treg are related to the secretion of pro-inflammatory factor IL-17A and anti-inflammatory factor IL-10, respectively. Spearman analysis showed that curcumin mainly relieved chronic inflammation caused by T2MD by increasing the level of Roseburia and decreasing the levels of Erysipelatoclostridum and norank_f_Oscillospiraceae (104). Tetrahydrocurcumin (THC) is the main metabolite of curcumin. THC improves diabetes in db/db mice by reducing the relative abundance of Proteus and actinomycetes and promoting the expression of GLP-1 in the pancreas (105).

Herbs such as Polygala, licorice, Scutellaria baicalensis and Lycium barbarum also have the potential to treat diabetes and chronic liver disease. Polygala polygala extract (PTE) inhibits fat accumulation by promoting the expression of PPAR α. In addition, PTE regulates metabolism by enriching Proteus and reducing deferrifying bacteria (106). Licorice extract can reduce intestinal inflammation by reducing the levels of NF- κ B, Toll-like receptor 4 (TLR4) and tumor necrosis factor-α (TNF- α) in the colon of diabetic mice. The recovery of intestinal microbiology by licorice extract is related to the decrease of Lachnospiraceae _ NK4A136 content at genus level (107). The water extract of Scutellaria baicalensis Georgi can treat diabetes and complications by regulating the interaction between intestinal flora and bile acid metabolism. FXR is highly expressed in liver and intestine and is the key receptor of bile acid. Scutellaria baicalensis water extract can inhibit the expression of FXR in diabetic rats. Water extract of Scutellaria baicalensis Georgi can reverse the low levels of thephylaTenericutes and Patescibacteria and decrease the abundance of Lactobacillus and feacalibaculum in diabetic rats (108). Lycium barbarum polysaccharides can increase the proportion of probiotics, such as Ackermania, Lactobacillus and Prevaceae; Lycium barbarum polysaccharides can reduce intestinal pH and regulate the intestinal environment; Lycium barbarum polysaccharides can also stimulate innate immunity in the intestinal mucosa, such as macrophages or lymphocytes (109, 110). In addition, some Chinese herbal and natural plant extracts, such as cinnamon, Dendrobium, Radix Astragali, rhubarb, Aristolochia manshuriensis, cichoric acid, inulin, polyphenols, Ganoderma lucidum and mulberry polysaccharides are also effective in preventing and treating T2DM, NAFLD and related metabolic diseases. More details are shown in Table 1.

4.2 Chinese herbal formulae

The formula of traditional Chinese medicine is another important means for the treatment of diseases in traditional Chinese medicine, and it is often used in the diagnosis and treatment of clinical diseases as a supplement to western medicine. The compatibility of traditional Chinese medicine is not random. On the contrary, it is necessary to follow the principle of compatibility of traditional Chinese medicine and the principle of diagnosis and treatment of traditional Chinese medicine (140).

Pi-Dan-Jian-Qingdecoction (PDJQ) contains Radix Astragali, Radix Pseudostellariae, Coptis chinensis, Scutellaria baicalensis, Rhizoma Atractylodes, Salvia miltiorrhiza and Litchi. PDJQ has a good intervention effect on the clinical treatment of diabetes. In addition to regulating intestinal flora and inhibiting inflammation, the mechanism of PDJQ in treating diabetes is also related to the regulation of tryptophan metabolism, histamine metabolism and tricarboxylic acid (TCA) circulation. The specific results were as follows: at the genus level, PDJQ increased the relative abundance of Lactobacillus, Brucella, Bacteroides, Vibrio Desulfuricus and Ackermania, and decreased the relative abundance of Prevos. In addition, correlation analysis showed that the regulatory effects of PDJQ on tryptophan metabolism, histidine metabolism and TCA cycle pathway were related to the abundance changes of Lactobacillus, Bacteroides and Ackermann bacteria (141).

Gegen Qinlian Decoction (GQD) is composed of seven traditional Chinese medicines: Pueraria lobata, Coptis chinensis, Scutellaria baicalensis, Anemarrhena anemarrhena, American ginseng, red peony root and dried ginger. The mechanism of GQD in the treatment of diabetes is similar to that of berberine. GQD restores glucose homeostasis by increasing butyrate-producing bacteria, such as Faecalibacterium and Roseburia (142).

LingguiZhugan (LGZG) formula, a traditional Chinese medicine formula composed of Poria cocos, cassia twig, Atractylodes macrocephala and licorice, plays a useful role in the treatment of obesity-related diabetes. LGZG plays a role in controlling blood glucose and reducing insulin resistance, which may be mediated by intestinal microorganism OscillospiraandHelicobacte (143).

The effective components of inQiJiangtangTablet (JQJT) tablets are berberine, chlorogenic acid, astragalus polysaccharides and astragaloside IV mainly from Coptis chinensis, astragalus membranaceus and honeysuckle. Studies have shown that these active components are related to intestinal bacteria relieving insulin resistance and low-grade host inflammation. JQJT can increase the concentration of SCFAs in T2DM mice, especially butyric acid. JQJT treatment group showed lower desulphurization vibrio and higher Ackermania (144).

Xiexin T ang was first recorded in the synopsis of the Golden Chamber, an ancient Chinese medical book, and consists of rhubarb, Scutellaria baicalensis and Coptis chinensis. In traditional medicine, diabetes is called diabetes. Xiexin T ang has a long history in the treatment of diabetes and its effect is obvious. The new study found that Xiexin T ang improved diabetic symptoms in rats by changing the levels of bacteria that produce SCFAs and anti-inflammatory bacteria, such as Adlercreutzia, Barnesiella, and Prevotellaceae NK3B31 group (145).

In addition, other TCM formulations and TCM preparations derived from TCM formulations, such as Simiao Wan, Qijian

TABLE 1 The mechanism of action of individual herb or herbal extracts.

Herb/Extract	Subjects	Results	Gut microbiota	Mechanisms	References
Resveratrol	db/db mice	BW, FBS↓	Bacteroides, Alistipes, Rikenella, Odoribacter, Parabacteroides, and Alloprevotella genera↑	1.Gut barrier: ZO-1 2.Inflammation: LPS, IFN-g, TNF-a, IL-6↓ 3.Gut–kidney axis	(87)
Resveratrol	SD rats	TG, T-CHO↓	Akkermansia muciniphila, Ruminococcaceae, and Lachnospiraceae↑; Desulfovibrio↓	1.Gut barrier: occludin, ZO1, claudin1↑; the endocannabinoid system(CB1) ↓ 2.Inflammation: FAK, MyD88, and IRAK4↓; the endocannabinoid system(CB2) ↓	(111)
Resveratrol	C57BL/6J mice	BW,AST, TG,CHOL, LDL-C↓; GSH↓	Olsenella,Hydrogenoanaerobacterium↑; Barnesiella, Parasutterella↓	1.Gut barrier: zo-1, occludin↑ 2.Oxidative stress↓ 3.Inflammation: TLR4, MyD88, IL-1, TNF-α↑ 4. Fatty acid metabolism: Fabp2, Fabp1, Cpt1, Acox1↓	(89)
Berberine	SD rats	HOMA-IR,OGTT, FBG↓	Lactobacillaceae↑; Proteobacteria, Verrucomicrobia↓	1.Energy metabolism: amino acids (AAAS) and lipids	(94)
Berberis kansuensis	Wistar rats	BW、FBG、GSP、HOMA-IR↓	phyla Bacteroidetes, genera Akkermansia↑	1. inflammation: LPS, TNF-α, IL-1β,IL-6 2. IR and IS	(112)
Berberrubine	C57BL/6J mice	BW,ALT, AST↓	Ileibacterium,Mucispirillum↑	1. lipid metabolism: ACC1,FAS,CD36↓; ATGL, GK,PPAR-α, CPT-1↑	(96)
Rg1	SD rats	BW, FBG, TC, TG, LDL-C, HOMA-IR↓; LDL-C, HOMA-IS↑	Lachnospiraceae_NK4A136_group, Lachnoclostridium↑	1. SCFAs 2. Oxidative stress: 3. IR and IS	(98)
Rg5	db/db mice	FBG, OGTT↓	Firmicutes, Verrucomicrobia↓	1.Gut barrier: Occludin, ZO-1 2.Inflammation: LPS/TLR4	(101)
T19	HepG2, HFD/STZ mice	FBG, TG, TC, LD↓; BW, HDL↑	Lachnospiraceae↑	1. Insulin Signal Pathway: AMPK and PI3K	(102)
Curcumin	C57BLKS/J(−/−)_mice	Blood glucose↓	Roseburia, Erysipelatoclostridum, norank_f_Oscillospiraceae	1. Th17/Treg: IL-17A, IL-10	(65)

(Continued)

TABLE 1 Continued

Herb/Extract	Subjects	Results	Gut microbiota	Mechanisms	References
Curcumin	specificpathogen-free(SPE) rats	BW, HOMA-IR↓	Bacteroidetes, Bifidobacterium↑ Enterobacterales, Firmicutes↓	1. Gut barrier: occluding, ZO-1 2.Insulin resistance 3. Inflammation: LPS, TNF-α, TLR4/NF-κB	(113)
Tetrahydrocurcumin	C57BL/6 J mice	Serum insulin and pancreatic GLP-1↑	Firmicutes↑, Actinobacteria↓	1. GLP-1	(105)
Polygala tenuifolia	ICR mice	BW, ALT, AST, triglycerides, glucose↓	Proteobacteria↑, Deferribacteres↓	1. Lipid and cholesterol biosynthesis: PPARα	(106)
Radix Scutellariae	SD rats	FBG, LDL-C, OGTT, HOMA-IR↓	phyla Tenericutes, Patescibacteria↑, Lactobacillus, feacalibaculum↓	1. Bile acid metabolism: CYP7A1	(108)
Lycium barbarum polysaccharides	C57BL/6 mice	BW, TC, TG, LDL-C↓	Proteobacteria↓, Lactobacillus spp↑	1. SCFAs	(110)
Lycium barbarum L. leaves	SPF-grade rats	FBG, TCHO, TG, LDL-C, FFA, ALT, AST, a↓	Marvinbryantia, Parasutterella, Prevotellaceae_NK3B31_group, Blautia, Ruminococcus_1, Coprococcus_2	1. Nicotinate and nicotinamide metabolism 2. Arachidonic acid metabolism	(114)
Cinnamaldehyde	C57 mice	OGTT, IPITTs, IGF1R, IRS1↓	Lactobacillus johnsonii↑, Lactobacillus murinus↓	1. Bile acid metabolism: Deoxycholic acid/ FXR/AMPK 2. Insulin sensitivity	(115)
Dendrobium	db/dbmice	BW, LDL-C, MDA↓ INS, SOD, CAT, GSH↑	Bacteroidetes/Firmicutes, Prevotella /Akkermansia, S24-7/Rikenella/Escherichia coli	1. Lipid metabolism 2. Inflammation 3. Oxidative stress	(116)
Astragaloside IV	Kunming mice	TG, LDL, MDA↓, HDL, SOD↑	Pelatoclostridum↑, Bacteroides, Oscillibacter, Parabacteroides, Roseburia↓	1. Signaling pathways: AMPK/SIRT1, PI3K/ AKT 2.SCFAs: Butyric acid 3. Oxidative stress 4.Lipid metabolism	(117)
Astragaloside IV	C57BL/6 mice	TC, TG, LDL-C,ALT, AST↓ GLP-1, HDL-C↑	Bacteroides, Lactobacillus, Streptococcus, Enterococcus, Lactococcus↓	1. Bile acid metabolis: FXR	(118)
Laminaria japonica polysaccharide	C57BL/6 mice	ITT, OGTT, HOMA-IR↓	Akkermansia	1. Insulin resistance 2.Inflammation:LPS, TLR4	(119)
Mulberry fruit polysaccharide	db/dbmice	TG, LDL-C, MDA, FFA ↓HDL-C, SOD, GSH-Px, CAT↑	Bacteroidales, Lactobacillus, Allobaculum, Bacteroides, and Akkermansia↑	1. Lipid metabolism	(120)
Chicoric Acid	C57BL/6 mice	BW, TC, TG, LDL-C, ROS, GPT-ALT, GOT-AST↓MDA, HDL-C IL-10	Lactoba- Callus, Turicibacter, Ruminococcaceae_ UCG-014, Alloprevotella, Candidatus_Saccharimonas	1. Signaling pathway: AMPK/Nrf2/NFκB	(121)
Inulin	C57BL/6 mice	ALT, AST, OGTT, HOMA-IR↓	Akkermansia, Bifidobacterium↑ Firmicutes/Bacteroidetes↓	1.SCFAs 2.Inflammation: (IL)-18, IL-1β, TNF-α, IL-6↓, IL-10↑	(122)
Rhubarb	C57BL/6J mice	BW, FBG, OGTT, IR, TC, TG, LDL-C↓	*Akkermansia muciniphila*	1. Insulin resistance 2. Inflammation: RANTES, TNF-α, IL-	(123)

(Continued)

TABLE 1 Continued

Herb/Extract	Subjects	Results	Gut microbiota	Mechanisms	References
				6, IFN-γ 3. Lipid metabolism	
Akebia saponin D	C57BL/6J mice	FBG, TC, TG, LDL-C, HOMA-IR↓	Alistipes, Prevotella↓ Butyricimonas, Ruminococcus, Bifidobacter↑	1.Signaling pathway: PPAR-γ/FABP4	(124)
Green Tea Polyphenols	C57BL/6J mice	TC, TG, LDL-C, INS↓	Bacteroidetes/Firmicutes	1.SCFAs: Acetic acid, butyric acid↑ 2.Lipid metabolism	(125)
Quercetin	C57BL/6J mice	BW, FBG, HOMA-IR↓	Akkermansia, Verrucomicrobia phylum↑	1. Lipid metabolism 2. Inflammation: TLR-4, NLRP3, TNF-α 3. SCFAs: Butyrate	(126)
Ganoderic acid A	Kunming mice	TC, TG, LDL-C, AST, ALT, MDA↓ SOD, GSH↑	Lactobacillus, Burkholderia_Caballeroria_Paraburkholderia, Escherichia_ Shigella, Erysipelatoclostridium↓Aerococcus, Bilophila, Bifidobacterium↑	1.Lipid metabolism 2. Inflammation	(127)
Ganoderma lucidum polysaccharides	SD rats	TC, TG, LDL-C, MDA ↓HDL-C, SOD, GSH↑	Proteus, Ruminococcus, Coprococcus↓	1.SCFAs: Acetic acid, propionic acid, butyric acid 2. Inflammation: IL-1β, IL-6	(128)
Morchella esculenta mushroom polysaccharide	BALB/c mice	BW, FBG, INS, HOMA-IR↓	Lactobacillus↑ Corynebacterium, Facklamia↓	1.Bile acid metabolis 2. Inflammation: IL-6, IL-1β, TNF-α	(129)
laurolitsine	db/db mice	FBG, TC, TG, LDL-C↓ HDL-C↑	Mucispirillum schaedleri, Anaerotruncus_sp_G3_2012↓	1.Signaling pathway: LKB1-AMPK 2. Inflammation: IL-1β, TNFα, IL-6, IL-18, IL-10 3.Lipid metabolism	(130)
Gynostemma pentaphyllum	SD rats	FBG, TC, TG, LDL-C, ALT, AST, HOMA-IR↓HDL-C↑	Elusimicrobia, Cyanobacteria, Lactococcus spp↑ Ruminococcus spp↓	1. Lipid metabolism 2. Gut barrier 3.Inflammation : TNF-α, IL-1β, IL-6, TLR4	(131)
Gynostemma pentaphyllum polysaccharides	C57BL/6 mice	TC, TG, LDL-C, ALT, AST↓ HDL-C↑	Lactobacillus, Akkermansia↑Clostridia_ uncultured↓	1.Signaling pathway: TLR2/NLRP3	(132)
Poria cocos polysaccharides	C57BL/6 mice	TC, TG, LDL-C, ALT, AST, MDA↓ HDL-C↑	Faecalibaculum, Escherichia_Shigella, unclassified Oscillospirales↑ Tuzzerella, Enterococcus, Staphylococcus↓	1. Signaling pathway: NF-κB/CCL3/CCR1	(133)
Astragalus mongholicus polysaccharides	SD rats	WB, TC, TG, LDL-C, ALT, AST, HOMA-IR↓ HDL-C↑	Proteobacteria, Epsilonbacteria↑Firmicutes/ Bacteroidetes↓	1. Signaling pathway: AMPK-PPAR-α, TLR4 - NLRP3, SCFAs-GPR 2. Gut barrier: ZO-1, Occludin	(134)
Pueraria lobata starch	C57BL/6J mice	TC, TG, LDL-C, ALT, AST↓	Lactobacillus, Bifidobacterium, Turicibacter↑Desulfovibrio↓	1. SCFAs 2. Lipid metabolism 3. Inflammation: IL-6, TNF-α	(135)
Salviae polysaccharide	C57/BL6 mice	BW, FBG, TC, TG, LDL-C↓	Ruminococcus_gnavus, Clostridium_cocleatum, Bifidobacterium_pseudolongum↓	1. Lipid metabolism 2. Inflammation: IL2, IL10, TGF-β, IL-6,	(136)

(Continued)

TABLE 1 Continued

Herb/Extract	Subjects	Results	Gut microbiota	Mechanisms	References
				IL23 3. Gut barrier:LPS	
Nuciferine	SD rats	Conjugated BA, Non-12OH BA↑ TC, TG↓	Akkermansiaceae, Akkermansia, norank_f_Erysipelotrichaceae, Lachnospiraceae_NK4A136_group↑	1. Bile acid metabolis	(137)
Nuciferine	SD rats	BW, TC, TG, LDL-C↓ HDL-C↑	Akkmensia muciniphila, Ruminococcaceae, Desulfovibrionaceae	1. Signaling pathway: TLR4/MyD88/NF-κB 2. Gut barrier: ZO-1, Occludin, Mucin2 3. SCFAs: Acetic acid, Propionic acid	(138)
Myristica fragrans	C57BL/6J mice	TC, TG, LDL-C↓	Akkermansia, Blautia, Bifidobacterium, Adlercreutzia↑	1. Signaling pathway: AhR-FAS, NF-κB	(139)

mixturen, Naoxintong capsule and Herbal formula LLKL, have also been found to play a therapeutic role through intestinal flora. More details are shown in Table 2.

5 Discussion

Traditional Chinese medicine can affect the abundance of intestinal microbiota at different levels (Tables 1, 2). Therefore, we believe that the role of traditional Chinese medicine in the treatment of T2DM and NAFLD is probably related to its role in mediating intestinal microbial changes. There are differences in intestinal flora changes and therapeutic mechanisms mediated by different Chinese medicines.

5.1 Intestinal barrier

The damage of intestinal mucosa and the increase of inflammatory factors are related to T2DM and T2DM related metabolic diseases. Traditional Chinese medicine alleviates metabolic inflammation by increasing intestinal mucus and tight connection (152).Restoratol, ginsenoside Rg5, Curcumin, Nuciferinehe and traditional Chinese medicine formula Si Miao maintain the integrity of intestinal barrier by promoting the expression of tight junction protein ZO-1 and blocking protein. Nuciferine also enhances the intestinal barrier by increasing the expression of goblet cells and mucin2 (138). intestinal epithelium from damage by producing certain enzymes in the intestine. Resveratrol, Inulin, Rhubarb, Quercetin, and traditional Chinese medicine formulas such as Simiao Wan, JinQi Jiangtang T ablet, Huang Lian Jie Du Division can increase the abundance of Akkermansia. Escherichia coli is not conducive to maintaining the integrity of the intestinal barrier. The metabolic enzyme StcE produced will break down mucin, increase intestinal permeability and induce intestinal inflammation. Dendrobium can reduce the content of Escherichia coli in the intestine of db/db mice (116). Oscillibacter belonging to Ruminal Cocci family can also increase intestinal permeability. Astragaloside IV inhibits the increase of intestinal permeability by reducing the abundance of Oscillibacter (117).

5.2 Inflammation

LPS entering the intestinal tract will induce intestinal inflammation, and LPS mainly comes from vibrio desulfuricus (153). Inverterol, Pueraria lobata starch and Nuciferine can reduce the abundance of harmful bacteria, Vibrio desulfurization. LPS combines with TLR of intestinal epithelial cells to induce the release of proinflammatory factors and aggravate the host's inflammatory response. Berberis kansuensis, Rhubarb, Quercetin, Morchella esculenta mushroom polysaccharide, Gynostemma pentaphyllum, Pueraria lobata start, as well as Chinese herbal formula Gegen Qinlian Reaction and JinQi Jiangtang T ablet can reduce TNF- α, IL-1 β And IL-6 levels, thereby relieving inflammation caused by bacterial endotoxin. Salviae polysaccharide, Laurolitsine, Inulin and Curcumin can increase the level of anti-inflammatory factor IL-10 (104, 122, 130, 136). In addition, Restoratol, Curcumin, Nuciferine and Chinese herbal formula LLKL can reduce TLR4/MyD88/NF- κ B pathway inhibits LPS induced inflammatory mediator production (89) (113, 138). In particular, Curcumin alleviates T2DM symptoms by maintaining the balance of immune cells Th17 and Treg, reducing intestinal mucosal damage and infiltration of inflammatory cells (104). Chiric Acid and laurolitsine regulate AMPK/NF- κ B signal pathway can reduce systemic inflammation caused by LPS (121) (130). Oxidative stress is another factor leading to inflammatory response. Astragaloside IV can reduce the level of oxidative stress through AMPK/SIRT1 and PI3K/AKT signaling

TABLE 2 The mechanism of action of Chinese Herbal Formulae.

Herbal Formula	Subjects	Results	Gut microbiota	Mechanisms	References
Pi-Dan-Jian-Qing decoction	SD rats	TG, TC, LDL, ALT, AST, MDA, HOMA-IR↓ HDL, SOD, GSH-Px↑	Prevotella↓ Lactobacill, Desulfovib, Akkerman, Bacteroides↑	1.Histamine metabolism 2.Tryptophan metabolism 3. TCA cycle 4.Oxidative stress 5. Inflammation	(141)
Gegen Qinlian Decoction	GK rats	BW, NFBG, HOMA-IR↓	Faecalibacterium, Roseburia↑	1.SCFAs: butyrate 2. Inflammation: IL-1β, IL-6, IL-17, TNF-α, IFN-γ, MCP-1 3. Lipid metabolism	(142)
Linggui Zhugan	C57BL/6 J mice	BW, FBG, TG, TC, LDL, FFA, HOMA-IR↓ HDL↑	Lactobacillus, Bacteroides↑ Helicobacter↓	1.Lipid metabolis 2.Insulin resistance	(143)
JinQi Jiangtang Tablet	C57BL/6J mice	FBG, HbA1c↓	Akkermansia↑ Desulfovibrio↓	1.SCFAs:Acetic acid, Propionic acid, Butyric acid 2. Insulin resistance: TNF-α, IL-6, MCP-1	(144)
Xiexin Tang	SD rats	TC, TG, LDL-C↓ HDL-C↑	Adlercreutzia, Alloprevotella, Barnesiella, Prevotellaceae NK3B31 group	1.Lipid metabolis 2. Inflammation:	(145)
Xiexin Tang	SD rats	TC, TG, LDL-C↓ HDL-C↑	Adlercreutzia Barnesiella, Blautia, Lachnospiraceae, Prevotellaceae NK3B31 group↑	1. SCFAs 2.Energy metabolism 3. Signal Pathway:PGC-1α/UCP-2, AMPK/mTOR	(146)
Simiao Wan	C57BL/6J mice	Primary BAs↑ Secondary BAs↓	Allobaculum, Clostridium, Akkermansia, Lactobacilus, Bilophila↑ Coprococcus, Halomonas↓	1. Bile acid metabolism	(147)
Si Miao	C57BL/6 mice	BW, ALT, AST, TC, LDL-C↓ HDL-C↑	Akkermansia, Bifidobacterium, Faecalibaculum↑	1.Lipid metabolism 2.Inflammation 3.Gut barrier	(10)
Qijian mixture	KKay mice	FBG, WB, TC, INS↓	Bacteroidetes, Lachnospiraceae NK4A136 group, Enterorhabdu, Lachnospiraceae, Prevotellacea, Parabacteroides↑	1.Signal Pathway: TP53, AKT1 and PPARA	(148)
Naoxintong capsule	SD rats	TG, TC, FFA, LDL-C↓ HDL-C↑	[Ruminococcus] gnavus group, Erysipelatoclostridium, Oscillibacter, Ruminiclostridium 9, Ruminococcus 1	1. Insulin resistance 2. Inflammation: IL-1β, TNF-α, IL-6↓ IL-4↑ 3. Lipid metabolism	(149)
LLKL	Zucker rats	FFA, TC, TG↓	Proteobacteria, Actinobacteria.	1. Signal Pathway: TLR4, MyD88, CTSK 2. Lipid metabolism 3. Inflammation: LPS, TNF-α, IL-6↓	(150)
Huang-Lian-Jie-Du-Decoction	SD rats	ALT, AST, TG, TC, LDL-C, HOMA-IR↓ SOD, CAT, GSH↑	Parabacteroides, Blautia, Akkermansia	1. SCFAs 2. Bile acid metabolism 3. Lipid metabolismI	(151)

pathways. Dendrobium, Mulberry fruit polysaccharide and Ganoderma lucidum extract have antioxidant capacity, which can reduce the level of malondialdehyde (MDA) and increase the content of superoxide dismutase (SOD), catalase (CA T) and glutathione (GSH) (116, 120, 127). The anti-inflammatory effect of traditional Chinese medicine may be mediated by increasing the abundance of anti-inflammatory bacteria Akkermania, Parabolides, Lactobacillus, Bacteroides and Blautia (141, 151).

5.3 SCFAs

TCM affects T2DM and NAFLD by affecting the abundance of SCFAs producing bacteria and the metabolism of SCFAs. SCFAs (acetate, propionate and butyrate) are produced by selective fermentation of intestinal microorganisms (154). Acetate participates in host energy metabolism by promoting the secretion of intestinal hormones (GLP-1 and PYY). Acetate is mainly produced by bifidobacteria and lactobacillus (155). Acetate can be converted to butyric acid by Firmicutes bacteria. Butyrate can protect the intestinal barrier and reduce inflammation (156). Clostridia, Bacteroides and Bifidobasteria are related to the production of butyric acid (157). Propionate is believed to reduce fat production, and serum cholesterol level has a beneficial effect on disorders of lipid metabolism (158). Green Tea Polyphenols increased the levels of acetic acid and butyric acid, which may be related to the increase of Clostridium populati, Blautia luti, Akkermania muciniphila and Thiothrix unzii (125). The increase of SCFAs content in Pueraria lobata star may be due to the increase of the content of Lactobacillus, Bifidobacterium and Turicibacte (135). Using Inulin in NAFLD treatment, it was found that SCFAs were positively correlated with Bacteroidetes, Akkermania and Bifidobasterium, and negatively correlated with Proteobasteria, Blautia and Ileiberium (122). In addition, the study also found that ginsenoside Rg1 can increase Lachnospiracea_ NK4A136_ The proportion of group, Roseburia and Romboutsia increases the content of SCFAs (98).

5.4 Bile acid metabolism

Bile acid metabolism, as an important part of the body's regulation of glucose and lipid metabolism, is mainly mediated by G-protein coupled BA receptor (TGR5) and nuclear receptor Farni X receptor (FXR) (159). TGR5 is expressed in intestinal epithelial cells. The activation of TGR5 is conducive to the renewal of intestinal epithelial cells and the repair of intestinal barrier function (160). Cholesterol - 7 α- Hydroxylase (CYP7A1) is the rate limiting enzyme for converting cholesterol into BA (161). The changes of intestinal flora involved in bile acid metabolism mainly include bile salt hydrolase (BSH) and α- Dehydroxylated genera decreased and taurine metabolism related genera increased (137). Radix Scutellariae, Cinnamaldehyde, Astragaloside IV, Morchella esculenta mushroom polysaccharide, Nuciferine and Simiao Wan can all improve glycolipid disorder through bile acid metabolism. Detailed mechanisms are shown in Tables 1 and 2.

6 Conclusion and prospect

Traditional Chinese medicine has the potential to treat metabolic diseases such as diabetes and non-alcoholic fatty liver. Reshaping intestinal flora and regulating intestinal microbial metabolism is the key for traditional Chinese medicine to play a therapeutic role. at the same time, intestinal flora also provides a new opportunity to clarify the mechanism of traditional Chinese medicine in the treatment of diseases. The main mechanisms of traditional Chinese medicine include: improving the proportion of thick-walled bacteria and Bacteroides, increasing dominant flora and reducing harmful flora; regulating intestinal microbial metabolites such as short-chain fatty acids and bile acids; and restoring intestinal barrier. Increase the expression of tight junction proteins and reduce the level of inflammatory factors. It can be seen that maintaining the stability of intestinal microecology is of great significance to human health. The intestinal microecology is stable and healthy, and the destruction of intestinal microecology leads to the occurrence of disease. Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus and/or Bifidobacterium can improve blood glucose levels in patients with diabetes. It can be inferred that dietary fiber, probiotics and probiotics are beneficial to the recovery of the disease. In addition, fecal microorganism transplantation has therapeutic potential in chronic inflammation, functional bowel disease, insulin resistance and morbid obesity. Herbs can be used as a treasure trove of potential probiotics for more in-depth research.

Author contributions

All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Li HS, Hu YY. Intestinal microecology: An important target for Chinese medicine treatment of non-alcoholic fatty liver disease. *Chin J Integr Med* (2020) 26 (10):723–8. doi: 10.1007/s11655-020-3268-3

2. Liu L, Zhang J, Cheng Y, Zhu M, Xiao Z, Ruan G, et al. Gut microbiota: A new target for T2DM prevention and treatment. *Front Endocrinol (Lausanne)* (2022) 13:958218. doi: 10.3389/fendo.2022.958218

3. Cobbina E, Akhlaghi F. Non-alcoholic fatty liver disease (NAFLD) - pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metab Rev* (2017) 49(2):197–211. doi: 10.1080/03602532.2017.1293683

4. Upadhyaya S, Banerjee G. Type 2 diabetes and gut microbiome: at the intersection of known and unknown. *Gut Microbes* (2015) 6(2):85–92. doi: 10.1080/19490976.2015.1024918

5. Zhang B, Liu K, Yang H, Jin Z, Ding Q, Zhao L. Gut microbiota: The potential key target of TCM's therapeutic effect of treating different diseases using the same method–UC and T2DM as examples. *Front Cell Infection Microbiol* (2022) 12:855075. doi: 10.3389/fcimb.2022.855075

6. Bril F, Cusi K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: A call to action. *Diabetes Care* (2017) 40(3):419–30. doi: 10.2337/dc16-1787

7. Blazina I, Selph S. Diabetes drugs for nonalcoholic fatty liver disease: a systematic review. *Syst Rev* (2019) 8(1):295. doi: 10.1186/s13643-019-1200-8

8. Kim KS, Lee BW. Beneficial effect of anti-diabetic drugs for nonalcoholic fatty liver disease. *Clin Mol Hepatol* (2020) 26(4):430–43. doi: 10.3350/cmh.2020.0137

9. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2013) 36 Suppl 1(Suppl 1):S67–74. doi: 10.2337/dc13-S067

10. Han R, Qiu H, Zhong J, Zheng N, Li B, Hong Y, et al. Si Miao formula attenuates non-alcoholic fatty liver disease by modulating hepatic lipid metabolism and gut microbiota. *Phytomedicine* (2021) 85:153544. doi: 10.1016/j.phymed.2021.153544

11. Bril F, Lomonaco R, Orsak B, Ortiz-Lopez C, Webb A, Tio F, et al. Relationship between disease severity, hyperinsulinemia, and impaired insulin clearance in patients with nonalcoholic steatohepatitis. *Hepatology* (2014) 59 (6):2178–87. doi: 10.1002/hep.26988

12. Parise ER. NONALCOHOLIC FATTY LIVER DISEASE (NAFLD), MORE THAN a LIVER DISEASE. *Arq Gastroenterol* (2019) 56(3):243–5. doi: 10.1590/s0004-2803.201900000-45

13. Chang Y, Jung HS, Yun KE, Cho J, Cho YK, Ryu S. Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. *Am J Gastroenterol* (2013) 108(12):1861–8. doi: 10.1038/ajg.2013.349

14. Movahedian M, Rahmani J, Hashemi Nazari SS, Mohamadi S, Naik G, Hekmatdoost A. Fatty liver index and risk of diabetes incidence: A systematic review and dose-response meta-analysis of cohort studies. *Prim Care Diabetes* (2020) 14(6):577–83. doi: 10.1016/j.pcd.2020.02.011

15. Chung SM, Kang MK, Jung J, Yoon JS, Won KC, Lee HW, et al. Long-term effects of the changes in hepatic steatosis status on the risk of incident type 2 diabetes mellitus: A 15-year community-based prospective cohort study. *Diabetes Res Clin Pract* (2022) 184:109208. doi: 10.1016/j.diabres.2022.109208

16. Abdelmalek MF, Liu C, Shuster J, Nelson DR, Asal NR. Familial aggregation of insulin resistance in first-degree relatives of patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* (2006) 4(9):1162–9. doi: 10.1016/j.cgh.2006.06.001

17. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* (2012) 56(3):943–51. doi: 10.1002/hep.25772

18. Hasanvand A. The role of AMPK-dependent pathways in cellular and molecular mechanisms of metformin: a new perspective for treatment and prevention of diseases. *Inflammopharmacology* (2022) 30(3):775–88. doi: 10.1007/s10787-022-00980-6

19. Woo SL, Xu H, Li H, Zhao Y, Hu X, Zhao J, et al. Metformin ameliorates hepatic steatosis and inflammation without altering adipose phenotype in diet-induced obesity. *PloS One* (2014) 9(3):e91111. doi: 10.1371/journal.pone.0091111

20. Zamani-Garmsiri F, Ghasempour G, Aliabadi M, Hashemnia SMR, Emamgholipour S, Meshkani R. Combination of metformin and chlorogenic acid attenuates hepatic steatosis and inflammation in high-fat diet fed mice. *IUBMB Life* (2021) 73(1):252–63. doi: 10.1002/iub.2424

21. Guo WR, Liu J, Cheng LD, Liu ZY, Zheng XB, Liang H, et al. Metformin alleviates steatohepatitis in diet-induced obese mice in a SIRT1-dependent way. *Front Pharmacol* (2021) 12:704112. doi: 10.3389/fphar.2021.704112

22. Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, et al. Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa zucker rats independent of glycemia and body weight. *Endocrinology* (2005) 146(4):2069–76. doi: 10.1210/en.2004-1349

23. Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* (2011) 31(9):1285–97. doi: 10.1111/j.1478-3231.2011.02462.x

24. Ding X, Saxena NK, Lin S, Gupta NA, Anania FA. Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* (2006) 43(1):173–81. doi: 10.1002/hep.21006

25. Lee J, Hong SW, Chae SW, Kim DH, Choi JH, Bae JC, et al. Exendin-4 improves steatohepatitis by increasing Sirt1 expression in high-fat diet-induced obese C57BL/6J mice. *PloS One* (2012) 7(2):e31394. doi: 10.1371/journal.pone.0031394

26. Mells JE, Fu PP, Sharma S, Olson D, Cheng L, Handy JA, et al. Glp-1 analog, liraglutide, ameliorates hepatic steatosis and cardiac hypertrophy in C57BL/6J mice fed a Western diet. *Am J Physiol Gastrointest Liver Physiol* (2012) 302(2):G225–235. doi: 10.1152/ajpgi.00274.2011

27. Tian F, Zheng Z, Zhang D, He S, Shen J. Efficacy of liraglutide in treating type 2 diabetes mellitus complicated with non-alcoholic fatty liver disease. *Biosci Rep* (2018) 38(6):1–9. doi: 10.1042/bsr20181304

28. Wu YR, Shi XY, Ma CY, Zhang Y, Xu RX, Li JJ. Liraglutide improves lipid metabolism by enhancing cholesterol efflux associated with ABCA1 and ERK1/2 pathway. *Cardiovasc Diabetol* (2019) 18(1):146. doi: 10.1186/s12933-019-0954-6

29. Liu L, Fan L, Chan M, Kraakman MJ, Yang J, Fan Y, et al. PPARγ deacetylation confers the antiatherogenic effect and improves endothelial function in diabetes treatment. *Diabetes* (2020) 69(8):1793–803. doi: 10.2337/db20-0217

30. Yang H, Suh DH, Kim DH, Jung ES, Liu KH, Lee CH, et al. Metabolomic and lipidomic analysis of the effect of pioglitazone on hepatic steatosis in a rat model of obese type 2 diabetes. *Br J Pharmacol* (2018) 175(17):3610–25. doi: 10.1111/bph.14434

31. Jiang L, Zhang H, Xiao D, Wei H, Chen Y. Farnesoid X receptor (FXR): Structures and ligands. *Comput Struct Biotechnol J* (2021) 19:2148–59. doi: 10.1016/j.csbj.2021.04.029

32. Han SY, Song HK, Cha JJ, Han JY, Kang YS, Cha DR. Farnesoid X receptor (FXR) agonist ameliorates systemic insulin resistance, dysregulation of lipid metabolism, and alterations of various organs in a type 2 diabetic kidney animal model. *Acta Diabetol* (2021) 58(4):495–503. doi: 10.1007/s00592-020-01652-z

33. Sun T, Xue M, Yang J, Pei Z, Zhang N, Qin K, et al. Metabolic regulation mechanism of fucoidan *via* intestinal microecology in diseases. *J Sci Food Agric* (2021) 101(11):4456–63. doi: 10.1002/jsfa.11202

34. Chen Y, Zhou J, Wang L. Role and mechanism of gut microbiota in human disease. *Front Cell Infect Microbiol* (2021) 11:625913. doi: 10.3389/fcimb.2021.625913

35. Wu J, Wang K, Wang X, Pang Y, Jiang C. The role of the gut microbiome and its metabolites in metabolic diseases. *Protein Cell* (2021) 12(5):360–73. doi: 10.1007/s13238-020-00814-7

36. Lembo E, Russo MF, Verrastro O, Anello D, Angelini G, Iaconelli A, et al. Prevalence and predictors of non-alcoholic steatohepatitis in subjects with morbid

obesity and with or without type 2 diabetes. *Diabetes Metab* (2022) 48(5):101363. doi: 10.1016/j.diabet.2022.101363

37. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity* (2022) 55(1):31–55. doi: 10.1016/j.immuni.2021.12.013

38. Tsai MC, Liu YY, Lin CC, Wang CC, Wu YJ, Yong CC, et al. Gut microbiota dysbiosis in patients with biopsy-proven nonalcoholic fatty liver disease: A cross-sectional study in Taiwan. *Nutrients* (2020) 12(3):820. doi: 10.3390/nu12030820

39. Cheng Z, Zhang L, Yang L, Chu H. The critical role of gut microbiota in obesity. *Front Endocrinol (Lausanne)* (2022) 13:1025706. doi: 10.3389/fendo.2022.1025706

40. Yang HT, Liu JK, Xiu WJ, Tian TT, Yang Y, Hou XG, et al. Gut microbiome-based diagnostic model to predict diabetes mellitus. *Bioengineered* (2021) 12(2):12521–34. doi: 10.1080/21655979.2021.2009752

41. Gulnaz A, Nadeem J, Han JH, Lew LC, Son JD, Park YH, et al. Lactobacillus sps in reducing the risk of diabetes in high-fat diet-induced diabetic mice by modulating the gut microbiome and inhibiting key digestive enzymes associated with diabetes. *Biol (Basel)* (2021) 10(4):348. doi: 10.3390/biology10040348

42. Kang H, You HJ, Lee G, Lee SH, Yoo T, Choi M, et al. Interaction effect between NAFLD severity and high carbohydrate diet on gut microbiome alteration and hepatic *de novo* lipogenesis. *Gut Microbes* (2022) 14(1):2078612. doi: 10.1080/19490976.2022.2078612

43. Sangineto M, Grander C, Grabherr F, Mayr L, Enrich B, Schwärzler J, et al. Recovery of bacteroides thetaiotaomicron ameliorates hepatic steatosis in experimental alcohol-related liver disease. *Gut Microbes* (2022) 14(1):2089006. doi: 10.1080/19490976.2022.2089006

44. Hu J, Lin S, Zheng B, Cheung PCK. Short-chain fatty acids in control of energy metabolism. *Crit Rev Food Sci Nutr* (2018) 58(8):1243–9. doi: 10.1080/10408398.2016.1245650

45. Chu H, Duan Y, Yang L, Schnabl B. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut* (2019) 68(2):359–70. doi: 10.1136/gutjnl-2018-316307

46. Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients* (2015) 7(4):2839–49. doi: 10.3390/nu7042839

47. Zhang S, Zhao J, Xie F, He H, Johnston LJ, Dai X, et al. Dietary fiber-derived short-chain fatty acids: A potential therapeutic target to alleviate obesity-related nonalcoholic fatty liver disease. *Obes Rev* (2021) 22(11):e13316. doi: 10.1111/obr.13316

48. Al Mahri S, Malik SS, Al Ibrahim M, Haji E, Dairi G, Mohammad S. Free fatty acid receptors (FFARs) in adipose: Physiological role and therapeutic outlook. *Cells* (2022) 11(4):750. doi: 10.3390/cells11040750

49. Secor JD, Fligor SC, Tsikis ST, Yu LJ, Puder M. Free fatty acid receptors as mediators and therapeutic targets in liver disease. *Front Physiol* (2021) 12:656441. doi: 10.3389/fphys.2021.656441

50. Priyadarshini M, Wicksteed B, Schiltz GE, Gilchrist A, Layden BT. SCFA receptors in pancreatic β cells: Novel diabetes targets? *Trends Endocrinol Metab* (2016) 27(9):653–64. doi: 10.1016/j.tem.2016.03.011

51. den Besten G, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* (2013) 305(12):G900–910. doi: 10.1152/ajpgi.00265.2013

52. Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. *Compr Physiol* (2017) 8(1):1–8. doi: 10.1002/cphy.c170012

53. Yoshida H, Ishii M, Akagawa M. Propionate suppresses hepatic gluconeogenesis *via* GPR43/AMPK signaling pathway. *Arch Biochem Biophys* (2019) 672:108057. doi: 10.1016/j.abb.2019.07.022

54. Hong YH, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, et al. Acetate and propionate short chain fatty acids stimulate adipogenesis *via* GPCR43. *Endocrinology* (2005) 146(12):5092–9. doi: 10.1210/en.2005-0545

55. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, et al. The gut microbiota suppresses insulin-mediated fat accumulation *via* the short-chain fatty acid receptor GPR43. *Nat Commun* (2013) 4:1829. doi: 10.1038/ncomms2852

56. Zhang L, Chu J, Hao W, Zhang J, Li H, Yang C, et al. Gut microbiota and type 2 diabetes mellitus: Association, mechanism, and translational applications. *Mediators Inflammation* (2021) 2021:5110276. doi: 10.1155/2021/5110276

57. Larraufie P, Martin-Gallausiaux C, Lapaque N, Dore J, Gribble FM, Reimann F, et al. SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Sci Rep* (2018) 8(1):74. doi: 10.1038/s41598-017-18259-0

58. Coutinho-Wolino KS, de Cardozo F.C.L.F.M., de Oliveira Leal V, Mafra D, Stockler-Pinto MB. Can diet modulate trimethylamine n-oxide (TMAO) production? what do we know so far? *Eur J Nutr* (2021) 60(7):3567–84. doi: 10.1007/s00394-021-02491-6

59. Arias N, Arboleya S, Allison J, Kaliszewska A, Higarza SG, Gueimonde M, et al. The relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. *Nutrients* (2020) 12(8):2340. doi: 10.3390/nu12082340

60. Li X, Hong J, Wang Y, Pei M, Wang L, Gong Z. Trimethylamine-N-Oxide pathway: A potential target for the treatment of MAFLD. *Front Mol Biosci* (2021) 8:733507. doi: 10.3389/fmolb.2021.733507

61. Kalagi NA, Thota RN, Stojanovski E, Alburikan KA, Garg ML. Association between plasma trimethylamine n-oxide levels and type 2 diabetes: A case control study. *Nutrients* (2022) 14(10):2093. doi: 10.3390/nu14102093

62. Li SY, Chen S, Lu XT, Fang AP, Chen YM, Huang RZ, et al. Serum trimethylamine-n-oxide is associated with incident type 2 diabetes in middle-aged and older adults: a prospective cohort study. *J Transl Med* (2022) 20(1):374. doi: 10.1186/s12967-022-03581-7

63. León-Mimila P, Villamil-Ramírez H, Li XS, Shih DM, Hui ST, Ocampo-Medina E, et al. Trimethylamine n-oxide levels are associated with NASH in obese subjects with type 2 diabetes. *Diabetes Metab* (2021) 47(2):101183. doi: 10.1016/j.diabet.2020.07.010

64. Krueger ES, Beales JL, Russon KB, Elison WS, Davis JR, Hansen JM, et al. Gut metabolite trimethylamine n-oxide protects INS-1 β-cell and rat islet function under diabetic glucolipotoxic conditions. *Biomolecules* (2021) 11(12):1892. doi: 10.3390/biom11121892

65. Shao JW, Ge TT, Chen SZ, Wang G, Yang Q, Huang CH, et al. Role of bile acids in liver diseases mediated by the gut microbiome. *World J Gastroenterol* (2021) 27(22):3010–21. doi: 10.3748/wjg.v27.i22.3010

66. Du L, Li Q, Yi H, Kuang T, Tang Y, Fan G. Gut microbiota-derived metabolites as key actors in type 2 diabetes mellitus. *BioMed Pharmacother* (2022) 149:112839. doi: 10.1016/j.biopha.2022.112839

67. Li R, Andreu-Sánchez S, Kuipers F, Fu J. Gut microbiome and bile acids in obesity-related diseases. *Best Pract Res Clin Endocrinol Metab* (2021) 35(3):101493. doi: 10.1016/j.beem.2021.101493

68. Trabelsi MS, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine l cells. *Nat Commun* (2015) 6:7629. doi: 10.1038/ncomms8629

69. Takahashi S, Luo Y, Ranjit S, Xie C, Libby AE, Orlicky DJ, et al. Bile acid sequestration reverses liver injury and prevents progression of nonalcoholic steatohepatitis in Western diet-fed mice. *J Biol Chem* (2020) 295(14):4733–47. doi: 10.1074/jbc.RA119.011913

70. Du C, Liu WJ, Yang J, Zhao SS, Liu HX. The role of branched-chain amino acids and branched-chain α-keto acid dehydrogenase kinase in metabolic disorders. *Front Nutr* (2022) 9:932670. doi: 10.3389/fnut.2022.932670

71. Gojda J, Cahova M. Gut microbiota as the link between elevated BCAA serum levels and insulin resistance. *Biomolecules* (2021) 11(10):1414. doi: 10.3390/biom11101414

72. Zhang R, Mu H, Li Z, Zeng J, Zhou Q, Li H, et al. Oral administration of branched-chain amino acids ameliorates high-fat diet-induced metabolic-associated fatty liver disease *via* gut microbiota-associated mechanisms. *Front Microbiol* (2022) 13:920277. doi: 10.3389/fmicb.2022.920277

73. Zakaria NF, Hamid M, Khayat ME. Amino acid-induced impairment of insulin signaling and involvement of G-protein coupling receptor. *Nutrients* (2021) 13(7):2229. doi: 10.3390/nu13072229

74. Cuomo P, Capparelli R, Iannelli A, Iannelli D. Role of branched-chain amino acid metabolism in type 2 diabetes, obesity, cardiovascular disease and non-alcoholic fatty liver disease. *Int J Mol Sci* (2022) 23(8):4325. doi: 10.3390/ijms23084325

75. Lo EKK, Felicianna, Xu JH, Zhan Q, Zeng Z, El-Nezami H. The emerging role of branched-chain amino acids in liver diseases. *Biomedicines* (2022) 10(6):1444. doi: 10.3390/biomedicines10061444

76. Iwao M, Gotoh K, Arakawa M, Endo M, Honda K, Seike M, et al. Supplementation of branched-chain amino acids decreases fat accumulation in the liver through intestinal microbiota-mediated production of acetic acid. *Sci Rep* (2020) 10(1):18768. doi: 10.1038/s41598-020-75542-3

77. Portincasa P, Bonfrate L, Khalil M, Angelis M, Calabrese FM, D'Amato M, et al. Intestinal barrier and permeability in health, obesity and NAFLD. *Biomedicines* (2021) 10(1):83. doi: 10.3390/biomedicines10010083

78. Murakami Y, Tanabe S, Suzuki T. High-fat diet-induced intestinal hyperpermeability is associated with increased bile acids in the Large intestine of mice. *J Food Sci* (2016) 81(1):H216–222. doi: 10.1111/1750-3841.13166

79. Cornell RP. Gut-derived endotoxin elicits hepatotrophic factor secretion for liver regeneration. *Am J Physiol* (1985) 249(5 Pt 2):R551–562. doi: 10.1152/ajpregu.1985.249.5.R551

80. Zheng Z, Wang B. The gut-liver axis in health and disease: The role of gut microbiota-derived signals in liver injury and regeneration. *Front Immunol* (2021) 12:775526. doi: 10.3389/fimmu.2021.775526

81. Guo S, Nighot M, Al-Sadi R, Alhmoud T, Nighot P, Ma TY. Lipopolysaccharide regulation of intestinal tight junction permeability is

mediated by TLR4 signal transduction pathway activation of FAK and MyD88. *J Immunol* (2015) 195(10):4999–5010. doi: 10.4049/jimmunol.1402598

82. Nolan JP. The role of intestinal endotoxin in liver injury: a long and evolving history. *Hepatology* (2010) 52(5):1829–35. doi: 10.1002/hep.23917

83. Ferro D, Baratta F, Pastori D, Cocomello N, Colantoni A, Angelico F, et al. New insights into the pathogenesis of non-alcoholic fatty liver disease: Gut-derived lipopolysaccharides and oxidative stress. *Nutrients* (2020) 12(9):2762. doi: 10.3390/nu12092762

84. Tian B, Zhao J, Zhang M, Chen Z, Ma Q, Liu H, et al. Lycium ruthenicum anthocyanins attenuate high-fat diet-induced colonic barrier dysfunction and inflammation in mice by modulating the gut microbiota. *Mol Nutr Food Res* (2021) 65(8):e2000745. doi: 10.1002/mnfr.202000745

85. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504(7480):451–5. doi: 10.1038/nature12726

86. Hoca M, Becer E, Vatansever HS. The role of resveratrol in diabetes and obesity associated with insulin resistance. *Arch Physiol Biochem* (2021) 1-7:1–7. doi: 10.1080/13813455.2021.1893338

87. Cai TT, Ye XL, Li RR, Chen H, Wang YY, Yong HJ, et al. Resveratrol modulates the gut microbiota and inflammation to protect against diabetic nephropathy in mice. *Front Pharmacol* (2020) 11:1249. doi: 10.3389/fphar.2020.01249

88. Andrade JM, Paraíso AF, de Oliveira MV, Martins AM, Neto JF, Guimarães AL, et al. Resveratrol attenuates hepatic steatosis in high-fat fed mice by decreasing lipogenesis and inflammation. *Nutrition* (2014) 30(7-8):915–9. doi: 10.1016/j.nut.2013.11.016

89. Du F, Huang R, Lin D, Wang Y, Yang X, Huang X, et al. Resveratrol improves liver steatosis and insulin resistance in non-alcoholic fatty liver disease in association with the gut microbiota. *Front Microbiol* (2021) 12:611323. doi: 10.3389/fmicb.2021.611323

90. Khoshandam A, Imenshahidi M, Hosseinzadeh H. Pharmacokinetic of berberine, the main constituent of berberis vulgaris l.: A comprehensive review. *Phytother Res* (2022) 36(11):4063–79. doi: 10.1002/ptr.7589

91. Yang Y, Cao S, Xu W, Zang C, Zhang F, Xie Y, et al. Dual modulation of gut bacteria and fungi manifests the gut-based anti-hyperlipidemic effect of coptidis rhizoma. *BioMed Pharmacother* (2022) 153:113542. doi: 10.1016/j.biopha.2022.113542

92. Wang H, Zhang H, Gao Z, Zhang Q, Gu C. The mechanism of berberine alleviating metabolic disorder based on gut microbiome. *Front Cell Infect Microbiol* (2022) 12:854885. doi: 10.3389/fcimb.2022.854885

93. Ruiz-Canela M, Guasch-Ferré M, Toledo E, Clish CB, Razquin C, Liang L, et al. Plasma branched chain/aromatic amino acids, enriched Mediterranean diet and risk of type 2 diabetes: case-cohort study within the PREDIMED trial. *Diabetologia* (2018) 61(7):1560–71. doi: 10.1007/s00125-018-4611-5

94. Yao Y, Chen H, Yan L, Wang W, Wang D. Berberine alleviates type 2 diabetic symptoms by altering gut microbiota and reducing aromatic amino acids. *BioMed Pharmacother* (2020) 131:110669. doi: 10.1016/j.biopha.2020.110669

95. Sun R, Kong B, Yang N, Cao B, Feng D, Yu X, et al. The hypoglycemic effect of berberine and berberrubine involves modulation of intestinal farnesoid X receptor signaling pathway and inhibition of hepatic gluconeogenesis. *Drug Metab Dispos* (2021) 49(3):276–86. doi: 10.1124/dmd.120.000215

96. Yang S, Cao S, Li C, Zhang J, Liu C, Qiu F, et al. Berberrubine, a main metabolite of berberine, alleviates non-alcoholic fatty liver disease *via* modulating glucose and lipid metabolism and restoring gut microbiota. *Front Pharmacol* (2022) 13:913378. doi: 10.3389/fphar.2022.913378

97. Chen Z, Zhang Z, Liu J, Qi H, Li J, Chen J, et al. Gut microbiota: Therapeutic targets of ginseng against multiple disorders and ginsenoside transformation. *Front Cell Infect Microbiol* (2022) 12:853981. doi: 10.3389/fcimb.2022.853981

98. Peng M, Wang L, Su H, Zhang L, Yang Y, Sun L, et al. Ginsenoside Rg1 improved diabetes through regulating the intestinal microbiota in high-fat diet and streptozotocin-induced type 2 diabetes rats. *J Food Biochem* (2022) 46(10):e14321. doi: 10.1111/jfbc.14321

99. Vacca M, Celano G, Calabrese FM, Portincasa P, Gobbetti M, De Angelis M. The controversial role of human gut lachnospiraceae. *Microorganisms* (2020) 8(4):573. doi: 10.3390/microorganisms8040573

100. Berger K, Burleigh S, Lindahl M, Bhattacharya A, Patil P, Stålbrand H, et al. Xylooligosaccharides increase bifidobacteria and lachnospiraceae in mice on a high-fat diet, with a concomitant increase in short-chain fatty acids, especially butyric acid. *J Agric Food Chem* (2021) 69(12):3617–25. doi: 10.1021/acs.jafc.0c06279

101. Wei Y, Yang H, Zhu C, Deng J, Fan D. Hypoglycemic effect of ginsenoside Rg5 mediated partly by modulating gut microbiota dysbiosis in diabetic db/db mice. *J Agric Food Chem* (2020) 68(18):5107–17. doi: 10.1021/acs.jafc.0c00605

102. Xu J, Li T, Xia X, Fu C, Wang X, Zhao Y. Dietary ginsenoside T19 supplementation regulates glucose and lipid metabolism *via* AMPK and PI3K pathways and its effect on intestinal microbiota. *J Agric Food Chem* (2020) 68(49):14452–62. doi: 10.1021/acs.jafc.0c04429

103. Filardi T, Varì R, Ferretti E, Zicari A, Morano S, Santangelo C. Curcumin: Could this compound be useful in pregnancy and pregnancy-related complications? *Nutrients* (2020) 12(10):3179. doi: 10.3390/nu12103179

104. Xiao QP, Zhong YB, Kang ZP, Huang JQ, Fang WY, Wei SY, et al. Curcumin regulates the homeostasis of Th17/Treg and improves the composition of gut microbiota in type 2 diabetic mice with colitis. *Phytother Res* (2022) 36(4):1708–23. doi: 10.1002/ptr.7404

105. Yuan T, Yin Z, Yan Z, Hao Q, Zeng J, Li L, et al. Tetrahydrocurcumin ameliorates diabetes profiles of db/db mice by altering the composition of gut microbiota and up-regulating the expression of GLP-1 in the pancreas. *Fitoterapia* (2020) 146:104665. doi: 10.1016/j.fitote.2020.104665

106. Wang CC, Yen JH, Cheng YC, Lin CY, Hsieh CT, Gau RJ, et al. Polygala tenuifolia extract inhibits lipid accumulation in 3T3-L1 adipocytes and high-fat diet-induced obese mouse model and affects hepatic transcriptome and gut microbiota profiles. *Food Nutr Res* (2017) 61(1):1379861. doi: 10.1080/16546628.2017.1379861

107. Zhang Y, Xu Y, Zhang L, Chen Y, Wu T, Liu R, et al. Licorice extract ameliorates hyperglycemia through reshaping gut microbiota structure and inhibiting TLR4/NF-kappaB signaling pathway in type 2 diabetic mice. *Food Res Int* (2022) 153:110945. doi: 10.1016/j.foodres.2022.110945

108. Zhao L, Ma P, Peng Y, Wang M, Peng C, Zhang Y, et al. Amelioration of hyperglycaemia and hyperlipidaemia by adjusting the interplay between gut microbiota and bile acid metabolism: Radix scutellariae as a case. *Phytomedicine* (2021) 83:153477. doi: 10.1016/j.phymed.2021.153477

109. Zhu W, Zhou S, Liu J, McLean RJC, Chu W. Prebiotic, immuno-stimulating and gut microbiota-modulating effects of lycium barbarum polysaccharide. *BioMed Pharmacother* (2020) 121:109591. doi: 10.1016/j.biopha.2019.109591

110. Yang Y, Chang Y, Wu Y, Liu H, Liu Q, Kang Z, et al. A homogeneous polysaccharide from lycium barbarum: Structural characterizations, anti-obesity effects and impacts on gut microbiota. *Int J Biol Macromol* (2021) 183:2074–87. doi: 10.1016/j.ijbiomac.2021.05.209

111. Chen M, Hou P, Zhou M, Ren Q, Wang X, Huang L, et al. Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. *Clin Nutr* (2020) 39(4):1264–75. doi: 10.1016/j.clnu.2019.05.020

112. Xu T, Ge Y, Du H, Li Q, Xu X, Yi H, et al. Berberis kansuensis extract alleviates type 2 diabetes in rats by regulating gut microbiota composition. *J Ethnopharmacol* (2021) 273:113995. doi: 10.1016/j.jep.2021.113995

113. Huang J, Guan B, Lin L, Wang Y. Improvement of intestinal barrier function, gut microbiota, and metabolic endotoxemia in type 2 diabetes rats by curcumin. *Bioengineered* (2021) 12(2):11947–58. doi: 10.1080/21655979.2021.2009322

114. Zhao XQ, Guo S, Lu YY, Hua Y, Zhang F, Yan H, et al. Lycium barbarum l. leaves ameliorate type 2 diabetes in rats by modulating metabolic profiles and gut microbiota composition. *BioMed Pharmacother* (2020) 121:109559. doi: 10.1016/j.biopha.2019.109559

115. Zhao H, Wu H, Duan M, Liu R, Zhu Q, Zhang K, et al. Cinnamaldehyde improves metabolic functions in streptozotocin-induced diabetic mice by regulating gut microbiota. *Drug Des Devel Ther* (2021) 15:2339–55. doi: 10.2147/DDDT.S288011

116. Li XW, Chen HP, He YY, Chen WL, Chen JW, Gao L, et al. Effects of rich-polyphenols extract of dendrobium loddigesii on anti-diabetic, anti-inflammatory, anti-oxidant, and gut microbiota modulation in db/db mice. *Molecules* (2018) 23(12):3245. doi: 10.3390/molecules23123245

117. Gong P, Xiao X, Wang S, Shi F, Liu N, Chen X, et al. Hypoglycemic effect of astragaloside IV *via* modulating gut microbiota and regulating AMPK/SIRT1 and PI3K/AKT pathway. *J Ethnopharmacol* (2021) 281:114558. doi: 10.1016/j.jep.2021.114558

118. Zhai Y, Zhou W, Yan X, Qiao Y, Guan L, Zhang Z, et al. Astragaloside IV ameliorates diet-induced hepatic steatosis in obese mice by inhibiting intestinal FXR *via* intestinal flora remodeling. *Phytomedicine* (2022) 107:154444. doi: 10.1016/j.phymed.2022.154444

119. Li QM, Zha XQ, Zhang WN, Liu J, Pan LH, Luo JP. Laminaria japonica polysaccharide prevents high-fat-diet-induced insulin resistance in mice *via* regulating gut microbiota. *Food Funct* (2021) 12(12):5260–73. doi: 10.1039/d0fo02100h

120. Chen C, You LJ, Huang Q, Fu X, Zhang B, Liu RH, et al. Modulation of gut microbiota by mulberry fruit polysaccharide treatment of obese diabetic db/db mice. *Food Funct* (2018) 9(7):3732–42. doi: 10.1039/c7fo01346a

121. Ding X, Jian T, Li J, Lv H, Tong B, Li J, et al. Chicoric acid ameliorates nonalcoholic fatty liver disease *via* the AMPK/Nrf2/NFκB signaling pathway and

restores gut microbiota in high-Fat-Diet-Fed mice. *Oxid Med Cell Longev* (2020) 2020:9734560. doi: 10.1155/2020/9734560

122. Bao T, He F, Zhang X, Zhu L, Wang Z, Lu H, et al. Inulin exerts beneficial effects on non-alcoholic fatty liver disease *via* modulating gut microbiome and suppressing the lipopolysaccharide-Toll-Like receptor 4-Mψ-Nuclear factor-κB-Nod-Like receptor protein 3 pathway *via* gut-liver axis in mice. *Front Pharmacol* (2020) 11:558525. doi: 10.3389/fphar.2020.558525

123. Regnier M, Rastelli M, Morissette A, Suriano F, Le Roy T, Pilon G, et al. Rhubarb supplementation prevents diet-induced obesity and diabetes in association with increased akkermansia muciniphila in mice. *Nutrients* (2020) 12 (10):2932. doi: 10.3390/nu12102932

124. Yang S, Hu T, Liu H, Lv YL, Zhang W, Li H, et al. Akebia saponin d ameliorates metabolic syndrome (MetS) *via* remodeling gut microbiota and attenuating intestinal barrier injury. *BioMed Pharmacother* (2021) 138:111441. doi: 10.1016/j.biopha.2021.111441

125. Wang L, Zeng B, Liu Z, Liao Z, Zhong Q, Gu L, et al. Green tea polyphenols modulate colonic microbiota diversity and lipid metabolism in high-fat diet treated HFA mice. *J Food Sci* (2018) 83(3):864–73. doi: 10.1111/1750-3841.14058

126. Porras D, Nistal E, Martínez-Flórez S, Olcoz JL, Jover R, Jorquera F, et al. Functional interactions between gut microbiota transplantation, quercetin, and high-fat diet determine non-alcoholic fatty liver disease development in germ-free mice. *Mol Nutr Food Res* (2019) 63(8):e1800930. doi: 10.1002/mnfr.201800930

127. Lv XC, Wu Q, Cao YJ, Lin YC, Guo WL, Rao PF, et al. Ganoderic acid a from ganoderma lucidum protects against alcoholic liver injury through ameliorating the lipid metabolism and modulating the intestinal microbial composition. *Food Funct* (2022) 13(10):5820–37. doi: 10.1039/d1fo03219d

128. Chen M, Xiao D, Liu W, Song Y, Zou B, Li L, et al. Intake of ganoderma lucidum polysaccharides reverses the disturbed gut microbiota and metabolism in type 2 diabetic rats. *Int J Biol Macromol* (2020) 155:890–902. doi: 10.1016/j.ijbiomac.2019.11.047

129. Rehman AU, Siddiqui NZ, Farooqui NA, Alam G, Gul A, Ahmad B, et al. Morchella esculenta mushroom polysaccharide attenuates diabetes and modulates intestinal permeability and gut microbiota in a type 2 diabetic mice model. *Front Nutr* (2022) 9:984695. doi: 10.3389/fnut.2022.984695

130. Yong Z, Ruiqi W, Yanan Y, Ning M, Zhi Z, Yinfeng T, et al. Laurolitsine ameliorates type 2 diabetes by regulating the hepatic LKB1-AMPK pathway and gut microbiota. *Phytomedicine* (2022) 106:154423. doi: 10.1016/j.phymed.2022.154423

131. Shen SH, Zhong TY, Peng C, Fang J, Lv B. Structural modulation of gut microbiota during alleviation of non-alcoholic fatty liver disease with gynostemma pentaphyllum in rats. *BMC Complement Med Ther* (2020) 20(1):34. doi: 10.1186/s12906-020-2835-7

132. Yue SR, Tan YY, Zhang L, Zhang BJ, Jiang FY, Ji G, et al. Gynostemma pentaphyllum polysaccharides ameliorate non-alcoholic steatohepatitis in mice associated with gut microbiota and the TLR2/NLRP3 pathway. *Front Endocrinol (Lausanne)* (2022) 13:885039. doi: 10.3389/fendo.2022.885039

133. Tan YY, Yue SR, Lu AP, Zhang L, Ji G, Liu BC, et al. The improvement of nonalcoholic steatohepatitis by poria cocos polysaccharides associated with gut microbiota and NF-κB/CCL3/CCR1 axis. *Phytomedicine* (2022) 103:154208. doi: 10.1016/j.phymed.2022.154208

134. Zhong M, Yan Y, Yuan H, Rong A, Xu G, Cai F, et al. Astragalus mongholicus polysaccharides ameliorate hepatic lipid accumulation and inflammation as well as modulate gut microbiota in NAFLD rats. *Food Funct* (2022) 13(13):7287–301. doi: 10.1039/d2fo01009g

135. Yang Y, Li M, Wang Q, Huang H, Zhao Y, Du F, et al. Pueraria lobata starch regulates gut microbiota and alleviates high-fat high-cholesterol diet induced non-alcoholic fatty liver disease in mice. *Food Res Int* (2022) 157:111401. doi: 10.1016/j.foodres.2022.111401

136. Li L, Lan X, Peng X, Shi S, Zhao Y, Liu W, et al. Polysaccharide from salviae miltiorrhizae radix et rhizoma attenuates the progress of obesity-induced non-alcoholic fatty liver disease through modulating intestinal microbiota-related gut-liver axis. *Int J Mol Sci* (2022) 23(18):10620. doi: 10.3390/ijms231810620

137. Sun J, Fan J, Li T, Yan X, Jiang Y. Nuciferine protects against high-fat diet-induced hepatic steatosis *via* modulation of gut microbiota and bile acid metabolism in rats. *J Agric Food Chem* (2022) 70(38):12014–28. doi: 10.1021/acs.jafc.2c04817

138. Fan J, Sun J, Li T, Yan X, Jiang Y. Nuciferine prevents hepatic steatosis associated with improving intestinal mucosal integrity, mucus-related microbiota and inhibiting TLR4/MyD88/NF-κB pathway in high-fat induced rats. *J Funct Foods* (2022) 88:104859. doi: 10.1016/j.jff.2021.104859

139. Zhao W, Guo M, Feng J, Gu Z, Zhao J, Zhang H, et al. Myristica fragrans extract regulates gut microbes and metabolites to attenuate hepatic inflammation and lipid metabolism disorders *via* the AhR-FAS and NF-kappaB signaling pathways in mice with non-alcoholic fatty liver disease. *Nutrients* (2022) 14 (9):1699. doi: 10.3390/nu14091699

140. Luan X, Zhang LJ, Li XQ, Rahman K, Zhang H, Chen HZ, et al. Compound-based Chinese medicine formula: From discovery to compatibility mechanism. *J Ethnopharmacol* (2020) 254:112687. doi: 10.1016/j.jep.2020.112687

141. Xie X, Liao J, Ai Y, Gao J, Zhao J, Qu F, et al. Pi-Dan-Jian-Qing decoction ameliorates type 2 diabetes mellitus through regulating the gut microbiota and serum metabolism. *Front Cell Infect Microbiol* (2021) 11:748872. doi: 10.3389/fcimb.2021.748872

142. Xu X, Gao Z, Yang F, Yang Y, Chen L, Han L, et al. Antidiabetic effects of gegen qinlian decoction *via* the gut microbiota are attributable to its key ingredient berberine. *Genomics Proteomics Bioinf* (2020) 18(6):721–36. doi: 10.1016/j.gpb.2019.09.007

143. Wu R, Zhao D, An R, Wang Z, Li Y, Shi B, et al. Linggui zhugan formula improves glucose and lipid levels and alters gut microbiota in high-fat diet-induced diabetic mice. *Front Physiol* (2019) 10:918. doi: 10.3389/fphys.2019.00918

144. Cao Y, Yao G, Sheng Y, Yang L, Wang Z, Yang Z, et al. JinQi jiangtang tablet regulates gut microbiota and improve insulin sensitivity in type 2 diabetes mice. *J Diabetes Res* (2019) 2019:1872134. doi: 10.1155/2019/1872134

145. Wei X, Tao J, Xiao S, Jiang S, Shang E, Zhu Z, et al. Xiexin tang improves the symptom of type 2 diabetic rats by modulation of the gut microbiota. *Sci Rep* (2018) 8(1):3685. doi: 10.1038/s41598-018-22094-2

146. Xiao S, Zhang Z, Chen M, Zou J, Jiang S, Qian D, et al. Xiexin tang ameliorates dyslipidemia in high-fat diet-induced obese rats *via* elevating gut microbiota-derived short chain fatty acids production and adjusting energy metabolism. *J Ethnopharmacol* (2019) 241:112032. doi: 10.1016/j.jep.2019.112032

147. Chen Y, Zhu L, Hu W, Wang Y, Wen X, Yang J. Simiao wan modulates the gut microbiota and bile acid metabolism during improving type 2 diabetes mellitus in mice. *Phytomedicine* (2022) 104:154264. doi: 10.1016/j.phymed.2022.154264

148. Gao K, Yang R, Zhang J, Wang Z, Jia C, Zhang F, et al. Effects of qijian mixture on type 2 diabetes assessed by metabonomics, gut microbiota and network pharmacology. *Pharmacol Res* (2018) 130:93–109. doi: 10.1016/j.phrs.2018.01.011

149. Yan Z, Wu H, Zhou H, Chen S, He Y, Zhang W, et al. Integrated metabolomics and gut microbiome to the effects and mechanisms of naoxintong capsule on type 2 diabetes in rats. *Sci Rep* (2020) 10(1):10829. doi: 10.1038/s41598-020-67362-2

150. Li M, Ding L, Hu YL, Qin LL, Wu Y, Liu W, et al. Herbal formula LLKL ameliorates hyperglycaemia, modulates the gut microbiota and regulates the gut-liver axis in zucker diabetic fatty rats. *J Cell Mol Med* (2021) 25(1):367–82. doi: 10.1111/jcmm.16084

151. Chen M, Liao Z, Lu B, Wang M, Lin L, Zhang S, et al. Huang-Lian-Jie-Du-Decoction ameliorates hyperglycemia and insulin resistant in association with gut microbiota modulation. *Front Microbiol* (2018) 9:2380. doi: 10.3389/fmicb.2018.02380

152. Gao Z, Li Q, Wu X, Zhao X, Zhao L, Tong X. New insights into the mechanisms of Chinese herbal products on diabetes: A focus on the "Bacteria-mucosal immunity-Inflammation-Diabetes" axis. *J Immunol Res* (2017) 2017:1813086. doi: 10.1155/2017/1813086

153. Cui HX, Zhang LS, Luo Y, Yuan K, Huang ZY, Guo Y. A purified anthraquinone-glycoside preparation from rhubarb ameliorates type 2 diabetes mellitus by modulating the gut microbiota and reducing inflammation. *Front Microbiol* (2019) 10:1423. doi: 10.3389/fmicb.2019.01423

154. Coppola S, Avagliano C, Calignano A, Berni Canani R. The protective role of butyrate against obesity and obesity-related diseases. *Molecules* (2021) 26(3):682. doi: 10.3390/molecules26030682

155. Hernández MAG, Canfora EE, Jocken JWE, Blaak EE. The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* (2019) 11 (8):1943. doi: 10.3390/nu11081943

156. Bach Knudsen KE, Lærke HN, Hedemann MS, Nielsen TS, Ingerslev AK, Gundelund Nielsen DS, et al. Impact of diet-modulated butyrate production on intestinal barrier function and inflammation. *Nutrients* (2018) 10(10):1499. doi: 10.3390/nu10101499

157. Fu X, Liu Z, Zhu C, Mou H, Kong Q. Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. *Crit Rev Food Sci Nutr* (2019) 59 (sup1):S130–s152. doi: 10.1080/10408398.2018.1542587

158. Hosseini E, Grootaert C, Verstraete W, Van de Wiele T. Propionate as a health-promoting microbial metabolite in the human gut. *Nutr Rev* (2011) 69 (5):245–58. doi: 10.1111/j.1753-4887.2011.00388.x

159. Shapiro H, Kolodziejczyk AA, Halstuch D, Elinav E. Bile acids in glucose metabolism in health and disease. *J Exp Med* (2018) 215(2):383–96. doi: 10.1084/jem.20171965

160. Sorrentino G, Perino A, Yildiz E, El Alam G, Bou Sleiman M, Gioiello A, et al. Bile acids signal *via* TGR5 to activate intestinal stem cells and epithelial regeneration. *Gastroenterology* (2020) 159(3):956–968.e958. doi: 10.1053/j.gastro.2020.05.067

161. Liu Y, Chen K, Li F, Gu Z, Liu Q, He L, et al. Probiotic lactobacillus rhamnosus GG prevents liver fibrosis through inhibiting hepatic bile acid synthesis and enhancing bile acid excretion in mice. *Hepatology* (2020) 71(6):2050–66. doi: 10.1002/hep.30975

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Gut microbiota and its metabolites – molecular mechanisms and management strategies in diabetic kidney disease

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Diabetic kidney disease (DKD) is one of the major microvascular complications of diabetes mellitus and is also one of the serious risk factors in cardiovascular events, end-stage renal disease, and mortality. DKD is associated with the diversified, compositional, and functional alterations of gut microbiota. The interaction between gut microbiota and host is mainly achieved through metabolites, which are small molecules produced by microbial metabolism from exogenous dietary substrates and endogenous host compounds. The gut microbiota plays a critical role in the pathogenesis of DKD by producing multitudinous metabolites. Nevertheless, detailed mechanisms of gut microbiota and its metabolites involved in the occurrence and development of DKD have not been completely elucidated. This review summarizes the specific classes of gut microbiota-derived metabolites, aims to explore the molecular mechanisms of gut microbiota in DKD pathophysiology and progression, recognizes biomarkers for the screening, diagnosis, and prognosis of DKD, as well as provides novel therapeutic strategies for DKD.

KEYWORDS

gut microbiota, metabolite, diabetic kidney disease, immunity, therapy

Introduction

Diabetic kidney disease (DKD) is a pivotal complication of diabetes mellitus and significantly increases the risk of cardiovascular disease and end-stage renal disease (ESRD), that ultimately results in dialysis or high-mortality and economic burdens (1). The increased number of DKD and ESRD is partially attributed to lifestyle and dietary habits associated with diabetes and hypertension (2). Management and treatment strategy of patients with DKD includes controlling blood glucose, blood lipid, and blood pressure as

well as blockade of the renin-angiotensin system (RAS); however, the risk of DKD still remains to be high (3) indicating the presence of unrecognized factors and mechanisms involved. The occurrence and progression of DKD is correlated to the interaction between gene and environment (4). Despite that hyperglycemia-induced metabolic alterations, hemodynamics changes, RAS activation, podocyte injury or loss, epithelial dysfunction, inflammation, and immunoreaction contributed to disease progression, specific molecular mechanisms and pathogenesis need to be explored (5).

The gut microbiota is powerful for maintaining host internal environmental homeostasis. For one thing, microbiome prevents infection caused by pathogens, promotes the digestion and absorption of nutrients, and synthesizes essential vitamins and amino acids (6). For another thing, it exerts an anti-inflammatory function (6), regulates fat metabolism (7), and participates in immune system development (8). And thirdly, gut microbiota-derived metabolites such as short-chain fatty acids (SCFAs), bile acids (BAs), lipopolysaccharide (LPS), and trimethylamine N-oxide (TMAO) are essential mediators of microbial-host crosstalk by interacting with host environment (9). The diversified, compositional, and functional alterations of gut microbiome are termed dysbiosis (10), which leads to a reduction in SCFAs and an increase in uremic toxins, activation of RAS, inflammation, and aggravated immune response. Nonetheless, specific mechanisms by which gut microbiota affects DKD have not been fully elucidated. This review summarized the role of gut microbiota and its metabolites in DKD, discussed underlying mechanisms of gut microbiota involved in DKD progression, and explored its potentials in DKD management and treatment.

Gut microbiota and its metabolites

Gut microbiota

The human gastrointestinal tract possesses a plentiful microbial community which collects approximately 100 trillion microorganisms, including bacteria, fungi, viruses, phages, and archaea (11). Commonly, the gut microbiota is comprised of 6 phyla incorporating with *Bacteroidetes*, *Frimicutes*, *Verrucomicrobia*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, in which *Bacteroidetes* and *Frimicutes* are the majority components (12). The stability of intestinal microbiota is closely related to host health and disease. What is more, gut microbiota is symbiotic with the host and participates in a variety of physiological activities, such as fermenting food, resisting pathogens and regulating immune function (13). The gut microbiota contributes to host physiology by producing a multitude of metabolites (14) (Figure 1). Numerous metabolites derived from gut microbiota fermentation are vital factors in host-microbiota cross-talk and have been shown to be correlated with kidney function.

16S rDNA, metagenomics, and mass spectrometry can be utilized to explore the diversity, composition, and function of gut microbiota as well as microbiota-related serum metabolites in patients with DKD. Interaction studies between plasma metabolomics and gut microbiome in experimental DKD mouse/rat model provided evidence for the gut-metabolism-kidney axis, and verified the involvement of gut microbiota and circulating metabolites in DKD progression (15, 16). DKD patients displayed dysbiosis with composition, richness and diversity in gut microbiota (17–19). *Roseburia intestinalis* was significantly decreased while *Bacteroides stercoris* was increased in DKD patients (20). Furthermore, studies in early DKD caused by type 1 diabetes indicated that differences in gut microbiota and serum metabolite profiles were dependent on albuminuria levels (21). Several studies also revealed diversity and species differences in gut microbiota between DKD patients and non-DKD patients (22–24).

SCFAs

SCFAs are produced by the fermentation of polysaccharides with the assistance of gut microbiota and are the main source of nutrition for colon epithelial cells. Acetate, propionate, and butyrate generated from the bacterial fermentation of dietary fiber are the predominant SCFAs (25). SCFAs have been shown to inhibit the activity of histone deacetylase (HDAC) and involve in G protein-coupled receptors (GPRs) mediated signaling pathway (26, 27). SCFAs can bind to GPRs such as GPR41, GPR43, GPR109A, and olfactory receptors (Olfr) 78, and then were absorbed into system circulation after reaching distant tissues. Furthermore, SCFAs were demonstrated to participate in the sustainment of intestinal barrier integrity (28), enhance glucose and lipid metabolism, restraint energy expenditure (29), and modulate immunoreaction and inflammatory responses (30). The reduction of SCFAs-producing bacteria as well as low serum and fecal SCFAs level may be correlated with kidney injury (31–33). Butyrate was reported to improve the intestinal barrier function by promoting the production of colonic mucin and tight junction proteins (ZO-1) (34). It could also mitigate oxidative stress, inflammation, and fibrosis in kidney disease through GPRs or HDAC (35–37). Serum valerate and caproate levels were negatively

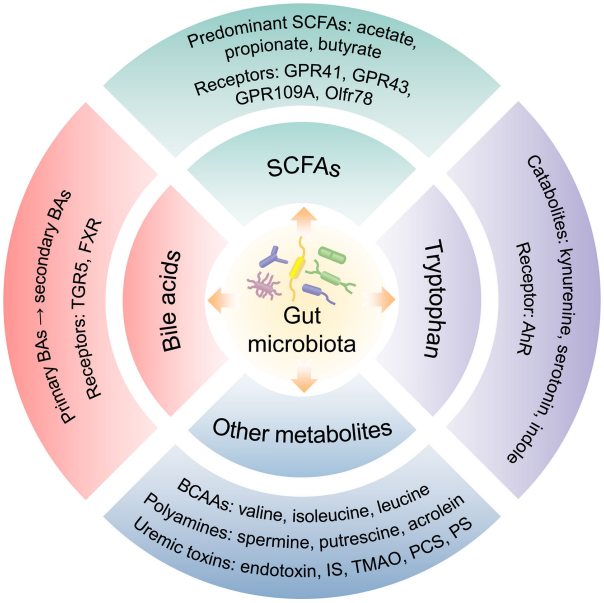

FIGURE 1
Gut microbiota-derived metabolites.

correlated with the progression of DKD to ESRD (38). It has been shown that acetate mediated the dysregulation of cholesterol homeostasis by activation of GPR43, thereby contributing to the tubulointerstitial injury of DKD (39).

Bile acids

BAs are synthesized from cholesterol in the hepatocytes and participates in the absorption of lipid as well as metabolic or inflammatory signaling pathways (40). The primary BAs including chenodeoxycholic acid (CDCA) and cholic acid (CA), are indispensable for lipid and vitamin digestion and absorption by conjugating to glycine or taurine (41). Primary BAs could transform and decompose into secondary BAs *via* gut microbiota. The gut microbiota modulates BA metabolism process through deconjugation, dehydrogenation, and dihydroxylation of primary BAs (42). Additionally, the synthesis of BAs is influenced by cholesterol 7α-hydroxylase (CYP7A1) and sterol 27-hydroxylase (CYP27A1) regulating *via* gut microbiota (14). BAs are ligands for G protein-coupled bile acid receptor (TGR5) and nuclear hormone receptor farnesoid X receptor (FXR). Moreover, the profiles of BAs and gut microbiota influence each other. BAs could alter the composition of intestinal microbiota. Conversely, microbiota modulates the size and composition of the BA pool as well as BA signaling (43). BAs combine with TGR5 to improve insulin sensitivity *via* glucagon-like peptide-1 (GLP-1) and regulate energy expenditure in muscle or brown adipose tissue (44). The activation of FXR decreases lipogenesis and hepatic gluconeogenesis, and inhibits bacterial overgrowth and translocation by producing antimicrobial peptides (45). FXR and TGR5 play a renal protective role in diabetes and obesity-related kidney disease by regulating renal signaling pathways (46). Gentiopicroside inhibits the NF-κB signaling pathway *via* TGR5 activation, thereby alleviating inflammation and fibrosis in DKD (47).

Tryptophan

An essential aromatic amino-acid, tryptophan, generally originates from daily diet such as fish, milk, oats, cheese. Besides the synthesis of proteins, dietary tryptophan could act as a precursor of critical metabolites including kynurenine, serotonin, indole, and its derivatives (48). Kynurenine, a tryptophan-derived metabolite produced by tryptophan 2,3-dioxygenase and indoleamine (2, 3)-dioxygenase, is correlated with kidney function (49, 50). Tryptophan is decomposed by bacterial tryptophanase into indole, which is a compound responsible for intercellular signal transduction, participating in the gene expression of intestinal epithelium connections and anti-inflammatory factors in intestinal epithelial cells, as well as maintaining host-microbiota homeostasis on the mucosa surface (51). As downstream critical metabolites, 3-(2-Hydroxyethyl) indole, 3-methylindole, and indoleacrylic acid were downregulated in the DKD model and were reinstated after treatment with Tangshen Formula (15). Some compounds produced by tryptophan metabolism are ligands for the aryl hydrocarbon receptor (AhR) and could induce AhR conformational changes. Moreover, these compounds are involved in the gene expression of pro-inflammatory factors, the metabolism of cytochrome P450 (CYP) superfamily CYP1A1, CYP1A2, CYP1B1 and cyclooxygenase-2 (COX-2), or the degradation of selective proteins (52). The deficient activation of AhR pathway could reduce the production of GLP-1 and interleukin (IL)-22, increase intestinal permeability and LPS translocation, which contribute to inflammation and insulin resistance (53). Based on the combined analysis of gut microbiota, serum metabolites and clinical indicators in DKD patients, phenylalanine and tryptophan metabolic pathways were demonstrated to be associated with the progression of DKD (54).

Other metabolites

Branched-chain amino acids (BCAAs) are essential amino-acids synthesized by gut microbiota, including valine, isoleucine, and leucine. BCAAs modulate protein synthesis, glucose/lipid metabolism, insulin resistance, and immunity, as well as maintain homeostasis (55). Polyamines, such as spermine, putrescine, polyamine oxidase and acrolein, are participated in the development of kidney disease by altering the metabolism of intestinal microbiota (56). The dysbiosis of gut microbiota promotes the production of bacteria-derived uremic toxins, such as indoxyl sulfate (IS), endotoxin, TMAO, and p-cresyl sulfate (PCS), which increase intestinal permeability and transfer into the systemic circulation through the damaged intestinal barrier. Accumulation of uremic toxins in kidneys could lead to kidney dysfunction (57). TMAO, a gut microbiota-derived metabolite, was associated with mortality and renal outcome in type 1 diabetes (58). Higher serum TMAO levels increased the risk of abdominal aortic venture in hemodialysis patients (59). Phenyl sulfate (PS) contributed to podocyte damage and albuminuria and was shown to be related to the progression of DKD (60). Imidazole propionate, a metabolite produced by the breakdown of histidine *via* gut microbiota, was increased in type 2 diabetes, affecting host inflammation and metabolism (61). Both PS and TMAO could be involved in the development of DKD through a secretory associated senescence phenotype and chronic low-grade inflammation (62). IS and PCS contributed to the nephrology and cardiovascular toxicities *via* the activation of inflammation and oxidative stress (63). Additionally, several uremic toxins such as urea, TMAO, PCS, and 3-carboxylic acid 4-methyl-5-propyl-2-furan propionic (CMPF) were associated with glucose homeostasis abnormalities and diabetes incidence (64). The dysbiosis of Gram-negative bacteria and increased LPS level were detected in type 2 diabetes related DKD (65).

Gut microbiota-related factors in DKD progression

Insulin resistance

DKD originates from metabolic dysregulation including hyperglycemia, hyperlipidemia, and insulin resistance (4). Hyperglycemia increases the generation of advanced glycation end products. The variance in insulin levels and insulin resistance might

be a significant factor in DKD. Severe albuminuria and glomerulosclerosis were occurred in animals with complete deletion of podocyte insulin receptor (66). The dysbiosis of gut microbiota is linked to insulin resistance (67, 68) (Figure 2). A few species of microbiota, especially *Prevotella copri* and *Bacteroides vulgatus* are associated with insulin resistance and then impact host metabolism (69). Gut commensal *Bacteroides acidifaciens* could improve insulin sensitivity and may have therapeutic potential for diabetes and obesity (70). Microbiota depletion such as antibiotic-treated or germ-free mice could enhance insulin sensitivity and glucose tolerance (71). Podocyte insulin resistance caused podocyte injury and led to albuminuria in early DKD. Dysregulated GPR43 by gut microbiota dysbiosis resulted in podocyte insulin resistance through the inhibition of adenosine monophosphate-activated protein kinase (AMPK)-α activity (72). Butyrate enhanced AMPK phosphorylation and increased GLP-1 secretion, thereby alleviating insulin resistance and renal failure (34). Imidazole propionate, a microbial histidine-derived metabolite, may contribute to insulin resistance through activation of mechanistic target of rapamycin complex1 (mTORC1) (73).

RAS

RAS is critical in the pathogenesis and progression of DKD. Moreover, local RAS might play a greater role than the circulating RAS (74). The secretion of renin in the juxtaglomerular apparatus plays an important role in the activation of intrarenal RAS by hyperglycemia. Olfr78 expressed in the renal juxtaglomerular afferent arteriole responded to signals from intestinal microbiota by mediating renin secretion, after that SCFAs could modulate blood pressure through Olfr78 and GPR41 (75). Succinate accumulated in the distal nephron-collecting duct, and activation of GPR91 responded to hyperglycemia through the stored (pro)renin and provoked tissue injury in DKD (76). The activation of intrarenal RAS by gut microbiota dysbiosis-derived excessive acetate was involved in the kidney injury of early DKD (77). Gut microbiota could promote angiotensin II (Ang II)-induced vascular dysfunction and hypertension by facilitating CCL2/IL-17-driven vascular immune cell infiltration and inflammation (78). Conversely, butyrate exerted an improvement for Ang II-induced renal injury and an antihypertension action by attenuating expression of (pro)renin receptor and renin as well as suppressing the (pro)renin receptor-mediated intrarenal RAS (79). During the fermentation of probiotics, angiotensin converting enzyme (ACE) inhibitory peptide and renin inhibitory peptide could be released, which are beneficial for lowering blood pressure (80, 81). In addition, ACE2 was associated with tryptophan metabolism and was sensitive to intestinal inflammation (82). A few uremic toxins such as IS and PCS are important stimulator of local RAS. Moreover, the inhibition of RAS ameliorated IS and PCS induced renal fibrosis (83).

Inflammation

Inflammation accompanies the pathogenesis and progression of DKD whereas anti-inflammatory therapies might be beneficial for alleviating renal damage in DKD. Several inflammatory pathways participate in the complicated molecular networks and processes in DKD, including chemokines (CCL2, CX3CL1 and CCL5), inflammatory cytokines (IL-1, IL-6, IL-18), adhesion molecules, E-selectin, α-actinin 4, transcription factor nuclear factor-kappa B (NF-κB), and tumor necrosis factor (84). The initial stage of the inflammatory response to injury or metabolic dysfunction involves the release of proinflammatory mediators and the recruitment of leukocytes. Therefore, targeting inflammatory-resolution pathways might contribute to impede the progression of DKD (85). SCFAs could be involved in the modulation of pro-inflammatory and anti-inflammatory responses by inhibiting HDAC directly and binding GPRs indirectly (86). SCFAs produced by dietary fiber fermentation

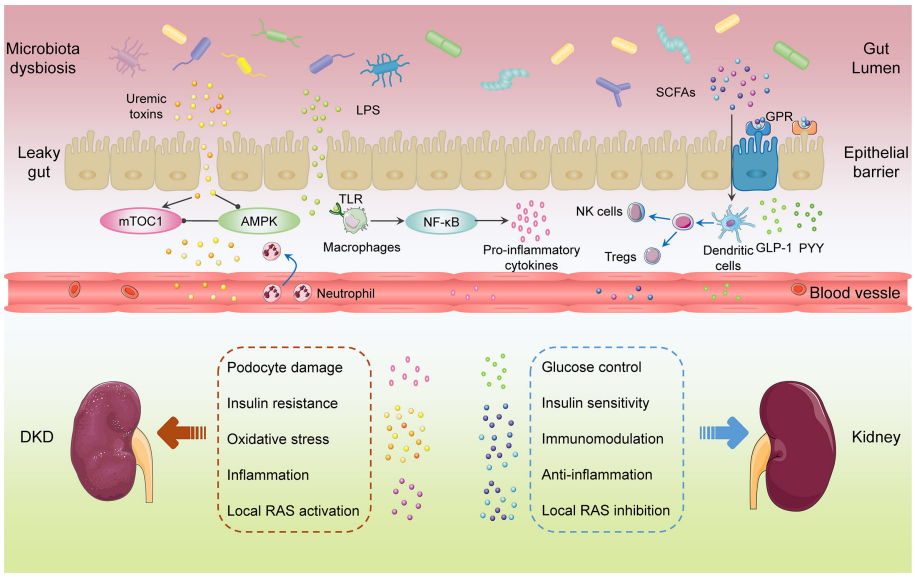

FIGURE 2
Gut microbiota-related factors in the progression of diabetic kidney disease.

decreased the expression of inflammatory cytokines, chemokines, and fibrosis-promoting proteins in experimental DKD, thereby reducing albuminuria, glomerular hypertrophy, podocyte injury, and interstitial fibrosis. Moreover, this process required the involvement of GPR43 or GPR109A (87). Host/gut microbiota-derived tryptophan metabolites regulated AhR and then affected oxidative stress and inflammation in DKD (88). TMAO and PS accelerated kidney inflammation and fibrosis, resulting in development of DKD (60, 89). LPS, combined with toll-like receptors (TLRs) TLR2 and TLR4, participated in the inflammatory process of DKD through NF-κB activation and pro-inflammatory cytokines release, leading to the renal injury (90). Obesity enhanced intestinal permeability and chronic low-grade inflammation by inducing gut microbiota dysbiosis, ultimately causing the exasperation of DKD (91)

Immunity

The activation of innate immunity through immune cells and resident renal cells contributed to the initiation and maintenance of inflammation (92). TLRs induced sterile tubulointerstitial inflammatory responses *via* NF-κB signaling pathway. The nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3 (NLRP3) inflammasome were associated with the connection of metabolic stress and pro-inflammatory cascades by inducing IL-1β and IL-18. The kallikrein-kinin system contributed to inflammatory progression by generating bradykinin and activating bradykinin receptors. Furthermore, coagulation enzymes promoted the activation of protease-activated receptors on kidney cells, leading to renal inflammation and fibrosis in DKD. Gut microbiota plays a significant role in maintaining host homeostasis as well as in modulating immune system (93). There have several studies characterizing the complex interaction between DKD, microbes and its metabolites, and immune responses. The microbiota colonized the intestinal tract after birth and regulated the antigenic responsiveness of lymphatic tissue (94). With the involvement of gut microbiota, the intestinal immune system started to build up and to be matured gradually. The dysbiosis of gut microbiota attracted immune cell activation and proinflammatory factors secretion, which led to immune dysregulation and inflammation (95). Mitochondrial antiviral signaling protein (MAVS), a component of innate immunity, was involved in maintaining intestinal integrity and barrier function. Damaged MAVS was conducive to the disrupted intestinal homeostasis, contributing to DKD progression (96). Microbiome-host interactions cooperatively maintained microbial community stability through metabolite-mediated innate immune modulation. What's more, metabolites could influence the host's immune homeostasis (97). Gut microbiota-derived metabolites passed through the intestinal barrier, accumulated in the circulation, recognized by immune system, and performed functions through gut-microbiome-immune axis (98). Bacteroids-derived SCFAs contributed to the activation of immune system by promoting neutrophil chemotaxis and inducing differentiation and proliferation of natural killer cells and Tregs (99).

Management and treatment options for gut microbiota in DKD

Clinical drugs

Various kinds of drug may alleviate DKD by affecting intestinal microbiota. Metformin was shown to contribute to several SCFAs-producing microbiota and increase the production of butyrate and propionate, thus participating in glucose homeostasis (100). Sodium-glucose cotransporter 2 inhibitor, as emerging antidiabetic drugs including empagliflozin, canagliflozin and dapagliflozin, restored the diversity of gut microbiota in experimental DKD mouse model. Moreover, reduced LPS production and increased SCFAs production by regulating the microbiota were observed in patients after inhibition of SGLT2 (101–103). Pirfenidone treatment increased gut microbial diversity in diabetic mouse model and reversed gut microbial dysbiosis and diabetic ketoacidosis biomarkers (104). Magnesium lithospermate B was found to ameliorate kidney injury by modulating gut microbiome dysbiosis and BAs metabolism (105). Abundant polysaccharides are beneficial for DKD. Polysaccharide from Armillariella tabescens mycelia, Cordyceps cicadae polysaccharide, and Bupleurum polysaccharide were demonstrated to modulate gut microbiota dysbiosis and inflammatory response (106–108). Traditional Chinese medicine such as Zicuiyin (109), Moutan Cortex polysaccharide (110), QiDiTangShen granules (111), Shenyan Kangfu tablet (112), and Tangshen Formula (113), have been used clinically to treat DKD. They had a significant curative role in regulating gut microbiota, eliminating intestinal toxins, inhibiting renal inflammation and immunity, alleviating renal injury, and protecting kidney function.

Dietary intervention

Diet is fundamental to support human growth, health, and reproduction. Furthermore, diet was also shown to modulate and maintain the symbiotic gut microbiota communities colonized the intestinal tract (114). Under multiple host-containing endogenous and exogenous factors, diet becomes a pivotal determinant of the structure and function in gut microbiota (115) (Figure 3). The latest review regarding the effect of dietary nutrient intake on gut microbiota indicated that diet-microbiota crosstalk and personalized nutrition strategies are associated with chronic kidney disease progression (116). Moreover, the variation in dietary protein sources affected the gut microbiota, microbiota-derived metabolites, immune cell activation, and production of inflammatory cytokines (117). Studies from human population with different diets showed that *Bacteroides* was enriched in a protein-rich diets while *Prevotella* was enriched in a carbohydrate-based diets (118). Whole-plant fibers from fresh vegetables contained a lot of necessary micronutrients compared with highly processed fibers or fibers from seed coats (119). Plant-based low-protein diets seemingly contributed to postpone kidney replacement therapy by disturbing RAS, reducing proteinuria, and decreasing insulin resistance (120). Fermented and germinated foxtail millet whole grain diet raised the bacterial diversity especially probiotics, thereby ameliorating kidney injury in

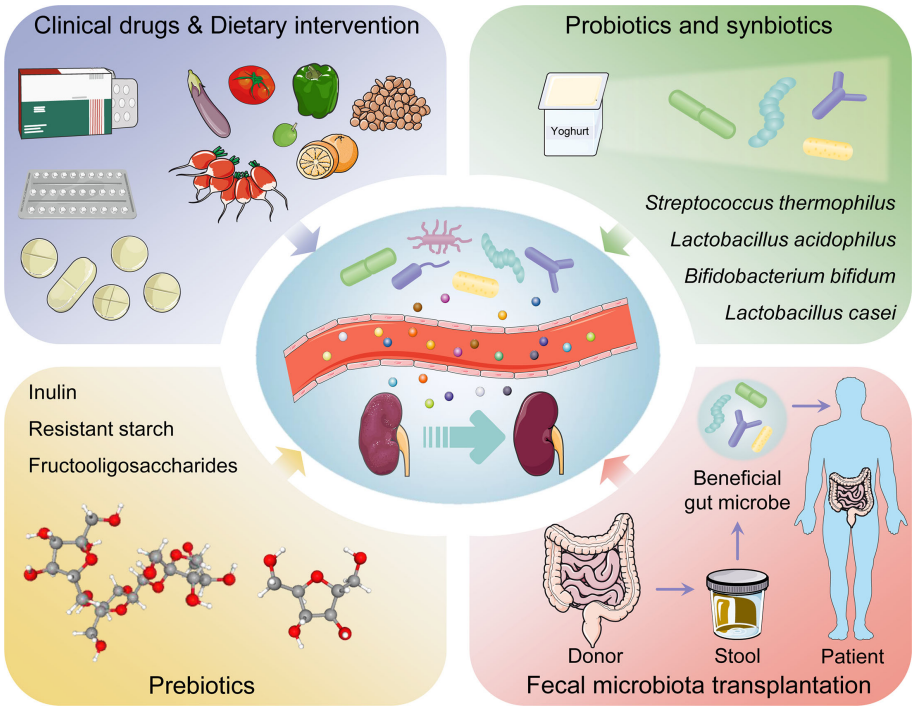

FIGURE 3
The management and therapeutic strategies of diabetic kidney disease based on gut microbiota.

experimental DKD mouse model through inhibition of inflammation and immunity signaling pathways (121). A high linolenic acid diet aggravated gut microbiota dysbiosis and inflammatory responses in diabetes mouse model. Conversely, a low n-6/n-3 ratio diet improved glucose homeostasis, inhibited systematic inflammation, and ameliorated DKD (122). Punicalagin from pomegranates, a prospective bioactive polyphenol, was shown to alleviate diabetic kidney injury through gut-kidney axis (123).

Probiotics and synbiotics

Probiotics contain live microorganisms that can change composition of microbiota and are supposed to provide health benefits to host (124). Synbiotics, a mixture comprising live microorganisms and substrates selectively utilized by host microorganisms confer a health benefit on the host (125) (Table 1). Probiotic and synbiotic supplementation, such as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium bifidum*, had beneficial effects on blood glucose and intestinal imbalance, production of uremic toxins, and inflammation or oxidative stress in diabetic hemodialysis patients (126–128). Furthermore, probiotics could ameliorate insulin resistance, stabilize fasting blood glucose levels, and improve antioxidant status (90, 129). Addition of probiotics such as *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium longum* reduced the blood urea nitrogen level and uric acid concentration in patients with stage 3 and stage 4 chronic kidney disease (130, 131). Systematic review and meta-analysis demonstrated that probiotics might ameliorate high sensitivity-C reactive protein and oxidative stress biomarkers, as well as regulate lipid profile and anthropometric indices in DKD patients (132, 133).

Prebiotics and postbiotics

Prebiotics such as noncarbohydrate food components, are substrates that are selectively used by host microorganisms for health benefits (134). The supplementation of prebiotics in daily dietary could exterminate pathogens, facilitate the growth of beneficial microorganisms, and regulate host intestinal microbiota (135). Moreover, prebiotic supplements might increase SCFAs levels (notably butyrate), restore intestinal barrier function, and relieve inflammatory response (136). Fructooligosaccharides could alleviate pathological changes in diabetes related kidney disease (137). Inulin-type fructans, a type of dietary fiber, was demonstrated to improve kidney diseases *via* modulating gut microbiota and SCFAs profile (138). Additionally, inulin-type fructans also decreased insulin resistance, serum insulin and fasting blood glucose levels, and increased fasting serum GLP-1 level in diabetes rats (139, 140). Resistant starch is a prebiotic compound that accelerates proliferation of health-promoting gut microbiota such as *Bifidobacteria* and *Lactobacilli*, increases the production of SCFAs, decreases the concentrations of uremic toxins and alleviates renal dysfunction (141). Postbiotics, defined as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" in 2019 (142), have appeared increasingly in the literature and products; however, their effects on DKD are insufficient in research. Postbiotics exert immunomodulatory and intestinal barrier protective roles by increasing anti-inflammatory cytokine secretion and ZO-1 expression (143). Postbiotic-GABA-salt,

TABLE 1 Differences between probiotics, synbiotics, prebiotics, and postbiotics.

Classification	Probiotics	Synbiotics	Prebiotics	Postbiotics
Definition	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host	A substrate that is selectively utilized by host microorganisms conferring a health benefit	Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host
Category	*Bifidobacterium* (*adolescentis*, *animalis*, *bifidum*, *breve* and *longum*); *Lactobacillus* (*acidophilus*, *casei*, *fermentum*, *gasseri*, *johnsonii*, *paracasei*, *plantarum*, *rhamnosus* and *salivarius*)	Complementary (prebiotic + probiotic); Synergistic (live microorganism + substrate)	Conjugated linoleic acids and polyunsaturated fatty acids; Oligosaccharides; Human milk oligosaccharides; Phenolics and phytochemicals; Readily fermentable	Inactivated strain (such as *Bacteroides xylanisolvens*, *Apilactobacillus kunkeei* and *Saccharomyces boulardii*); Bacterial lysates; Spirulina formulations
Health benefit	Healthy digestive tract construction (such as infectious diarrhoea, antibiotic-associated diarrhoea, and ulcerative colitis); Healthy immune system construction (including preventing allergic disease, decreasing inflammation, and enhancing anti-infection activities)	Treatment of NAFLD, obesity and metabolic syndrome, T2DM and glycaemia, IBS, CKD, dyslipidaemia, PCOS, AD, and inflammation; Prevention of surgical infections and complications, sepsis in infants, and AD; Eradication of *Helicobacter pylori*	Metabolic health; Satiety; Improved absorption of calcium and other minerals, bone health; Skin health; Digestive tract health; Allergy; Constipation; Immune function in elderly individuals	New antimicrobials; Targeted anti-inflammatory, immunoregulatory, and enhance vaccination efficacy agents; Novel signaling molecules that affect gut pain, sensation, secretion, and motility; Fermented infant formulas and bacterial lysates
Mechanism	Colonization resistance; Normalization of perturbed microbiota; SCFA production; Increased turnover of enterocytes; Regulation of intestinal transit; Competitive exclusion of pathogen; Vitamin synthesis; Bile salt metabolism; Gut barrier reinforcement	Complementary approach combines prebiotic (targets autochthonous beneficial microorganisms) and probiotic; Synergistic approach selects substrate that is utilized by the co-administered live microorganism, enhancing its functionality	Modulation of SCFA production; Promotion of beneficial microbiota; Bile salt metabolism; Alteration of bacterial growth and interaction with immune system; Enhanced secretion of satiety hormones peptide YY and GLP-1; Immunological modulation	Modulation of resident microbiota, immune responses, and systemic metabolic responses; Enhancement of epithelial barrier functions; Regulation of systemic signaling *via* the nervous system

NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; IBS, irritable bowel syndrome; CKD, chronic kidney disease; PCOS, polycystic ovarian syndrome; AD, atopic dermatitis; SCFA, short-chain fatty acid; GLP-1, glucagon-like peptide1.

spirulina formulations, sonicated *Lactobacillus paracasei* and *O. formigenes lysates* contribute to improve renal outcomes (144, 145).

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is a treatment in which the microbial community from a healthy donor's stool was minimally transplanted into the patient's intestinal tract (146). FMT is implemented with the purpose of restoring normal function of the gut microbiota and has generally been adapted into treatment for *Clostridium difficile* infection (147). Faecal microbiota is separated cautiously from selected donor's stool, quantified in accordance with viable bacteria, and cryopreservation (148). Transplantable materials can be delivered in the form of encapsulated oral medication (149). As a true organ, gut microbiota is indispensable to human pathophysiology, suggesting that FMT might be an advantageous treatment for problems with metabolism, autoimmunity, and system development (150). Body weight gain, insulin resistance, albuminuria, and tumor necrosis factor-α levels in experimental DKD mouse model could be prevented by FMT (151). After six weeks post-FMT using stool derived from lean donors, the peripheral insulin sensitivity was significantly improved in male patients with metabolic syndrome, although the result was not sustained in following few weeks (152). Another double blind randomized controlled trial demonstrated that TMAO or proxies of vascular inflammation was undifferentiated in patients with metabolic syndrome received FMT from either lean donors or autologous (153). Hence, abundant experiments are needed to explore these potential therapeutic indications.

Conclusion and perspective

The pathogenesis and pathophysiology of DKD incorporate not only hyperglycemia-induced metabolic alterations, hemodynamics changes, RAS activation, podocyte injury or loss, epithelial dysfunction, inflammation, and immune dysregulation, but also the influences of environmental factors and interactions between host and gut microbiota as well as its metabolites. Gut microbiota is associated with kidney disease, confirming the presence of gut-kidney axis through the involvement of genetic, immunity and dietary approaches. The gut microbiota participates in host homeostasis by producing a myriad of metabolites, which act ss key signaling molecules and substrates for metabolic reactions. The combination of metagenomics and metabolomics could help to investigate the relationship between dysbiosis of gut microbiota and metabolic disorders. Nonetheless, there are still complexities to overcome in identifying the potential causality of some metabolites from fully microbiota-derived or diet and host itself. High-quality microbiome analysis workflow is important to obtain reliable and repeatable results (154).

Dietary intervention, probiotics, synbiotics, and prebiotics are widely acceptable to patients in relative safety and traditional concept. However, various intestinal bacteria and metabolites have

heterogeneous effects on host, some of which are beneficial to human health and others contribute to pathophysiology of diseases. Hence, it is necessary to investigate the signals and effects mediated by different bacteria and metabolites as well as reasonable application of bacteria community in the treatment strategies. Gut Microbiota-derived metabolites could act as biomarkers of DKD. Identification of biomarkers for screening, diagnosis, and prognosis of DKD as well as exploration of molecular mechanisms or pathways involved in DKD can facilitate individualized prevention and treatment. However, further studies involving human trials are needed to investigate the beneficial role of prebiotics, probiotics, synbiotics or FMT in DKD management by regulating gut microbiota. The therapeutic strategy targeting intestinal microbiota has prodigious potential in the future and will open an emerging perspective and orientation for DKD treatment.

Author contributions

PW and Z-SL conceived the idea. Z-HM prepared the figures and tables, and drafted the manuscript. PW, Z-XG, D-WL and Z-SL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KAM, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primers* (2015) 1:15018. doi: 10.1038/nrdp.2015.18

2. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. *Lancet* (2013) 382:260–72. doi: 10.1016/S0140-6736(13)60687-X

3. Lytvyn Y, Bjornstad P, van Raalte DH, Heerspink HL, Cherney DZI. The new biology of diabetic kidney disease-mechanisms and therapeutic implications. *Endocr Rev* (2020) 41:202–231. doi: 10.1210/endrev/bnz010

4. Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. *J Clin Invest* (2014) 124:2333–40. doi: 10.1172/JCI72271

5. DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: Impact of SGLT2 inhibitors. *Nat Rev Nephrol* (2021) 17:319–34. doi: 10.1038/s41581-021-00393-8

6. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* (2009) 461:1282–6. doi: 10.1038/nature08530

7. Frick J-S, Autenrieth IB. The gut microflora and its variety of roles in health and disease. *Curr Top Microbiol Immunol* (2013) 358:273–89. doi: 10.1007/82_2012_217

8. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science* (2016) 352:539–44. doi: 10.1126/science.aad9378

9. Blander JM, Longman RS, Iliev ID, Sonnenberg GF, Artis D. Regulation of inflammation by microbiota interactions with the host. *Nat Immunol* (2017) 18:851–60. doi: 10.1038/ni.3780

10. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nat Rev Immunol* (2017) 17:219–32. doi: 10.1038/nri.2017.7

11. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature* (2012) 489:220–30. doi: 10.1038/nature11550

12. Shanahan F, Ghosh TS, O'Toole PW. The healthy microbiome-what is the definition of a healthy gut microbiome? *Gastroenterology* (2021) 160:483–94. doi: 10.1053/j.gastro.2020.09.057

13. Hou K, Wu Z-X, Chen X-Y, Wang J-Q, Zhang D, Xiao C, et al. Microbiota in health and diseases. *Signal Transduct Target Ther* (2022) 7:135. doi: 10.1038/s41392-022-00974-4

14. Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. *Nat Rev Microbiol* (2021) 19:77–94. doi: 10.1038/s41579-020-0438-4

15. Zhang B, Wan Y, Zhou X, Zhang H, Zhao H, Ma L, et al. Characteristics of serum metabolites and gut microbiota in diabetic kidney disease. *Front Pharmacol* (2022) 13:872988. doi: 10.3389/fphar.2022.872988

16. Wu C, Fei J, Xu Q, Tao Y, Zhou Z, Wang Y, et al. Interaction between plasma metabolomics and intestinal microbiome in db/db mouse, an animal model for study of type 2 diabetes and diabetic kidney disease. *Metabolites* (2022) 12:775. doi: 10.3390/metabo12090775

17. Du X, Liu J, Xue Y, Kong X, Lv C, Li Z, et al. Alteration of gut microbial profile in patients with diabetic nephropathy. *Endocrine* (2021) 73:71–84. doi: 10.1007/s12020-021-02721-1

18. Tao S, Li L, Li L, Liu Y, Ren Q, Shi M, et al. Understanding the gut-kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: An analysis of the gut microbiota composition. *Acta Diabetol* (2019) 56:581–92. doi: 10.1007/s00592-019-01316-7

19. Wang Y, Zhao J, Qin Y, Yu Z, Zhang Y, Ning X, et al. The specific alteration of gut microbiota in diabetic kidney diseases-a systematic review and meta-analysis. *Front Immunol* (2022) 13:908219. doi: 10.3389/fimmu.2022.908219

20. Zhang L, Wang Z, Zhang X, Zhao L, Chu J, Li H, et al. Alterations of the gut microbiota in patients with diabetic nephropathy. *Microbiol Spectr* (2022) 10:e0032422. doi: 10.1128/spectrum.00324-22

21. Winther SA, Henriksen P, Vogt JK, Hansen TH, Ahonen L, Suvitaival T, et al. Gut microbiota profile and selected plasma metabolites in type 1 diabetes without and with stratification by albuminuria. *Diabetologia* (2020) 63:2713–24. doi: 10.1007/s00125-020-05260-y

22. Chen R, Zhu D, Yang R, Wu Z, Xu N, Chen F, et al. Gut microbiota diversity in middle-aged and elderly patients with end-stage diabetic kidney disease. *Ann Transl Med* (2022) 10:750. doi: 10.21037/atm-22-2926

23. He X, Sun J, Liu C, Yu X, Li H, Zhang W, et al. Compositional alterations of gut microbiota in patients with diabetic kidney disease and type 2 diabetes mellitus. *Diabetes Metab Syndr Obes* (2022) 15:755–65. doi: 10.2147/DMSO.S347805

24. Han S, Chen M, Cheng P, Zhang Z, Lu Y, Xu Y, et al. A systematic review and meta-analysis of gut microbiota in diabetic kidney disease: Comparisons with diabetes mellitus, non-diabetic kidney disease, and healthy individuals. *Front Endocrinol (Lausanne)* (2022) 13:1018093. doi: 10.3389/fendo.2022.1018093

25. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* (2015) 11:577–91. doi: 10.1038/nrendo.2015.128

26. Nogal A, Valdes AM, Menni C. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes* (2021) 13:1–24. doi: 10.1080/19490976.2021.1897212

27. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio* (2019) 10:e02566–18. doi: 10.1128/mBio.02566-18

28. Kim CH. Microbiota or short-chain fatty acids: which regulates diabetes? *Cell Mol Immunol* (2018) 15:88–91. doi: 10.1038/cmi.2017.57

29. Hu J, Lin S, Zheng B, Cheung PCK. Short-chain fatty acids in control of energy metabolism. *Crit Rev Food Sci Nutr* (2018) 58:1243–9. doi: 10.1080/10408398.2016.1245650

30. Kim CH. Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. *Cell Mol Immunol* (2021) 18:1161–71. doi: 10.1038/s41423-020-00625-0

31. Zhong C, Dai Z, Chai L, Wu L, Li J, Guo W, et al. The change of gut microbiota-derived short-chain fatty acids in diabetic kidney disease. *J Clin Lab Anal* (2021) 35:e24062. doi: 10.1002/jcla.24062

32. Cai K, Ma Y, Cai F, Huang X, Xiao L, Zhong C, et al. Changes of gut microbiota in diabetic nephropathy and its effect on the progression of kidney injury. *Endocrine* (2022) 76:294–303. doi: 10.1007/s12020-022-03002-1

33. Li Y, Su X, Gao Y, Lv C, Gao Z, Liu Y, et al. The potential role of the gut microbiota in modulating renal function in experimental diabetic nephropathy murine models established in same environment. *Biochim Biophys Acta Mol Basis Dis* (2020) 1866:165764. doi: 10.1016/j.bbadis.2020.165764

34. Gonzalez A, Krieg R, Massey HD, Carl D, Ghosh S, Gehr TWB, et al. Sodium butyrate ameliorates insulin resistance and renal failure in CKD rats by modulating intestinal permeability and mucin expression. *Nephrol Dial Transplant* (2019) 34:783–94. doi: 10.1093/ndt/gfy238

35. Cheng X, Zhou T, He Y, Xie Y, Xu Y, Huang W. The role and mechanism of butyrate in the prevention and treatment of diabetic kidney disease. *Front Microbiol* (2022) 13:961536. doi: 10.3389/fmicb.2022.961536

36. Lu Y, Fan C, Li P, Lu Y, Chang X, Qi K. Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. *Sci Rep* (2016) 6:37589. doi: 10.1038/srep37589

37. Lin MY, de Zoete MR, van Putten JPM, Strijbis K. Redirection of epithelial immune responses by short-chain fatty acids through inhibition of histone deacetylases. *Front Immunol* (2015) 6:554. doi: 10.3389/fimmu.2015.00554

38. Zhong C, Bai X, Chen Q, Ma Y, Li J, Zhang J, et al. Gut microbial products valerate and caproate predict renal outcome among the patients with biopsy-confirmed diabetic nephropathy. *Acta Diabetol* (2022) 59:1469–77. doi: 10.1007/s00592-022-01948-2

39. Hu ZB, Lu J, Chen PP, Lu CC, Zhang JX, Li XQ, et al. Dysbiosis of intestinal microbiota mediates tubulointerstitial injury in diabetic nephropathy *via* the disruption of cholesterol homeostasis. *Theranostics* (2020) 10:2803–16. doi: 10.7150/thno.40571

40. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discovery* (2008) 7:678–93. doi: 10.1038/nrd2619

41. Just S, Mondot S, Ecker J, Wegner K, Rath E, Gau L, et al. The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism. *Microbiome* (2018) 6:134. doi: 10.1186/s40168-018-0510-8

42. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall H-U, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* (2013) 17:225–35. doi: 10.1016/j.cmet.2013.01.003

43. Jia W, Xie G, Jia W. Bile acid–microbiota cross-talk in gastrointestinal inflammation and carcinogenesis. *Nat Rev Gastroenterol Hepatol* (2018) 15:111–28. doi: 10.1038/nrgastro.2017.119

44. Schaap FG, Trauner M, Jansen PLM. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* (2014) 11:55–67. doi: 10.1038/nrgastro.2013.151

45. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science* (2006) 312:233–6. doi: 10.1126/science.1121435

46. Wang XX, Wang D, Luo Y, Myakala K, Dobrinskikh E, Rosenberg AZ, et al. FXR/TGR5 dual agonist prevents progression of nephropathy in diabetes and obesity. *J Am Soc Nephrol* (2018) 29:118–37. doi: 10.1681/ASN.2017020222

47. Xiao H, Sun X, Liu R, Chen Z, Lin Z, Yang Y, et al. Gentiopicroside activates the bile acid receptor Gpbar1 (TGR5) to repress NF-kappaB pathway and ameliorate diabetic nephropathy. *Pharmacol Res* (2020) 151:104559. doi: 10.1016/j.phrs.2019.104559

48. Comai S, Bertazzo A, Brughera M, Crotti S. Tryptophan in health and disease. *Adv Clin Chem* (2020) 95:165–218. doi: 10.1016/bs.acc.2019.08.005

49. Liu J-R, Miao H, Deng D-Q, Vaziri ND, Li P, Zhao Y-Y. Gut microbiota-derived tryptophan metabolism mediates renal fibrosis by aryl hydrocarbon receptor signaling activation. *Cell Mol Life Sci* (2021) 78:909–22. doi: 10.1007/s00018-020-03645-1

50. Pawlak K, Myśliwiec M, Pawlak D. Kynurenine pathway - a new link between endothelial dysfunction and carotid atherosclerosis in chronic kidney disease patients. *Adv Med Sci* (2010) 55:196–203. doi: 10.2478/v10039-010-0015-6

51. Lee J-H, Lee J. Indole as an intercellular signal in microbial communities. *FEMS Microbiol Rev* (2010) 34:426–44. doi: 10.1111/j.1574-6976.2009.00204.x

52. Safe S, Han H, Goldsby J, Mohankumar K, Chapkin RS. Aryl hydrocarbon receptor (AhR) ligands as selective AhR modulators: Genomic studies. *Curr Opin Toxicol* (2018) 11–12:10–20. doi: 10.1016/j.cotox.2018.11.005

53. Taleb S. Tryptophan dietary impacts gut barrier and metabolic diseases. *Front Immunol* (2019) 10:2113. doi: 10.3389/fimmu.2019.02113

54. Zhang Q, Zhang Y, Zeng L, Chen G, Zhang L, Liu M, et al. The role of gut microbiota and microbiota-related serum metabolites in the progression of diabetic kidney disease. *Front Pharmacol* (2021) 12:757508. doi: 10.3389/fphar.2021.757508

55. Tajiri K, Shimizu Y. Branched-chain amino acids in liver diseases. *Transl Gastroenterol Hepatol* (2018) 3:47. doi: 10.21037/tgh.2018.07.06

56. Feng Y-L, Cao G, Chen D-Q, Vaziri ND, Chen L, Zhang J, et al. Microbiome-metabolomics reveals gut microbiota associated with glycine-conjugated metabolites and polyamine metabolism in chronic kidney disease. *Cell Mol Life Sci* (2019) 76:4961–78. doi: 10.1007/s00018-019-03155-9

57. Nallu A, Sharma S, Ramezani A, Muralidharan J, Raj D. Gut microbiome in chronic kidney disease: challenges and opportunities. *Transl Res* (2017) 179:24–37. doi: 10.1016/j.trsl.2016.04.007

58. Winther SA, Øllgaard JC, Tofte N, Tarnow L, Wang Z, Ahluwalia TS, et al. Utility of plasma concentration of trimethylamine n-oxide in predicting cardiovascular and renal complications in individuals with type 1 diabetes. *Diabetes Care* (2019) 42:1512–20. doi: 10.2337/dc19-0048

59. He L, Yang W, Yang P, Zhang X, Zhang A. Higher serum trimethylamine-n-oxide levels are associated with increased abdominal aortic calcification in hemodialysis patients. *Ren Fail* (2022) 44:2019–27. doi: 10.1080/0886022X.2022.2145971

60. Kikuchi K, Saigusa D, Kanemitsu Y, Matsumoto Y, Thanai P, Suzuki N, et al. Gut microbiome-derived phenyl sulfate contributes to albuminuria in diabetic kidney disease. *Nat Commun* (2019) 10:1835. doi: 10.1038/s41467-019-09735-4

61. Molinaro A, Lassen PB, Henricsson M, Wu H, Adriouch S, Belda E, et al. Imidazole propionate is increased in diabetes and associated with dietary patterns and altered microbial ecology. *Nat Commun* (2020) 11:5881. doi: 10.1038/s41467-020-19589-w

62. Fernandes R, Viana SD, Nunes S, Reis F. Diabetic gut microbiota dysbiosis as an inflammaging and immunosenescence condition that fosters progression of retinopathy and nephropathy. *Biochim Biophys Acta Mol Basis Dis* (2019) 1865:1876–97. doi: 10.1016/j.bbadis.2018.09.032

63. Rossi M, Campbell KL, Johnson DW, Stanton T, Vesey DA, Coombes JS, et al. Protein-bound uremic toxins, inflammation and oxidative stress: a cross-sectional study in stage 3-4 chronic kidney disease. *Arch Med Res* (2014) 45:309–17. doi: 10.1016/j.arcmed.2014.04.002

64. Koppe L, Fouque D, Soulage CO. Metabolic abnormalities in diabetes and kidney disease: Role of uremic toxins. *Curr Diabetes Rep* (2018) 18:97. doi: 10.1007/s11892-018-1064-7

65. Salguero MV, Al-Obaide MAI, Singh R, Siepmann T, Vasylyeva TL. Dysbiosis of gram-negative gut microbiota and the associated serum lipopolysaccharide exacerbates inflammation in type 2 diabetic patients with chronic kidney disease. *Exp Ther Med* (2019) 18:3461–9. doi: 10.3892/etm.2019.7943

66. Welsh GI, Hale LJ, Eremina V, Jeansson M, Maezawa Y, Lennon R, et al. Insulin signaling to the glomerular podocyte is critical for normal kidney function. *Cell Metab* (2010) 12:329–40. doi: 10.1016/j.cmet.2010.08.015

67. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* (2016) 14:20–32. doi: 10.1038/nrmicro3552

68. Vatanen T, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature* (2018) 562:589–94. doi: 10.1038/s41586-018-0620-2

69. Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BAH, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* (2016) 535:376–81. doi: 10.1038/nature18646

70. Yang J-Y, Lee Y-S, Kim Y, Lee S-H, Ryu S, Fukuda S, et al. Gut commensal bacteroides acidifaciens prevents obesity and improves insulin sensitivity in mice. *Mucosal Immunol* (2017) 10:104–16. doi: 10.1038/mi.2016.42

71. Suárez-Zamorano N, Fabbiano S, Chevalier C, Stojanović O, Colin DJ, Stevanović A, et al. Microbiota depletion promotes browning of white adipose tissue and reduces obesity. *Nat Med* (2015) 21:1497–501. doi: 10.1038/nm.3994

72. Lu J, Chen PP, Zhang JX, Li XQ, Wang GH, Yuan BY, et al. GPR43 deficiency protects against podocyte insulin resistance in diabetic nephropathy through the restoration of AMPKα activity. *Theranostics* (2021) 11:4728–42. doi: 10.7150/thno.56598

73. Koh A, Molinaro A, Ståhlman M, Khan MT, Schmidt C, Mannerås-Holm L, et al. Microbially produced imidazole propionate impairs insulin signaling through mTORC1. *Cell* (2018) 175:947–961.e17. doi: 10.1016/j.cell.2018.09.055

74. Wysocki J, Ye M, Khattab AM, Fogo A, Martin A, David NV, et al. Angiotensin-converting enzyme 2 amplification limited to the circulation does not protect mice from development of diabetic nephropathy. *Kidney Int* (2017) 91:1336–46. doi: 10.1016/j.kint.2016.09.032

75. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci U.S.A.* (2013) 110:4410–5. doi: 10.1073/pnas.1215927110

76. Peti-Peterdi J. High glucose and renin release: the role of succinate and GPR91. *Kidney Int* (2010) 78:1214–7. doi: 10.1038/ki.2010.333

77. Lu C-C, Hu Z-B, Wang R, Hong Z-H, Lu J, Chen P-P, et al. Gut microbiota dysbiosis-induced activation of the intrarenal renin-angiotensin system is involved in kidney injuries in rat diabetic nephropathy. *Acta Pharmacol Sin* (2020) 41:1111–8. doi: 10.1038/s41401-019-0326-5

78. Karbach SH, Schönfelder T, Brandão I, Wilms E, Hörmann N, Jäckel S, et al. Gut microbiota promote angiotensin II-induced arterial hypertension and vascular dysfunction. *J Am Heart Assoc* (2016) 5:e003698. doi: 10.1161/JAHA.116.003698

79. Wang L, Zhu Q, Lu A, Liu X, Zhang L, Xu C, et al. Sodium butyrate suppresses angiotensin II-induced hypertension by inhibition of renal (pro)renin receptor and intrarenal renin-angiotensin system. *J Hypertens* (2017) 35:1899–908. doi: 10.1097/HJH.0000000000001378

80. Dave LA, Hayes M, Montoya CA, Rutherfurd SM, Moughan PJ. Human gut endogenous proteins as a potential source of angiotensin-i-converting enzyme (ACE-i)-, renin inhibitory and antioxidant peptides. *Peptides* (2016) 76:30–44. doi: 10.1016/j.peptides.2015.11.003

81. Gonzalez-Gonzalez C, Gibson T, Jauregi P. Novel probiotic-fermented milk with angiotensin I-converting enzyme inhibitory peptides produced by bifidobacterium bifidum MF 20/5. *Int J Food Microbiol* (2013) 167:131–7. doi: 10.1016/j.ijfoodmicro.2013.09.002

82. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* (2012) 487:477–81. doi: 10.1038/nature11228

83. Sun C-Y, Chang S-C, Wu M-S. Uremic toxins induce kidney fibrosis by activating intrarenal renin-angiotensin-aldosterone system associated epithelial-to-mesenchymal transition. *PloS One* (2012) 7:e34026. doi: 10.1371/journal.pone.0034026

84. Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, García-Pérez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* (2011) 7:327–40. doi: 10.1038/nrneph.2011.51

85. Brennan E, Kantharidis P, Cooper ME, Godson C. Pro-resolving lipid mediators: regulators of inflammation, metabolism and kidney function. *Nat Rev Nephrol* (2021) 17:725–39. doi: 10.1038/s41581-021-00454-y

86. Dupraz L, Magniez A, Rolhion N, Richard ML, Da Costa G, Touch S, et al. Gut microbiota-derived short-chain fatty acids regulate IL-17 production by mouse and human intestinal γδ T cells. *Cell Rep* (2021) 36:109332. doi: 10.1016/j.celrep.2021.109332

87. Li YJ, Chen X, Kwan TK, Loh YW, Singer J, Liu Y, et al. Dietary fiber protects against diabetic nephropathy through short-chain fatty acid-mediated activation of G protein-coupled receptors GPR43 and GPR109A. *J Am Soc Nephrol* (2020) 31:1267–81. doi: 10.1681/ASN.2019101029

88. Tan Y-Q, Wang Y-N, Feng H-Y, Guo Z-Y, Li X, Nie X-L, et al. Host/microbiota interactions-derived tryptophan metabolites modulate oxidative stress and inflammation *via* aryl hydrocarbon receptor signaling. *Free Radic Biol Med* (2022) 184:30–41. doi: 10.1016/j.freeradbiomed.2022.03.025

89. Fang Q, Zheng B, Liu N, Liu J, Liu W, Huang X, et al. Trimethylamine n-oxide exacerbates renal inflammation and fibrosis in rats with diabetic kidney disease. *Front Physiol* (2021) 12:682482. doi: 10.3389/fphys.2021.682482

90. He C, Shan Y, Song W. Targeting gut microbiota as a possible therapy for diabetes. *Nutr Res* (2015) 35:361–7. doi: 10.1016/j.nutres.2015.03.002

91. Li J, Lv J-L, Cao X-Y, Zhang H-P, Tan Y-J, Chu T, et al. Gut microbiota dysbiosis as an inflammaging condition that regulates obesity-related retinopathy and nephropathy. *Front Microbiol* (2022) 13:1040846. doi: 10.3389/fmicb.2022.1040846

92. Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. *Nat Rev Nephrol* (2020) 16:206–22. doi: 10.1038/s41581-019-0234-4

93. Mertowska P, Mertowski S, Wojnicka J, Korona-Głowniak I, Grywalska E, Błażewicz A, et al. A link between chronic kidney disease and gut microbiota in immunological and nutritional aspects. *Nutrients* (2021) 13:3637. doi: 10.3390/nu13103637

94. Cerf-Bensussan N, Eberl G. The dialog between microbiota and the immune system: shaping the partners through development and evolution. *Semin Immunol* (2012) 24:1–2. doi: 10.1016/j.smim.2011.11.007

95. Chi M, Ma K, Wang J, Ding Z, Li Y, Zhu S, et al. The immunomodulatory effect of the gut microbiota in kidney disease. *J Immunol Res* (2021) 2021:5516035. doi: 10.1155/2021/5516035

96. Linh HT, Iwata Y, Senda Y, Sakai-Takemori Y, Nakade Y, Oshima M, et al. Intestinal bacterial translocation contributes to diabetic kidney disease. *J Am Soc Nephrol* (2022) 33:1105–19. doi: 10.1681/ASN.2021060843

97. Levy M, Thaiss CA, Zeevi D, Dohnalová L, Zilberman-Schapira G, Mahdi JA, et al. Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* (2015) 163:1428–43. doi: 10.1016/j.cell.2015.10.048

98. Ma N, Ma X. Dietary amino acids and the gut-Microbiome-Immune axis: Physiological metabolism and therapeutic prospects. *Compr Rev Food Sci Food Saf* (2019) 18:221–42. doi: 10.1111/1541-4337.12401

99. Kim CH, Park J, Kim M. Gut microbiota-derived short-chain fatty acids, T cells, and inflammation. *Immune Netw* (2014) 14:277–88. doi: 10.4110/in.2014.14.6.277

100. Vallianou NG, Stratigou T, Tsagarakis S. Metformin and gut microbiota: their interactions and their impact on diabetes. *Hormones (Athens)* (2019) 18:141–4. doi: 10.1007/s42000-019-00093-w

101. Deng L, Yang Y, Xu G. Empagliflozin ameliorates type 2 diabetes mellitus-related diabetic nephropathy *via* altering the gut microbiota. *Biochim Biophys Acta Mol Cell Biol Lipids* (2022) 1867:159234. doi: 10.1016/j.bbalip.2022.159234

102. Mishima E, Fukuda S, Kanemitsu Y, Saigusa D, Mukawa C, Asaji K, et al. Canagliflozin reduces plasma uremic toxins and alters the intestinal microbiota composition in a chronic kidney disease mouse model. *Am J Physiol Renal Physiol* (2018) 315:F824–33. doi: 10.1152/ajprenal.00314.2017

103. Lee DM, Battson ML, Jarrell DK, Hou S, Ecton KE, Weir TL, et al. SGLT2 inhibition *via* dapagliflozin improves generalized vascular dysfunction and alters the gut microbiota in type 2 diabetic mice. *Cardiovasc Diabetol* (2018) 17:62. doi: 10.1186/s12933-018-0708-x

104. Singh H, Miyamoto S, Darshi M, Torralba MG, Kwon K, Sharma K, et al. Gut microbial changes in diabetic db/db mice and recovery of microbial diversity upon pirfenidone treatment. *Microorganisms* (2020) 8:E1347. doi: 10.3390/microorganisms8091347

105. Zhao J, Zhang Q-L, Shen J-H, Wang K, Liu J. Magnesium lithospermate b improves the gut microbiome and bile acid metabolic profiles in a mouse model of diabetic nephropathy. *Acta Pharmacol Sin* (2019) 40:507–13. doi: 10.1038/s41401-018-0029-3

106. Yang R, Li Y, Mehmood S, Yan C, Huang Y, Cai J, et al. Polysaccharides from armillariella tabescens mycelia ameliorate renal damage in type 2 diabetic mice. *Int J Biol Macromol* (2020) 162:1682–91. doi: 10.1016/j.ijbiomac.2020.08.006

107. Yang J, Dong H, Wang Y, Jiang Y, Zhang W, Lu Y, et al. Cordyceps cicadae polysaccharides ameliorated renal interstitial fibrosis in diabetic nephropathy rats by repressing inflammation and modulating gut microbiota dysbiosis. *Int J Biol Macromol* (2020) 163:442–56. doi: 10.1016/j.ijbiomac.2020.06.153

108. Feng Y, Weng H, Ling L, Zeng T, Zhang Y, Chen D, et al. Modulating the gut microbiota and inflammation is involved in the effect of bupleurum polysaccharides against diabetic nephropathy in mice. *Int J Biol Macromol* (2019) 132:1001–11. doi: 10.1016/j.ijbiomac.2019.03.242

109. Liu J, Gao L-D, Fu B, Yang H-T, Zhang L, Che S-Q, et al. Efficacy and safety of zicuiyin decoction on diabetic kidney disease: A multicenter, randomized controlled trial. *Phytomedicine* (2022) 100:154079. doi: 10.1016/j.phymed.2022.154079

110. Zhang M, Yang L, Zhu M, Yang B, Yang Y, Jia X, et al. Moutan cortex polysaccharide ameliorates diabetic kidney disease *via* modulating gut microbiota dynamically in rats. *Int J Biol Macromol* (2022) 206:849–60. doi: 10.1016/j.ijbiomac.2022.03.077

111. Wei H, Wang L, An Z, Xie H, Liu W, Du Q, et al. QiDiTangShen granules modulated the gut microbiome composition and improved bile acid profiles in a mouse model of diabetic nephropathy. *BioMed Pharmacother* (2021) 133:111061. doi: 10.1016/j.biopha.2020.111061

112. Chen Q, Ren D, Wu J, Yu H, Chen X, Wang J, et al. Shenyan kangfu tablet alleviates diabetic kidney disease through attenuating inflammation and modulating the gut microbiota. *J Nat Med* (2021) 75:84–98. doi: 10.1007/s11418-020-01452-3

113. Zhao T, Zhang H, Yin X, Zhao H, Ma L, Yan M, et al. Tangshen formula modulates gut microbiota and reduces gut-derived toxins in diabetic nephropathy rats. *BioMed Pharmacother* (2020) 129:110325. doi: 10.1016/j.biopha.2020.110325

114. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe* (2018) 23:705–15. doi: 10.1016/j.chom.2018.05.012

115. 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. doi: 10.1038/s41575-018-0061-2

116. Koppe L, Soulage CO. The impact of dietary nutrient intake on gut microbiota in the progression and complications of chronic kidney disease. *Kidney Int* (2022) 102:728–39. doi: 10.1016/j.kint.2022.06.025

117. Mattson DL, Dasinger JH, Abais-Battad JM. Gut-Immune-Kidney axis: Influence of dietary protein in salt-sensitive hypertension. *Hypertension* (2022) 79:2397–408. doi: 10.1161/HYPERTENSIONAHA.122.18556

118. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature* (2012) 486:222–7. doi: 10.1038/nature11053

119. Padayachee A, Netzel G, Netzel M, Day L, Zabaras D, Mikkelsen D, et al. Binding of polyphenols to plant cell wall analogues - part 2: Phenolic acids. *Food Chem* (2012) 135:2287–92. doi: 10.1016/j.foodchem.2012.07.004

120. Mocanu C-A, Simionescu TP, Mocanu AE, Garneata L. Plant-based versus animal-based low protein diets in the management of chronic kidney disease. *Nutrients* (2021) 13:3721. doi: 10.3390/nu13113721

121. Liu X, Qiu B, Liu W, Zhang Y, Wang X, Li X, et al. The preventive effects of fermented and germinated foxtail millet whole grain on kidney damage in a diabetic mouse model. *Front Nutr* (2022) 9:940404. doi: 10.3389/fnut.2022.940404

122. Lee H-C, Yu S-C, Lo Y-C, Lin I-H, Tung T-H, Huang S-Y. A high linoleic acid diet exacerbates metabolic responses and gut microbiota dysbiosis in obese rats with diabetes mellitus. *Food Funct* (2019) 10:786–98. doi: 10.1039/c8fo02423e

123. Hua Q, Han Y, Zhao H, Zhang H, Yan B, Pei S, et al. Punicalagin alleviates renal injury *via* the gut-kidney axis in high-fat diet-induced diabetic mice. *Food Funct* (2022) 13:867–79. doi: 10.1039/d1fo03343c

124. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* (2014) 11:506–14. doi: 10.1038/nrgastro.2014.66

125. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol* (2020) 17:687–701. doi: 10.1038/s41575-020-0344-2

126. Soleimani A, Zarrati Mojarrad M, Bahmani F, Taghizadeh M, Ramezani M, Tajabadi-Ebrahimi M, et al. Probiotic supplementation in diabetic hemodialysis patients has beneficial metabolic effects. *Kidney Int* (2017) 91:435–42. doi: 10.1016/j.kint.2016.09.040

127. Liu S, Liu H, Chen L, Liang S-S, Shi K, Meng W, et al. Effect of probiotics on the intestinal microbiota of hemodialysis patients: a randomized trial. *Eur J Nutr* (2020) 59:3755–66. doi: 10.1007/s00394-020-02207-2

128. Zheng HJ, Guo J, Jia Q, Huang YS, Huang W-J, Zhang W, et al. The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials. *Pharmacol Res* (2019) 142:303–13. doi: 10.1016/j.phrs.2019.02.016

129. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* (2012) 28:539–43. doi: 10.1016/j.nut.2011.08.013

130. Ranganathan N, Friedman EA, Tam P, Rao V, Ranganathan P, Dheer R. Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada. *Curr Med Res Opin* (2009) 25:1919–30. doi: 10.1185/03007990903069249

131. Miranda Alatriste PV, Urbina Arronte R, Gómez Espinosa CO, de los Ángeles Espinosa Cuevas M. Effect of probiotics on human blood urea levels in patients with chronic renal failure. *Nutr Hosp* (2014) 29:582–90. doi: 10.3305/nh.2014.29.3.7179

132. Bohlouli J, Namjoo I, Borzoo-Isfahani M, Hojjati Kermani MA, Balouch Zehi Z, Moravejolahkami AR. Effect of probiotics on oxidative stress and inflammatory status in diabetic nephropathy: A systematic review and meta-analysis of clinical trials. *Heliyon* (2021) 7:e05925. doi: 10.1016/j.heliyon.2021.e05925

133. Moravejolahkami AR, Hojjati Kermani MA, Balouch Zehi Z, Mirenayat SMS, Mansourian M. The effect of probiotics on lipid profile & anthropometric indices in diabetic nephropathy; a systematic review and meta-analysis of clinical trials. *J Diabetes Metab Disord* (2021) 20:893–904. doi: 10.1007/s40200-021-00765-8

134. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* (2017) 14:491–502. doi: 10.1038/nrgastro.2017.75

135. Zoumpopoulou G, Pot B, Tsakalidou E, Papadimitriou K. Dairy probiotics: Beyond the role of promoting gut and immune health. *Int Dairy J* (2017) 67:46–60. doi: 10.1016/j.idairyj.2016.09.010

136. Snelson M, de Pasquale C, Ekinci EI, Coughlan MT. Gut microbiome, prebiotics, intestinal permeability and diabetes complications. *Best Pract Res Clin Endocrinol Metab* (2021) 35:101507. doi: 10.1016/j.beem.2021.101507

137. Pengrattanachot N, Thongnak L, Lungkaphin A. The impact of prebiotic fructooligosaccharides on gut dysbiosis and inflammation in obesity and diabetes related kidney disease. *Food Funct* (2022) 13:5925–45. doi: 10.1039/d1fo04428a

138. Luo L, Luo J, Cai Y, Fu M, Li W, Shi L, et al. Inulin-type fructans change the gut microbiota and prevent the development of diabetic nephropathy. *Pharmacol Res* (2022) 183:106367. doi: 10.1016/j.phrs.2022.106367

139. Zaky A, Glastras SJ, Wong MYW, Pollock CA, Saad S. The role of the gut microbiome in diabetes and obesity-related kidney disease. *Int J Mol Sci* (2021) 22:9641. doi: 10.3390/ijms22179641

140. Zhang Q, Yu H, Xiao X, Hu L, Xin F, Yu X. Inulin-type fructan improves diabetic phenotype and gut microbiota profiles in rats. *PeerJ* (2018) 6:e4446. doi: 10.7717/peerj.4446

141. Snelson M, Kellow NJ, Coughlan MT. Modulation of the gut microbiota by resistant starch as a treatment of chronic kidney diseases: Evidence of efficacy and mechanistic insights. *Adv Nutr* (2019) 10:303–20. doi: 10.1093/advances/nmy068

142. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol* (2021) 18:649–67. doi: 10.1038/s41575-021-00440-6

143. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol* (2020) 72:558–77. doi: 10.1016/j.jhep.2019.10.003

144. Gargouri M, Hamed H, Akrouti A, Dauvergne X, Magné C, El Feki A. Effects of spirulina platensis on lipid peroxidation, antioxidant defenses, and tissue damage in kidney of alloxan-induced diabetic rats. *Appl Physiol Nutr Metab* (2018) 43:345–54. doi: 10.1139/apnm-2017-0461

145. Favero C, Giordano L, Mihaila SM, Masereeuw R, Ortiz A, Sanchez-Niño MD. Postbiotics and kidney disease. *Toxins (Basel)* (2022) 14:623. doi: 10.3390/toxins14090623

146. Smith MB, Kelly C, Alm EJ. Policy: How to regulate faecal transplants. *Nature* (2014) 506:290–1. doi: 10.1038/506290a

147. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. *Nat Rev Gastroenterol Hepatol* (2016) 13:508–16. doi: 10.1038/nrgastro.2016.98

148. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent clostridium difficile infection. *Am J Gastroenterol* (2012) 107:761–7. doi: 10.1038/ajg.2011.482

149. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing clostridium difficile infection. *JAMA* (2014) 312:1772–8. doi: 10.1001/jama.2014.13875

150. Smits LP, Bouter KEC, de Vos WM, Borody TJ, Nieuwdorp M. Therapeutic potential of fecal microbiota transplantation. *Gastroenterology* (2013) 145:946–53. doi: 10.1053/j.gastro.2013.08.058

151. Bastos RMC, Simplício-Filho A, Sávio-Silva C, Oliveira LFV, Cruz GNF, Sousa EH, et al. Fecal microbiota transplant in a pre-clinical model of type 2 diabetes mellitus, obesity and diabetic kidney disease. *Int J Mol Sci* (2022) 23:3842. doi: 10.3390/ijms23073842

152. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFWM, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* (2012) 143:913–916.e7. doi: 10.1053/j.gastro.2012.06.031

153. Smits LP, Kootte RS, Levin E, Prodan A, Fuentes S, Zoetendal EG, et al. Effect of vegan fecal microbiota transplantation on carnitine- and choline-derived trimethylamine-N-Oxide production and vascular inflammation in patients with metabolic syndrome. *J Am Heart Assoc* (2018) 7:e008342. doi: 10.1161/JAHA.117.008342

154. Krukowski H, Valkenburg S, Madella A-M, Garssen J, van Bergenhenegouwen J, Overbeek SA, et al. Gut microbiome studies in CKD: Opportunities, pitfalls and therapeutic potential. *Nat Rev Nephrol* (2022). doi: 10.1038/s41581-022-00647-z

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The association between altered intestinal microbiome, impaired systemic and ocular surface immunity, and impaired wound healing response after corneal alkaline-chemical injury in diabetic mice

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Purpose: We aim to investigate the effect of sustained hyperglycemia on corneal epithelial wound healing, ocular surface and systemic immune response, and microbiome indices in diabetic mice compared to controls after alkaline chemical injury of the eye.

Methods: Corneal alkaline injury was induced in the right eye of $Ins2^{Akita}$ (Akita) mice and wild-type mice. The groups were observed at baseline and subsequently days 0, 3, and 7 after injury. Corneal re-epithelialization was observed under slit lamp with fluorescein staining using a cobalt blue light filter. Enucleated cornea specimens were compared at baseline and after injury for changes in cornea thickness under hematoxylin and eosin staining. Tear cytokine and growth factor levels were measured using protein microarray assay and compared between groups and time points. Flow cytometry was conducted on peripheral blood and ocular surface samples to determine CD3+CD4+ cell count. Fecal samples were collected, and gut microbiota composition and diversity pattern were measured using shotgun sequencing.

Results: Akita mice had significantly delayed corneal wound healing compared to controls. This was associated with a reduction in tear levels of vascular endothelial growth factor A, angiopoietin 2, and insulin growth factor 1 on days 0, 3, and 7 after injury. Furthermore, there was a distinct lack of upregulation of peripheral blood and ocular surface CD3+CD4+ cell counts in response to injury in Akita mice compared to controls. This was associated with a reduction in intestinal microbiome diversity indices in Akita mice compared to controls after injury.

Specifically, there was a lower abundance of Firmicutes bacterium M10-2 in Akita mice compared to controls after injury.

Conclusion: In diabetic mice, impaired cornea wound healing was associated with an inability to mount systemic and local immune response to ocular chemical injury. Baseline and post-injury differences in intestinal microbial diversity and abundance patterns between diabetic mice and controls may potentially play a role in this altered response.

KEYWORDS

intestinal microbiome, diabetes, corneal wound healing, alkaline chemical injury, T-cell mediated immunity, ocular surface

1 Introduction

Diabetes mellitus (DM) is a significant health problem worldwide. It is associated with sight-threatening ocular complications and represents the most common causes of blindness in working age populations. Apart from retinal diseases, corneal disease is another major complication of DM, affecting up to 70% of all diabetic patients (1, 2). Clinically, diabetic keratopathy is characterized by an impaired cornea epithelial wound healing response, which leads to higher risks of recurrent cornea erosion syndrome, infectious keratitis, sterile corneal ulcers, and ultimately corneal blindness due to scarring. The clinical manifestations of diabetic keratopathy can be explained by the detrimental effect of sustained hyperglycemia on the cornea, resulting in reduced secretion of growth factors on the ocular surface during corneal injury, damaged corneal sub-basal nerves with loss of reduced levels of neurotrophic factor, and increased apoptosis of cornea epithelial cell (3, 4). Despite major leaps in our understanding of the pathogenesis of diabetic keratopathy in the past decade, there remains a lack of therapeutic strategies in clinical practice. While published *in vivo* and *in vitro* studies have examined the use of topical therapeutic agents, including substance P (5) and aloe vera (6), in diabetic cornea disorders, there is still a need to explore glucose-independent systemic therapies that may have ameliorated long-term complications of diabetes across multiple body systems.

With the advent of next-generation deep sequencing in metagenomics over the last two decades, we now understand the importance of gut microbiome in the development and programming of host metabolism and immunity (7). From new evidence gathered, the composition of our gut microbiome plays an important role in the development of autoimmune diseases, including demyelinating disease, inflammatory bowel disease (IBD), and autoimmune uveitis (8). The gut microbiome reportedly acts a key mediator in balancing the immune response between the anti-inflammatory regulatory T (Treg) cells and the pro-inflammatory helper T (Th) 17 cells at the mucosal surface (9–13). Recent interest has been focused on the metabolic effects of gut commensals, particularly in their role in the development of obesity, insulin resistance, and metabolic syndrome. Researchers discovered that high-fat diets were associated with particular detrimental patterns of intestinal microbiota, termed dysbiosis (14). This conferred a pro-metabolic syndrome state to the host, who subsequently developed obese and insulin-resistant clinical phenotypes. These metabolic effects could be transferred to healthy lean individuals through fecal transplantation techniques to give rise to a similar pro-insulin resistant state. Thus, the intestinal microbiome may be an important lever for manipulation in the management of metabolic syndrome and type 2 diabetes. Considering the potential of the gut microbiome to alter both host metabolism and immunity concurrently, it is important to point out that diabetic patients have been shown to have altered intestinal T-cell immunity, potentially as a result of endotoxemia (15). Thus, the development of microvascular complications, e.g., diabetic keratopathy, and the altered immune response in diabetic patients may be potentially correlated with the altered gut microbiome composition in diabetics.

To further investigate this relationship between gut microbiome, immunity, and complications in diabetics, we employed a diabetic cornea wound healing model in rodents. We used a controlled alkaline burn injury of consistent concentration, exposure time, and surface area on the corneas of heterozygous Akita mice. Wild-type mice were used as controls. Chemical injury was selected as a method of insult as it creates a cornea epithelial wound and induces an inflammatory response on the ocular surface, thereby allowing us to investigate both corneal epithelial wound healing and ocular surface immunity (16–18).

2 Methodology

2.1 Breeding and selection of heterozygous Akita mice

Mice heterozygous for the Akita spontaneous mutation ($Ins2^{Akita}$) were used in experiments. This is a model of type I diabetes, with heterozygous Akita mice developing hyperglycemia, hypo-insulinemia, polydipsia, and polyuria starting at 3–4 weeks of age (19). Wild-type (WT) female and heterozygous Akita male mice aged 8–12 weeks were used for mouse breeding. The animals were kept in a temperature-controlled animal room subjected to a 12-h light/12-h dark cycle provided with water supply and sufficient food. All the animal care and experimental procedures conformed to the

Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR, 4696-18) of the University of Hong Kong. Blood glucose was initially measured when the mice reached 5 weeks of age to determine whether the offspring phenotype was Akita or WT.

2.2 Blood glucose measurement

Sufficient amount of blood sample (~5 µl) was collected from the saphenous vein. Briefly, hair around the saphenous vein was removed and a puncture is made to obtain a sufficient blood sample with a capillary tube. A blood glucose test strip together with a blood glucose meter (Contour plus) was used to conduct the measurement. Hemostasis was achieved by applying gentle pressure with a sterile cotton bud to the puncture wound. Blood glucose was measured at 5 weeks after birth in order to identify Akita mice and WT mice, and was further determined at day 0, day 3, and day 7 after alkaline injury of the cornea, as previously described (19).

2.3 Induction of corneal alkaline injury

To induce chemical injury, an alkaline burn was conducted on the mouse cornea. For general anesthesia, the animal will receive intraperitoneal injection of ketamine (0.6 mg/100 µl/10 g body wt) and xylazine (0.15 mg/100 µl/10 g body wt). Local analgesic was applied on the mouse cornea prior to the injury. A 1.5-mm-diameter filter paper was soaked with 0.1 M NaOH and covered the center of the cornea for 10 s. Then, the cornea and conjunctival sac were rinsed with filtered water for 30 s immediately. Antibiotics were applied to avoid infection. Corneal re-epithelialization was examined under a slit lamp with fluorescein stain at day 0, day 3, and day 7 after the injury, as previously described (20).

2.4 Hematoxylin and eosin staining

The excised corneas were immediately fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight. The corneas were then dehydrated with increased gradient of ethanol and finally with chloroform overnight. After that, the corneas were infiltrated with paraffin wax [Tissue PrepTM Embedding Media (Certified), Fisher Chemical] and embedded using the Shandon Histocentre2 Embedding Station, Midwest. Sagittal corneal sections with 5 µm thickness were prepared using a microtome (HM 315 Microtome, Microm). Sections were mounted on the positively charged microscope slides (Lab'IN Co) and dried. Corneal sections were deparaffinized and stained with hematoxylin for 30 s and eosin for 5 s. The sections were dehydrated and mounted with DPX mountant (06522, Sigma-Aldrich). Only central corneal sections were chosen for measuring corneal thickness, and the average of the two measurements was used for analysis, as previously described (20).

2.5 Tear protein microarray assay

Mouse tear samples were collected by placing a Schirmer's strip at the mouse conjunctiva for 5 min. The wet part of the strip was cut off and the length was measured. The surface area of the cut-out pieces and the respective tear volume were calculated on ImageJ. The total protein concentration was measured with a Nanodrop. Total protein concentration was used as a normalization value and calculated all data points per total protein and generated Prism graphs (21). An unpaired *t*-test was conducted to compare the tear protein levels between WT and Akita samples, as well as between time points.

2.6 Flow cytometry

Peripheral blood was collected from the mouse tail vein with a restrainer. Blood (150 to 200 µl) was collected in 1.5-ml Eppendorf tubes containing EDTA. Red blood cells were lysed with Red Blood Cell lysis buffer and centrifuged at 250*g* for 5 min. Ocular surface samples were excised from the mice and were digested in collagenase at 37°C overnight. The cells were filtered with a 100-µm cell strainer. Then, the lymphocyte cells or ocular surface cell samples were washed three times with flow cytometry buffer (Invitrogen) and were incubated with rat anti-mouse CD4 antibody attached to FITC fluorophore and anti-mouse CD3 antibody (Invitrogen) attached to PE for 1 h. Then, the cells were washed three times with flow cytometry buffer for and then the samples were subjected to flow cytometry immediately. The flow cytometry was conducted with BD FACSCanto II (HKU core facility). Compensation was calculated by single-stain sample with FITC and PE. A CD3+CD4+ T-cell prevalence was obtained by FlowJo software for the diabetic and WT samples, as previously described (22).

2.7 Shotgun sequencing of intestinal microbiome

Mouse fecal samples were collected at baseline, day 3, and day 7 by putting the mice individually in single cages for 30 min. DNA samples were extracted, and microbiota species present in the sample were identified by shotgun sequencing followed by bioinformatic analysis using statistical and computational methods (23). Raw, unassembled reads were used as input and matched the sequence against reference databases of known bacteria, viruses, fungi, protists, and antibiotic resistance genes. Microbiota diversity pattern was presented by heat map and alpha diversity index.

2.8 Genotyping with polymerase chain reaction gel electrophoresis

Tail tips (1-3cm in length) were collected after the mice were sacrificed. The tips were cut into tiny pieces (approximately 2 mm in length). The DNA samples from each mouse was extracted from the samples, and polymerase chain reaction (PCR) was conducted for DNA amplification and measurement (19).

2.9 Statistical analysis

The data were presented as mean and standard deviation. A Pearson chi-squared test was used to compare differences in percentage of diabetic and WT mice healed at day 3 and day 7. An unpaired *t*-test was used to compare differences in blood glucose level, protein level in the tear sample, and alpha diversity pattern of intestinal microbiome and between WT and Akita mice; a paired *t*-test was performed to compare changes in tear protein concentration between baseline level and day 0, day 3, and day 7 after the injury. One-way ANOVA was used to analyze longitudinal changes of microbiota composition and tear protein level. When $p < 0.05$, the samples were considered significantly different. All statistical analyses were conducted using GraphPad Prism 9 (California, USA). Additionally, the gut microbiota diversity pattern was analyzed using the alpha diversity indices, Chao1, Simpson, and Shannon.

3 Results

3.1 Blood glucose measurement and genotyping of WT and Akita mice

Blood glucose was measured before induction of the corneal alkaline burn injury, at day 3 and day 7 after the injury, respectively. Compared to WT mice at baseline, Akita mice had a significantly higher blood glucose level (27.95 ± 1.446 mmol/L) compared to WT mice (11.42 ± 0.4621 mmol/L) at baseline as well as at day 3 (28.95 ± 1.522 mmol/L vs. 11.17 ± 1.477 mmol/L) and day 7 (28.09 ± 0.6987 mmol/L vs. 11.71 ± 0.3653 mmol/L) after injury (Figure 1A) ($p < 0.001$, $n = 55$). Additionally, genotyping was conducted with the genomic DNA of WT and Akita mice to confirm the presence of the mutated insulin 2 gene (*Ins2*) (Figure 1B).

3.2 Significantly delayed cornea epithelial wound healing after corneal alkaline injury in Akita mice compared to WT mice

Differences in degree of re-epithelialization between Akita mice and WT mice were compared with the help of fluorescein staining and examination under cobalt blue filter immediately after injury (day 0) as well as on day 3 and day 7 after injury. Fluorescein dye stained the de-epithelialized cornea surface. The representative photos of slit lamp examination showed that alkaline burn with 0.1 M NaOH and 10 s injury time induced significant cornea epithelial injury as indicated by the presence of green fluorescein under cobalt blue light (Figure 2A-i, iv) and the cornea opacity in the bright-field images (Figure 2B-i, iv). The presence of green fluorescein under cobalt blue light for Akita cornea was clearly visible till day 3 after injury (Figure 2A-v), whereas for WT mice, the green fluorescein was not present at day 3 after injury (Figure 2A-ii). The presence of corneal opacification was visible from the bright-field images for the cornea from Akita mouse (Figure 2B-v), whereas no opacification was visible since day 3 after injury for WT mice (Figure 2B-ii, iii).

A chi-square test was performed to determine an association between the healing rate of corneal epithelium and the presence of sustained hyperglycemia. Contingency tables for the number and percentage of WT and Akita mice healed at day 3 and day 7, respectively, are as shown in Tables 1 and 2. Results of the chi-square test analysis demonstrated that Akita mice exhibited significantly delayed wound healing compared to WT mice after corneal alkaline burn injury; i.e., at day 3 after the corneal alkali injury, significantly more WT mice (88.9%) had achieved complete corneal re-epithelialization than Akita mice (22.2%) ($n = 9$, $p = 0.0044$) (Figure 3A; Table 1), proving that diabetes significantly impaired cornea wound healing.

Furthermore, at day 7 after the corneal alkali injury, more WT mice (100%) had achieved complete corneal re-epithelialization than Akita mice (88.9%) (Figure 3B; Table 2); however, this difference was not statistically significant ($n = 9$, $p > 0.9999$).

Slit lamp images of corneas from the left uninjured eye of the mice showed that no impairment was present at baseline, day 3, and day 7 after injury of the corneas from the right eyes for both WT and Akita mice. Bright-field images showed transparent corneas with no

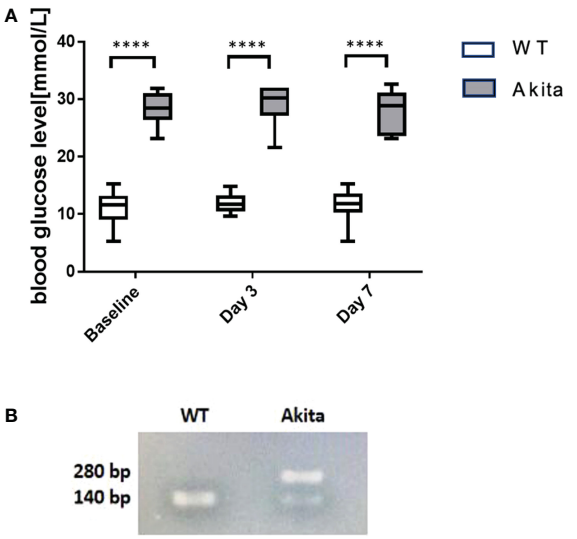

FIGURE 1
Result of blood glucose level monitoring of WT and Akita mice. **(A)** Result of blood glucose level monitoring of WT and Akita mice. **(A)** Blood glucose measurement at baseline, day 3, and day 7. Unpaired *t*-test shows that Akita mice have significantly higher blood glucose level as compared to WT mice at baseline (before injury), day 3, and day 7 after injury. **(B)** Confirmation of WT and Akita mice by genotyping with PCR gel electrophoresis;, two DNA strands of WT mice are both able to be digested by enzyme Fnu4HI while heterozygous Akita mice have one mutated DNA strand that cannot be digested; thus, one band is present in the lane containing WT mice DNA sample whereas two bands present in that containing Akita mice DNA samples ($n = 50$ in WT, $n = 50$ in Akita). ****p<0.0001.

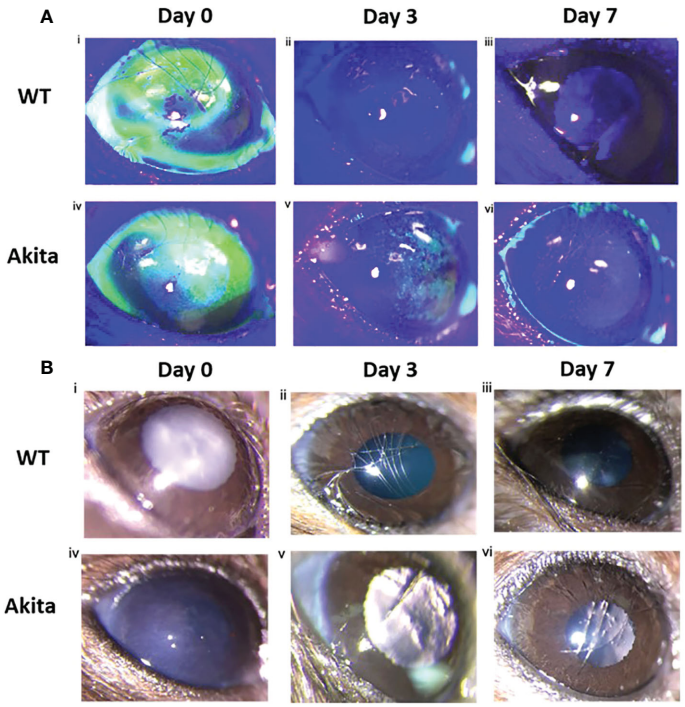

FIGURE 2
Wound healing analysis for WT and Akita mice with slit lamp images. **(A)** Injured cornea from WT and Akita mice at days 0, 3, and 7 after injury using 0.1 M NaOH and 10 s injury time, with fluorescein stain under cobalt blue light. Photos of the corneas taken immediately after injury showed significantly impaired corneal epithelium for both WT and Akita mice. (i, iv) The cornea from WT mouse was fully re-epithelized at day 3 after injury (ii); the cornea from Akita mouse was not fully re-epithelized at day 3 (v) and day 7 (vi) after injury. Magnification:40×. **(B)** Bright-field images of the right (injured) cornea from WT and Akita mice taken at day 0 (i, iv), day 3 (ii, v), and day 7 (iii, vi) after corneal alkaline injury (n = 55 in WT, n = 55 in Akita).

epithelial defect for all corneas (Supplementary Figure 1A), whereas fluorescein stain under cobalt blue light indicated that the cornea showed no absorption of the dye, meaning that the corneas were intact (Supplementary Figure 1B)

To further characterize the effectiveness of the mouse corneal alkaline burn model, hematoxylin and eosin (H&E) staining was conducted on corneal sections, and there was a significantly reduced corneal epithelium thickness between the injury and control group (n = 7, p = 0.0129), while no significant changes in thickness were found between the injured and the uninjured group in total corneal thickness (n = 7, p = 0.6725) and stromal thickness (n = 7, p = 0.4906) (Figure 4E). Meanwhile, there was no significant changes between epithelial thickness of WT and Akita mice at baseline (Figures 4A, B).

To further confirm the delayed cornea wound healing in Akita mice, cornea thickness was checked between the WT and Akita groups at day 3 after injury. There was significantly reduced cornea epithelium thickness at day 3 after injury (n = 7, p = 0.0498), whereas no significant changes were found between the WT and Akita groups in total corneal thickness (n = 7, p = 0.4586) and stromal thickness (n= 7, p = 0.3654) (Figures 4C, D, F).

3.3 Tear protein analysis of diabetic and WT mice at baseline and after corneal alkaline burn injury

3.3.1 Diabetic mice had altered baseline tear protein chemokine and growth factor levels compared to wild-type mice

To analyze the level of cytokine secretion on the ocular surface, tear samples were collected at baseline, day 0, day 3, and day 7 after the corneal alkaline burn, and a protein micro-array assay was

TABLE 1 Contingency table for Pearson chi-square test analysis of number and percentage (in brackets) of WT and Akita mice healed at day 3 after corneal alkaline injury (degrees of freedom = 1, significance level = 0.05).

		Healed	Not Healed	Total
Akita	Observed number	2(22.2%)	7(77.8%)	9
	Expected number	4.5(50%)	4.5(50%)	
WT	Observed number	8(88.9%)	1(11.1%)	9
	Expected number	4.5(50%)	4.5(50%)	
Total		9(100%)	9(100%)	18

TABLE 2 Contingency table for Pearson chi-square test analysis of number and percentage (in brackets) of WT and Akita mice healed at day 7 after corneal alkaline injury (degrees of freedom = 1, significance level = 0.05).

		Healed	Not Healed	Total
Akita	Observed number	8(88.9%)	1(11.1%)	9
	Expected number	4.5(50%)	4.5(50%)	
WT	Observed number	9(100%)	0	9
	Expected number	4.5(50%)	4.5(50%)	
Total		9	9	18

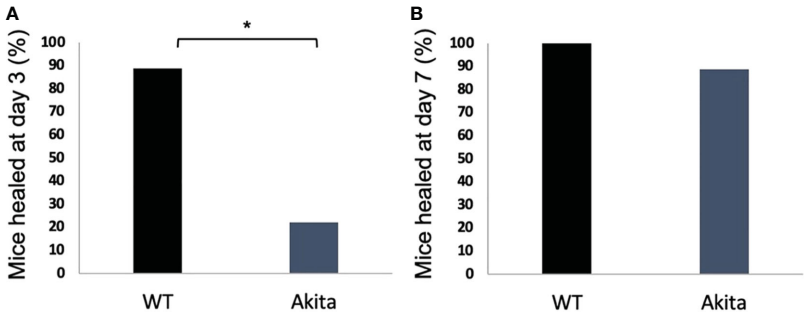

FIGURE 3
Percentage of diabetic and WT mice healed at day 3 after the corneal alkaline injury. **(A)** Pearson chi-squared test was performed (confidence level = 95% and p =0.0044, *n = 9*) and indicated that a significantly greater percentage of WT mice were healed than diabetic mice at day 3. **(B)** Pearson chi-squared test was performed and indicated no significant difference between the percentage of WT mice and diabetic mice healed at day 7 (p > 0.9999, *n = 9* for both the WT and Akita groups). *p<0.05.

conducted with a panel of cytokines related to ocular surface immunity, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), C-C motif chemokine ligand 2 (CCL2), insulin growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), and vascular endothelial growth factor A (VEGF-A) (Supplementary Table 6). At baseline before injury, tear concentration of the Ang-2 (1,062 ± 371.5 vs. 402.4 ± 69.35 pg/ml, p = 0.0075, n = 6) was significantly higher in Akita mice compared to WT mice (Figure 5B). In addition, CCL2 showed elevated tear level in Akita mice compared to WT mice (37.9 ± 5.253 vs. 15.9 ± 4.687 pg/ml, p = 0.0189, n = 6) at baseline (Figure 5C). Yet, no significant difference was found for baseline levels of Ang-1, IGF-1, PDGF, and VEGF-A in Akita mice tear samples as compared to WT ones, as indicated by an unpaired *t*-test (Figures 5A, C–F).

3.3.2 Diabetic mice had significant impairment of tear growth factor and chemokine secretion after corneal alkaline burn injury

To compare whether there is a significant change of tear protein level at day 0, day 3, and day 7 after injury as compared with baseline level, one-way ANOVA was performed to compare the tear levels of Ang-1, Ang-2, CCL2, IGF-1, PDGF, and VEGF-A at baseline with each post-injury time point. For WT samples, the result showed no significant changes in tear levels of Ang-2, CCL2, IGF-1, PDGF, and VEGF-A between baseline and at day 0, day 3, and day 7, respectively, after the corneal alkaline burn Supplementary Tables 2–6). However, tear Ang-1 showed an increased level from 910.5 ± 216.3 pg/ml to 2,967 ± 1,337 pg/ml at day 0 after injury in WT mice (p = 0.0412, n = 8), but not in Akita mice. Among the result presented for Akita mice samples, there was a significant decrease in tear levels of Ang-2, IGF-1, and VEGF-A immediately (day 0) after the chemical injury in comparison with baseline measurement (Supplementary Tables 2, 4, 6) (Figures 5B, D, F). In particular, the tear IGF-1 level from Akita mice decreased from a baseline level of 520.1 ± 118.3 pg/ml to 77.65 ± 22.04 pg/ml (p = 0.0018, n = 8), whereas the tear VEGF-A level from Akita mice decreased from 3,423 ± 772.8 pg/ml to 143.3 ± 59.82 pg/ml (p = 0.0016, n = 8). Yet, no significant changes were found in tear samples of WT mice immediately after injury except for Ang-1.

At day 7 after injury, the tear concentration of Ang-2 decreased from a baseline level of 1,062 ± 172.4 pg/ml to 201.7 ± 87.21 pg/ml (p = 0.0243, n = 6) (Figure 5D; Supplementary Table 2). Meanwhile, there was also a significant decrease in tear levels of IGF-1 and VEGF-A both at day 3 after the injury and at day 7 after the CABI in Akita mice, in comparison with the baseline level (Figures 5D; F; Supplementary Tables 4, 6). The tear concentration of IGF-1 in Akita mice decreased from a baseline level of 520.1 ± 118.3 pg/ml to 75.46 ± 31.04 pg/ml at day 3 after injury (p = 0.0026, n = 8) and to 185.7 ± 72.96 at day 7 after the injury (p = 0.0147, n = 8). Additionally, the tear concentration of VEGF-A in Akita mice decreased from a baseline level of 3,423 ± 772.8 pg/ml to 805.6 ± 573.1 pg/ml at day 3 after injury (p = 0.0063, n = 8) and to 601.6 ± 243.8 pg/ml at day 7 after injury (p = 0.0032, n = 8) (Figure 5F; Supplementary Table 6). Although without significance, the tear CCL2 level in diabetic mice decreased from a baseline level of 37.9 ± 5.253 pg/ml to a day 3 level of 14.1 ± 9.15 pg/ml (p = 0.1308, n = 8), whereas no apparent decrease was observed for WT mice (p = 0.9991, n = 8) (Figure 5C; Supplementary Table 3). Furthermore, in diabetic Akita mice, there was also a trend of decrease of tear expression of Ang-2 at day 0 and day 3 after injury and of PDGF-BB at day 0, day 3, and day 7 after injury (Figures 5B, E; Supplementary Tables 2, 5).

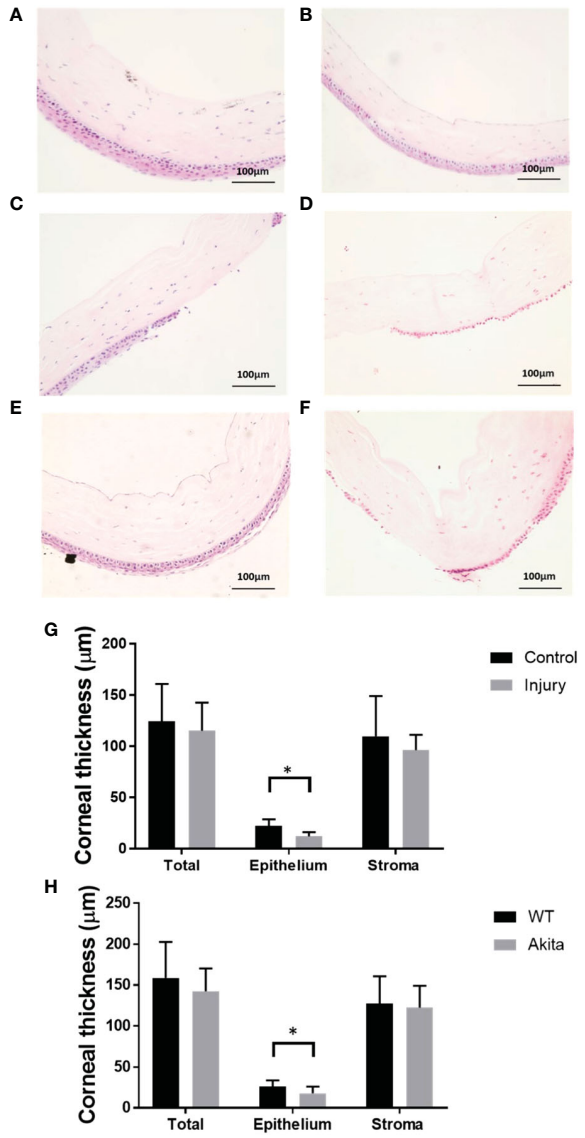

FIGURE 4
H&E staining of the cornea cross section and thickness measurements. Representative images of corneas were selected from **(A)** a WT mouse at baseline, **(B)** an Akita mouse at baseline, **(C)** a WT mouse immediately after corneal alkaline injury, **(D)** an Akita mouse immediately after corneal alkaline injury, **(E)** a WT mouse immediately at day 3 after corneal alkaline injury, and **(F)** an Akita mouse immediately at day 3 after corneal alkaline injury. **(G)** Corneal thickness measurement for total cornea, corneal epithelium, and corneal stroma for mouse corneas without injury (control group) and those immediately after injury; significantly reduced cornea thicknesses were found in the injured group compared with controls (n = 7, p = 0.0129). **(H)** Corneal thickness measurement for total cornea, corneal epithelium, and corneal stroma for corneas from WT mice and Akita mice at day 3 after injury. Significantly reduced cornea thickness was found for Akita mice at day 3 after injury compared to the WT group (n = 7 for both the WT and Akita groups, p = 0.0498). *p<0.05.

3.4 Diabetic mice showed altered intestinal microbiome composition compared to WT mice

The microbiome diversity pattern was compared between groups using an unpaired t-test of the CHAO1, Simpson, and Shannon indices at baseline, day 3, and day 7 after the corneal alkali injury. Akita mice were found to have higher diversity in microbiota composition at baseline (n = 7, p = 0.0370) and day 3 (n = 7, p = 0.0376) after corneal alkaline injury, as compared to WT mice from the CHAO1 index (Figure 6A), whereas the difference was insignificant with the Shannon and Simpson indices (Figures 6B, C). At day 7 after the alkaline injury, the difference in gut microbiome diversity was insignificant between WT and Akita mice with the CHAO1, Shannon, and Simpson index (n = 7, p = 0.1048) (Figure 6A).

Additionally, longitudinal changes in gut microbiome diversity pattern at day 3 and day 7 after injury were measured. The results showed that Akita mice have significantly reduced gut microbiota diversity at day 3 in the post-injury period compared to baseline from the CHAO1 index (n = 7, p = 0.0137) (Figure 6A) based on a one-way ANOVA test (p = 0.2894). Comparatively, there were no significant changes in gut microbiome diversity pattern in WT mice over time (Figure 6A).

Individually, *Firmicutes bacterium M10-2* was found to be significantly more abundant in WT mice at day 7 after the corneal alkaline injury (n = 5, p = 0.0164), while an increase in abundance level was observed at day 7 after injury in WT mice only, but not in Akita mice (n = 5, p = 0.0215) (Figure 6D).The means of relative abundance of bacteria present in the intestinal microbiome of WT and Akita mice were acquired at the phylum, genus, and species level and compared in the form of heat maps, as shown in Figures 7B, C. At the phylum level, Bacteroidetes were in higher relative abundance of 0.52 in WT mice as compared to Akita mice, with a relative abundance of 0.35. Conversely, Firmicutes were in lower relative abundance at 0.35 in WT mice as compared to Akita mice with a relative abundance of 0.52. Additionally, Proteobacteria were in higher relative abundance in WT mice compared to Akita mice whereas Verrucomicrobia were in higher relative abundance in Akita mice compared to WT mice. There were no significant differences in relative abundance between WT and Akita mice for Actinobacteria, Deferribacteres, and Tenericutes (n = 5) (Figure 7A).

At the genus level, Bacteroides were in higher relative abundance at 0.35 in WT mice than in Akita mice at 0.23; Lachnospiraceae_u_g were in higher relative abundance at 0.23 in Akita mice than in WT mice at 0.12; Parabacteroides, Lactobacillus, and Firmicutes_u_g were all in higher abundance in WT mice than in Akita mice (n = 5) (Figure 7B).

At the species level, *Bacteroides sartorii*, *Helicobacter typhlonius*, *Parabacteroides distasonis*, *Bacteroides* sp. *2_1_33B*, *Parabacteroides* sp. *20_3*, *Bacteroides uniformis*, *Lactobacillus johnsonii*, and *Firmicutes bacterium M10-2* were in higher relative abundance in WT mice than in Akita mice, whereas *Akkermansia muciniphila*, *Lachnospiraceae bacterium 10-1*, *Lachnospiraceae bacterium A4*, *Oscillibacter* sp. *1-3*, and Clostridioides were in higher relative abundance in Akita mice compared to WT mice (n = 5) (Figure 7C).

3.5 Diabetic mice showed altered T-cell profile at baseline and on post-injury day 3

3.5.1 Diabetic mice exhibited an impaired systemic adaptive immune response towards cornea injury compared to wild-type mice

Peripheral blood samples of WT and Akita mice were collected at baseline and day 3 after injury and flow cytometry was used to analyze the levels of CD3+CD4+ T cells. All gates were set on the FlowJo

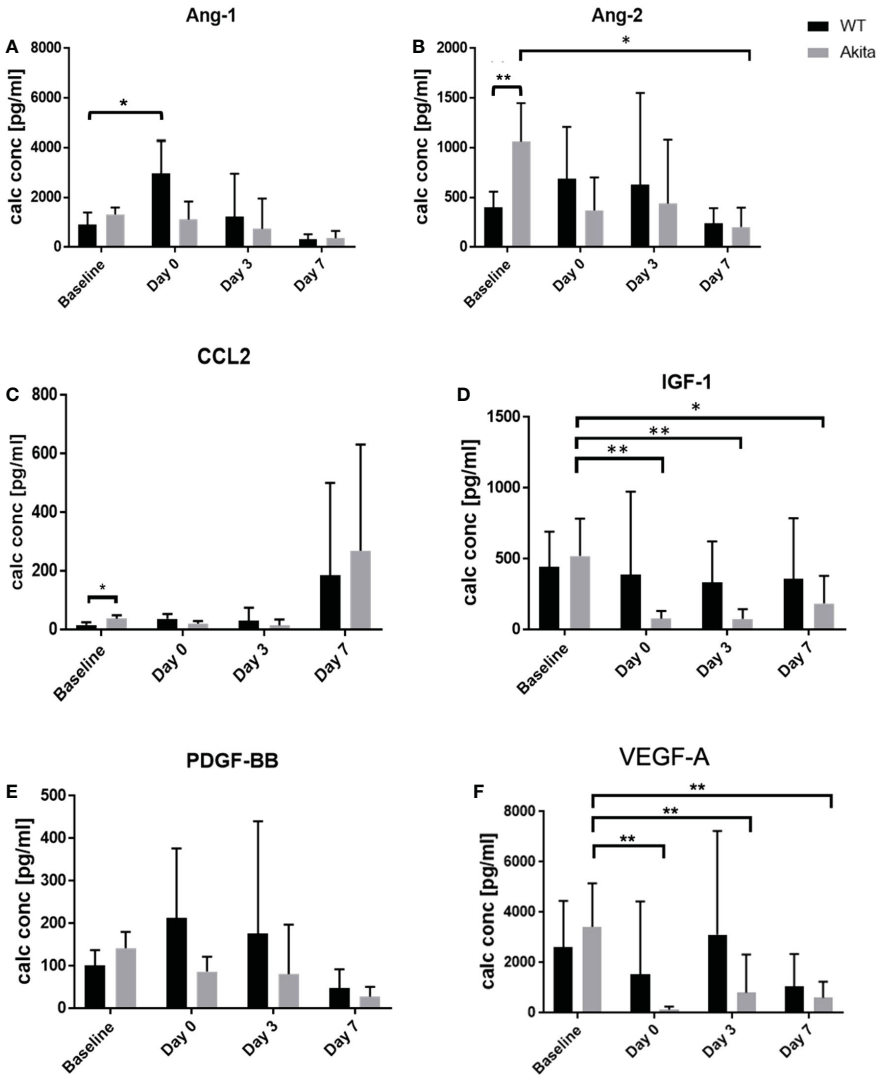

FIGURE 5
Comparison of tear protein concentration of the right (injured) eye from WT and Akita mice measured at baseline (before injury) with day 0, day 3, and day 7 after corneal alkaline injury. **(A)** Tear level of Ang-1 showed significantly elevated level immediately after injury WT mice (n = 6, p = 0.0412). **(B)** Tear level of Ang-2 showed significantly higher tear level at baseline and has significantly decreased at day 7 after injury for Akita mice (n = 6, p = 0.0243). **(C)** Tear level of CCL2 showed significantly elevated level in Akita mice at baseline. **(D)** Tear level of IGF-1 has significantly decreased immediately after injury (n = 8, p = 0.0018), and on post-injury day 3 (n = 8, p = 0.0026) and day 7 (n = 8, p = 0.0147) in Akita mice. **(E)** Tear level of PDGF-BB showed a trend of decrease from baseline level to the post-injury period in tear levels of Akita mice; however, there was no statistical significance. **(F)** Tear level of VEGF-A for Akita mice has significantly decreased immediately after injury (n = 8, p = 0.0016), on day 3 (n = 8, p = 0.0063), and on post-injury day 7 (n = 8, p = 0.0032) compared to baseline level (n number means the number of eyes/mice from both the WT and Akita groups, same for all measurements). *p<0.05; **p<0.01.

software, with P1 gating for lymphocytes, P2 gating for single cells against doublets or clusters of multiple cells, and Q2 gating for both FITC and PE fluorescently labeled cells (Figures 8A–C). The proportions of CD3+CD4+ cells from WT and Akita mice at baseline were analyzed by an unpaired t-test with a significance level of 0.05. Akita mice were found to have a significantly greater proportion of CD3+CD4+ cells as compared to WT mice at baseline (n = 7, p = 0.0146) (Figure 8D). However, the difference between groups became insignificant at day 3 after injury (Figure 8D). Additionally, the proportions of CD3+CD4+ cells from WT and Akita mice at baseline were compared by a paired t-test with a significance level of 0.05 for longitudinal analysis. WT mice had significantly increased CD3+CD4+ cells in the peripheral blood at day 3 after injury compared to baseline (n = 7, p = 0.0022), whereas Akita mice had no significant changes in CD3+CD4+ cells in the peripheral blood before and after injury (Figure 8D).

3.5.2 Diabetic mice exhibited an impaired ocular surface adaptive immune response towards cornea injury compared to WT mice

In addition, the proportion of CD4+CD3+ T cells were tested with ocular surface samples taken from the mice at day 3 after the chemical injury. The gates were set on FlowJo as shown in Figures 9A–C. Using a paired t-test, we found that WT mice have a significant increase of CD3+CD4+ T cells on the ocular surface at day 3 after corneal alkali injury as compared to baseline (n = 7, p = 0.0471), whereas no such significant change was found in CD3+CD4+ T cells on the ocular surface of Akita mice after injury (n = 7, p = 0.0941) (Figure 9D). Moreover, Akita mice

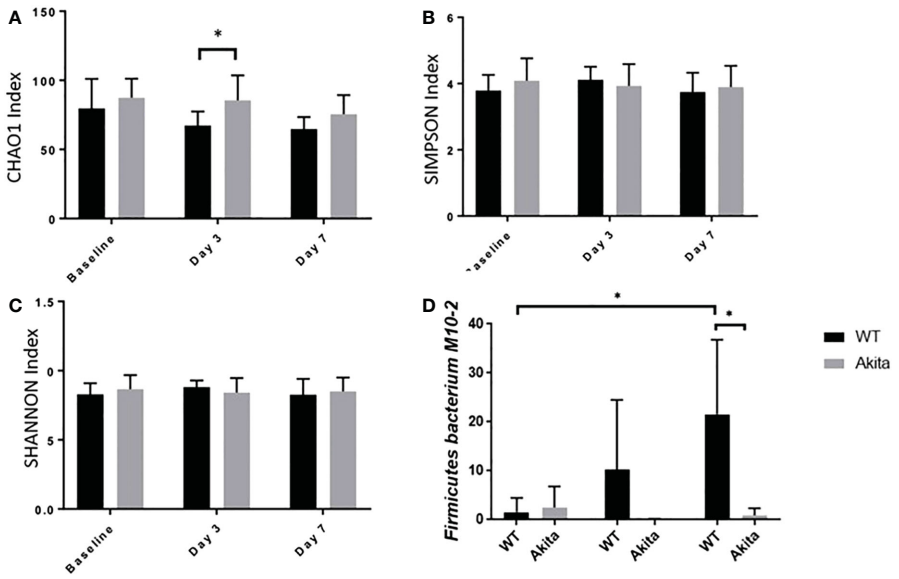

FIGURE 6
Comparison of intestinal microbiome diversity pattern of WT and Akita mice measured at baseline (before injury) with day 0, day 3, and day 7 aftercorneal alkaline injury. **(A)** Comparison of alpha diversity index at baseline, day 3, and day 7 after injury. Akita mice were found to have higher abundance ofmicrobiota composition as compared to WT mice from the CHAO1 index at day 3 after injury (n = 7, p = 0.0376). **(B, C)** Comparison of alpha diversity index at baseline, day 3, and day 7 after injury by the SIMPSON index **(B)** and SHANNON index **(C)** at day 3 after injury. No significance difference was found at baseline, day 3, or day 7 after injury after one-way ANOVA test (*n* = 7). **(D)** *Firmicutes bacterium M10-2* had higher abundance in WT mice as compared to diabetic mice at day 7 after cornea alkaline injury (*n* = 5, *p* = 0.0164); there was a significant increase of Firmicutes bacterium M10-2 at day 7 after injury as compared to baseline level for WT mice (*n* = 5, *p* = 0.0215) (*n* number means the number of mice from both the WT and Akita groups, same for all measurements). *p<0.05.

showed more abundant CD3+CD4+ T cells as compared to WT mice at baseline, yet the difference is insignificant using an unpaired *t*-test (*n* = 7, *p* = 0.0639) (Figure 9D).

4 Discussion

4.1 We report a highly repeatable animal model of diabetic cornea wound healing

For our experiments, the Akita mouse was selected to model a persistent hyperglycemic state. This is an animal model of T1DM that exhibits significant hyperglycemia starting from 3 to 4 weeks of age. While it has been previously used as an animal model of diabetic retinopathy complications, our group is the first to use this model to investigate impaired cornea wound healing after injury in diabetic mice versus controls (24). In our experiments, Akita mice showed significantly slower corneal re-epithelialization rates compared to WT mice after alkaline chemical injury. This is consistent with findings from diabetic cornea wound healing studies using other types of animal models (25). Meanwhile, the result also demonstrates that alkaline burn injury induced on Akita mouse cornea is an excellent model to investigate molecular pathogenic and therapeutic aspects of corneal wound healing in diabetes.

4.2 Diabetic mice exhibited impaired ocular surface immune response to corneal injury

The results of protein micro-array assay of tear cytokines reported that diabetic mice had reduced tear levels of VEGF-A, Ang2, and IGF-1 after injury compared to baseline. The components of tear fluid are secreted from several sources including conjunctival goblet cells, the lacrimal gland, the cornea epithelium, and the episcleral vascular system in close proximity (26). The altered tear cytokine levels suggest an inability of the ocular surface to mount an immune response to promote cornea wound healing after injury. The importance of VEGF in diabetic complications has been previously comprehensively established (27, 28). VEGF is known to promote vascular endothelial cell proliferation, migration, and vasopermeability. VEGF levels were reported to be elevated in the blood of diabetic patients, playing a key role in diabetes-related morbidity such as diabetic retinopathy, age-related macular degeneration, and a variety of cardiovascular diseases. In ocular surface diseases such as DED, VEGF is known as an inflammatory marker that impairs the surface epithelium of the eye (29). However, VEGF is also recognized as a pleiotropic factor with a broad effect on endothelial, neuronal, and glial behaviors (30). Because of the above-mentioned importance of VEGF in diabetes and in corneal wound healing response, VEGF level was measured in our experiments and was found to be suppressed in diabetic mice after injury, suggesting that while sustained high levels of VEGF in the serum may correlate with the development of microvascular complications, post-injury tear VEGF levels may be important in the cornea wound healing response.

Our study reported an elevated baseline level of CCL2 in tear samples of Akita mice. CCL2, also known as MCP-1, is a proinflammatory chemokine and has a controversial role in diabetes and wound healing. Previous studies suggested that CCL2 is beneficial in wound healing by restoring the macrophage response (31). On the other hand, it is also a risk factor for worsening diabetic nephropathy due to its role in promoting inflammation (32). In

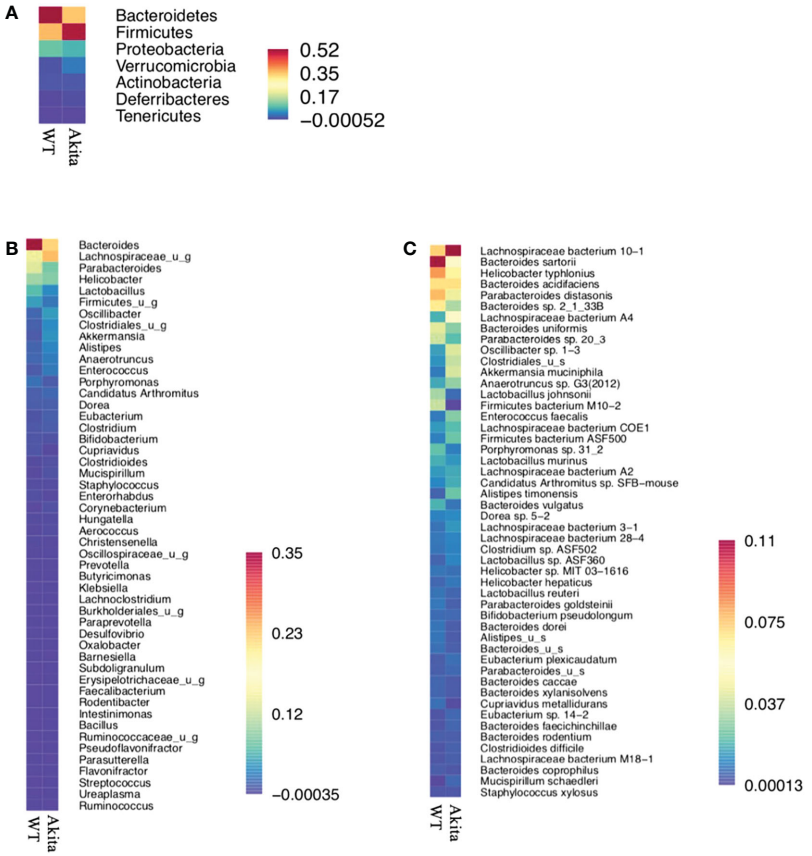

FIGURE 7
Heat maps for relative abundance of bacteria present in the intestinal microbiome of WT and Akita mice at the phylum, species, and genus level. **(A)** Heat map of bacteria present in intestinal microbiome of WT and Akita mice generated at the phylum level based on mean relative abundance. **(B)** Heat map of bacteria present in the intestinal microbiome of WT and Akita mice generated at the genus level based on mean relative abundance. **(C)** Heat map of bacteria present in the intestinal microbiome of WT and Akita mice generated at the species level based on mean relative abundance. Values of relative abundance are presented with corresponding colors as shown in the legend for B to D (n = 5 in WT and Akita).

previous ocular studies, overexpression of the CCL2 was involved in the pathogenesis of diabetic retinopathy (33). In our experiments, tear secretion of CCL2 diminished sequentially after injury in diabetic mice but not in WT mice, suggesting its importance in the cornea wound healing response.

Previous reports have shown higher blood levels of Ang-2 in diabetic mice compared to controls (34). The function of Ang-2 in the integrity of the blood–retinal barrier was previously highlighted in studies of diabetic retinopathy (35). In our experiments, tear Ang-2 was lower in diabetic mice compared to controls immediately after injury (day 0), suggesting an important role in the initiation of the cornea wound healing response.

The association between IGF and metabolic syndromes has been explored extensively in previous experiments (36). Patients with very high or very low levels of serum IGF-1 are both at increased risk of diabetes (37, 38). Meanwhile, animal studies indicate that IGF-1 has an essential inhibitory effect on cell apoptosis and has been shown to promote wound healing (39, 40). This is consistent with our finding of diminished tear IGF-1 after injury in diabetic mice, confirming its importance in the cornea wound healing response.

Taken together, our findings show that cytokines are key mediators of corneal wound healing after injury, despite the fact that they are normally detrimental in the pathogenesis of common microvascular complications of diabetes, including retinopathy and nephropathy. The fact that tear levels of these cytokines are suppressed in diabetic mice following corneal alkaline burn injury demonstrates the importance of the local inflammatory response in initiating and propagating cornea re-epithelialization.

4.3 Altered gut microbial diversity in response to corneal injury and in diabetic mice

In our study, diabetic mice and WT mice each had distinct microbial abundance patterns at baseline. In response to cornea injury, both diabetic and WT mice demonstrated changes in alpha diversity patterns compared to baseline. Such a response has been previously documented in other forms of severe non-abdominal injuries, including traumatic brain injury and spinal cord injury resulting in intestinal dysbiosis, which subsequently was followed by impaired systemic immunity (41, 42). Such changes may represent a stress response resulting in a temporary alteration in gut microbiome composition. This is consistent with previous findings that psychological stress or depression may cause intestinal dysbiosis (43). The resulting altered intestinal microbiome may, in turn, impact

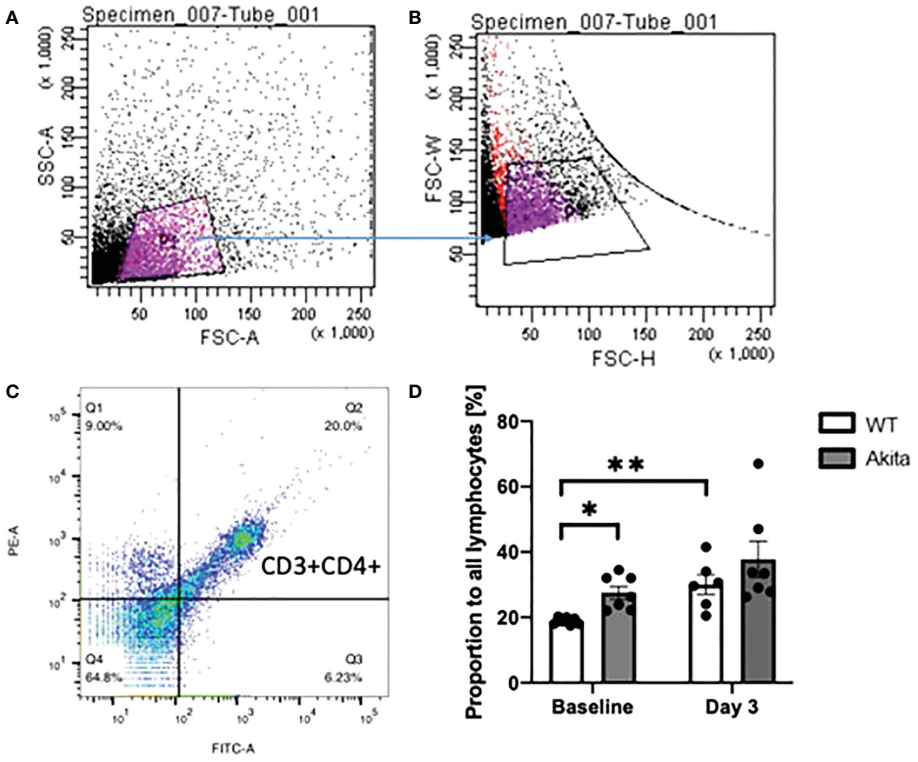

FIGURE 8
Proportion of CD3+CD4+ T cells in lymphocytes in peripheral blood samples of WT mice and Akita mice at baseline and at day 3 after corneal alkaline injury **(A)** Gating of lymphocytes (P1). **(B)** Gating of single cells against double or multiple cells (P2). **(C)** Gating of CD3+CD4+ T cells. **(D)** Akita mice had a higher abundance of CD3+CD4+ T cells in peripheral blood as compared to WT mice at baseline as indicated by an unpaired *t*-test (n = 7, p = 0.0146). WT mice had a significant increase of CD3+CD4+ cells in the peripheral blood at day 3 after the injury as indicated by a paired *t*-test (n = 7 for both the WT and Akita groups, p = 0.0022). *p<0.05; **p<0.01.

the adaptive immune system, making it less effective in promoting healing after an insult.

Previous studies have reported the close association between intestinal dysbiosis and type 2 diabetes. The gut microbiota contributes to maintenance of intestinal integrity by modulating tight junction proteins between gut epithelial cells (44). Alterations in intestinal microbiome have been shown to enhance systemic inflammation (45) due to the increased abundance of pathogenic bacteria. In diabetes, intestinal dysbiosis may further promote microvascular complications through endotoxemia. This can be ameliorated through the use of probiotic treatment, which has been shown to effectively reverse both intestinal dysbiosis and diabetic progression in T2DM (45).

When considering the microbiome response on an individual species level, our study identified several potential targets for manipulation in future studies (46). In our study, the abundance of *Bacteroides* was initially lower in diabetic mice compared to controls at baseline, but was significantly elevated after cornea injury. A previous study demonstrated a reduced abundance of *Bacteroides* in Bio-Breeding diabetes-prone (BB-DP) rats (47). Furthermore, in our experiments, *A. muciniphila* is present only in diabetic mice after the injury and not in controls. In previous studies, *A. muciniphila* was shown to have beneficial effects in attenuating diabetic complications in humans (48). It has also been shown to have a protective role against immune-mediated liver injury in mice (49). We also observed lower abundance levels of *H. typhlonius* in diabetic mice compared to controls. This was consistent with the findings of previous published results in humans (50). Its role in diabetic complications, however, has not been fully elucidated. Finally, the abundance level of *Firmicutes bacterium M10-2* was significantly reduced in diabetic mice after injury. It was previously reported that that the ratio of Firmicutes and Bacteroidetes correlated with body mass index (51). However, no published study has investigated the role of firmicutes bacterium in diabetes pathogenesis and complications.

4.4 Diabetic mice have impaired systemic and local immune response to corneal injury

Upon stimulation by the innate immune response, CD4+ cells differentiate into T helper (Th) cells and regulatory T (Treg) cells, facilitating adaptive immunity. Th and Treg cells play important roles in response to insult, including acute kidney injury (52) and skin injury (53).

In our study, Akita mice had higher peripheral blood CD4+ T-cell counts compared to WT mice at baseline. This finding may represent a persistent inflammatory response towards intestinal dysbiosis and endotoxemia in the Akita mice. This, however, changed significantly in Akita and WT mice groups after cornea injury. While WT mice had an upregulation of CD3+CD4+ T-cell count after injury, diabetic

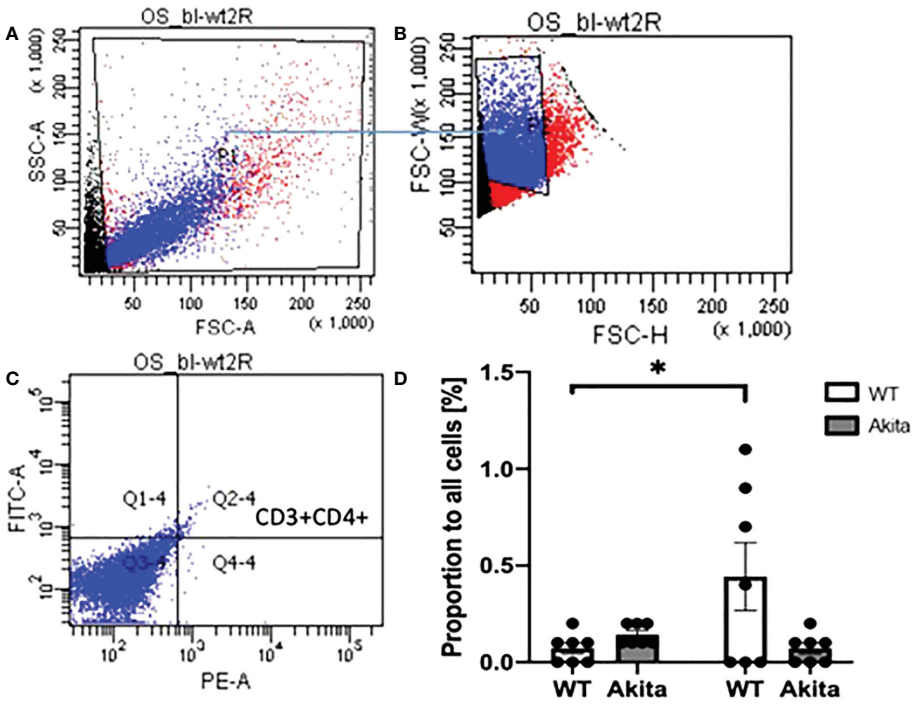

FIGURE 9
Proportion of CD3+CD4+ T cells in lymphocytes in ocular surface samples of WT and Akita mice at baseline and day 3 after corneal alkaline injury. **(A)** Gating of lymphocytes (P1). **(B)** Gating of single cells against double or multiple cells (P2). **(C)** Gating of CD3+CD4+ T cells (Q2–4). **(D)** Using a paired *t*-test, WT mice had a significant increase of CD3+CD4+ T cells on the ocular surface at day 3 after CABI as compared to baseline (n = 7, p = 0.0471), whereas no significant change was observed in CD3+CD4+ T cells on Akita mice ocular surface after injury (n = 7 for both the WT and Akita groups, p = 0.0941). *p<0.05.

mice had no significant changes, both on the ocular surface and in peripheral blood. This highly suggests that diabetic mice are incapable of initiating a proper adaptive immune response towards injury. This finding may partially explain impaired ocular surface immune response and wound healing after injury under diabetic conditions. Furthermore, given our understanding of the link between intestinal microbiome and T-cell-mediated immunity, the differences in immune response between WT and Akita mice may be a consequence of the observed differences in microbiome diversity and abundance patterns.

4.5 Limitations of the study

Our study had several limitations. Firstly, in our experiments, we used the Akita mouse as the animal model of diabetes. It is important to note that there are etiological differences between the diabetes in this model and that in human patients, which may serve as confounding factors, particularly in regard to the non-glycemic pathways for pathogenesis and complications of diabetes. It may be worthwhile to confirm whether our observations are present in other animal models of diabetes and cornea injury. Furthermore, differences in the gut microbiome profile between mouse and human serve another limitation, as our target bacteria and pathways may not be applicable to the human gut. Thirdly, this is an observational study rather than a therapeutic trial. We will next need to examine the impact of treating the intestinal dysbiosis in diabetic mice, either through probiotics or fecal transplantation, on cornea wound healing outcomes.

4.6 Clinical implications

Our results demonstrated a potential link between gut microbiome, systemic and local immunity, and cornea wound healing, suggesting that further exploration of this relationship in diabetes is worthwhile. Keratopathy is a common and sight-threatening complication of diabetes, manifested by delayed cornea wound healing, recurrent corneal erosion syndrome, and neurotrophic ulcers. Current treatment options are limited and do not address the underlying pathological changes caused by DM. Our continued research in this area may eventually pave the way for specific dietary modifications, fecal transplantation, and/or probiotic therapy as promising and more cost-effective options in ameliorating complications of DM.

5 Conclusion

In summary, we demonstrated that in diabetic mice, impaired cornea wound healing was associated with an inability to mount a systemic and local immune response to ocular chemical injury. The lack of ability of the immune system in diabetic mice to respond to the ocular insult, both locally and systemically, was evidenced by a lack of peripheral blood T-cell proliferation and lack of tear chemokine and growth factor production. This is in stark contrast to the immune system in WT controls, where a swift immune response facilitated prompt cornea wound healing. Our study further postulated that baseline differences in intestinal microbial diversity and abundance

patterns may account for the observed differences in immune response between diabetic mice and controls. While further in vivo and in vitro experiments are needed to clarify the relationship between gut microbiome and systemic and ocular surface immunity, our results add to the growing amount of evidence of the intimate link between the two systems. The gut microbiome may provide a useful and non-invasive target for manipulation in diabetic patients with reduced morbidity and mortality.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR, 4696-18).

Author contributions

All authors attest that they meet the current ICMJE criteria for authorship. YB, KS, and AC-YL were involved in the study design, data collection, data analysis, manuscript writing, and editing. JY-KC, HLW, A-KN, TC-YC, VJ, and LT were involved in data collection, data analysis, manuscript writing, and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

1. Schultz RO, Van Horn DL, Peters MA, Klewin KM, Schutten WH. Diabetic keratopathy. *Trans Am Ophthalmol Soc* (1981) 79:180–99.

2. Vieira-Potter VJ, Karamichos D, Lee DJ. Ocular complications of diabetes and therapeutic approaches. *BioMed Res Int* (2016) 2016:3801570. doi: 10.1155/2016/3801570

3. Shih KC, Lam KS, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. *Nutr Diabetes* (2017) 7(3):e251. doi: 10.1038/nutd.2017.4

4. Ljubimov AV. Diabetic complications in the cornea. *Vision Res* (2017) 139:138–52. doi: 10.1016/j.visres.2017.03.002

5. Yang L, Di G, Qi X, Qu M, Wang Y, Duan H, et al. Substance p promotes diabetic corneal epithelial wound healing through molecular mechanisms mediated *via* the neurokinin-1 receptor. *Diabetes* (2014) 63(12):4262–74. doi: 10.2337/db14-0163

6. Atiba A, Wasfy T, Abdo W, Ghoneim A, Kamal T, Shukry M. Aloe vera gel facilitates re-epithelialization of corneal alkali burn in normal and diabetic rats. *Clin Ophthalmol (Auckland NZ)* (2015) 9:2019–26. doi: 10.2147/OPTH.S90778

7. Segre JA. MICROBIOME. microbial growth dynamics and human disease. *Sci (New York NY)* (2015) 349(6252):1058–9. doi: 10.1126/science.aad0781

8. Chu H, Khosravi A, Kusumawardhani IP, Kwon AH, Vasconcelos AC, Cunha LD, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Sci (New York NY)* (2016) 352(6289):1116–20. doi: 10.1126/science.aad9948

9. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, et al. MUCOSAL IMMUNOLOGY. individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Sci (New York NY)* (2015) 349 (6251):993–7. doi: 10.1126/science.aaa9420

10. Kim J, Choi SH, Kim YJ, Jeong HJ, Ryu JS, Lee HJ, et al. Clinical effect of IRT-5 probiotics on immune modulation of autoimmunity or alloimmunity in the eye. *Nutrients* (2017) 9(11):1166. doi: 10.3390/nu9111166

11. Lu LJ, Liu J. Human microbiota and ophthalmic disease. *Yale J Biol Med* (2016) 89 (3):325–30.

12. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. et al: Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* (2009) 139 (3):485–98. doi: 10.1016/j.cell.2009.09.033

13. Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, et al. et al: ATP drives lamina propria T(H)17 cell differentiation. *Nature* (2008) 455(7214):808–12. doi: 10.1038/nature07240

14. Murphy EA, Velazquez KT, Herbert KM. Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care* (2015) 18(5):515–20. doi: 10.1097/MCO.0000000000000209

15. Li Q, Gao Z, Wang H, Wu H, Liu Y, Yang Y, et al. Intestinal immunomodulatory cells (T lymphocytes): A bridge between gut microbiota and diabetes. *Mediators Inflammation* (2018) 2018:9830939. doi: 10.1155/2018/9830939

16. Shin YJ, Hyon JY, Choi WS, Yi K, Chung ES, Chung TY, et al. Chemical injury-induced corneal opacity and neovascularization reduced by rapamycin *via* TGF-beta1/ERK pathways regulation. *Invest Ophthalmol Vis Sci* (2013) 54(7):4452–8. doi: 10.1167/iovs.13-11684

17. Nakano Y, Uchiyama M, Arima T, Nagasaka S, Igarashi T, Shimizu A, et al. PPARalpha agonist suppresses inflammation after corneal alkali burn by suppressing proinflammatory cytokines, MCP-1, and nuclear translocation of NF-kappaB. *Molecules (Basel Switzerland)* (2018) 24(1). doi: 10.3390/molecules24010114

18. Chen Y, Yang W, Zhang X, Yang S, Peng G, Wu T, et al. MK2 inhibitor reduces alkali burn-induced inflammation in rat cornea. *Sci Rep* (2016) 6:28145. doi: 10.1038/srep28145

19. Wang W, Tam KC, Ng TC, Goit RK, Chan KLS, Lo ACY. Long-term lutein administration attenuates retinal inflammation and functional deficits in early diabetic retinopathy using the Ins2(Akita/+) mice. *BMJ Open Diabetes Res Care* (2020) 8(1). doi: 10.1136/bmjdrc-2020-001519

20. Wong HL, Bu Y, Chan YK, Shih KC. Lycium barbarum polysaccharide promotes corneal re-epithelialization after alkaline injury. *Exp Eye Res* (2022) 221:109151. doi: 10.1016/j.exer.2022.109151

21. Soria J, Acera A, Merayo LJ, Durán JA, González N, Rodriguez S, et al. Tear proteome analysis in ocular surface diseases using label-free LC-MS/MS and multiplexed-microarray biomarker validation. *Sci Rep* (2017) 7(1):17478. doi: 10.1038/s41598-017-17536-2

22. Singh A, Schabath R, Ratei R, Stroux A, Klemke CD, Nebe T, et al. Peripheral blood $sCD3^-$ CD4+ T cells: A useful diagnostic tool in angioimmunoblastic T cell lymphoma. *Hematol Oncol* (2014) 32(1):16–21. doi: 10.1002/hon.2080

23. Yan J, Liao C, Taylor BP, Fontana E, Amoretti LA, Wright RJ, et al. A compilation of fecal microbiome shotgun metagenomics from hematopoietic cell transplantation patients. *Sci Data* (2022) 9(1):219. doi: 10.1038/s41597-022-01302-9

24. Barber AJ, Antonetti DA, Kern TS, Reiter CE, Soans RS, Krady JK, et al. The Ins2Akita mouse as a model of early retinal complications in diabetes. *Invest Ophthalmol Visual Sci* (2005) 46(6):2210–8. doi: 10.1167/iovs.04-1340

25. Xu K, Yu FS. Impaired epithelial wound healing and EGFR signaling pathways in the corneas of diabetic rats. *Invest Ophthalmol Vis Sci* (2011) 52(6):3301–8. doi: 10.1167/iovs.10-5670

26. Chng C-L, Seah LL, Yang M, Shen SY, Koh SK, Gao Y, et al. Tear proteins calcium binding protein A4 (S100A4) and prolactin induced protein (PIP) are potential biomarkers for thyroid eye disease. *Sci Rep* (2018) 8(1):16936. doi: 10.1038/s41598-018-35096-x

27. Wirostko B, Wong TY, Simo R. Vascular endothelial growth factor and diabetic complications. *Prog retinal eye Res* (2008) 27(6):608–21. doi: 10.1016/j.preteyeres.2008.09.002

28. Aiello LP, Wong JS. Role of vascular endothelial growth factor in diabetic vascular complications. *Kidney Int Supplement* (2000) 77:S113–119. doi: 10.1046/j.1523-1755.2000.07718.x

29. Laude A, Lim JW, Srinagesh V, Tong L. The effect of intravitreal injections on dry eye, and proposed management strategies. *Clin Ophthalmol (Auckland NZ)* (2017) 11:1491–7. doi: 10.2147/OPTH.S136500

30. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, Hartnett ME. Vascular endothelial growth factor in eye disease. *Prog retinal eye Res* (2008) 27(4):331–71. doi: 10.1016/j.preteyeres.2008.05.001

31. Wood S, Jayaraman V, Huelsmann EJ, Bonish B, Burgad D, Sivaramakrishnan G, et al. Pro-inflammatory chemokine CCL2 (MCP-1) promotes healing in diabetic wounds by restoring the macrophage response. *PLos One* (2014) 9(3):e91574. doi: 10.1371/journal.pone.0091574

32. Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. *Am J Physiology-Renal Physiol* (2008) 294(4):F697–701. doi: 10.1152/ajprenal.00016.2008

33. Taghavi Y, Hassanshahi G, Kounis NG, Koniari I, Khorramdelazad H. Monocyte chemoattractant protein-1 (MCP-1/CCL2) in diabetic retinopathy: latest evidence and clinical considerations. *J Cell Commun Signal* (2019) 13:451–462. doi: 10.1007/s12079-018-00500-8

34. Lim HS, Lip GY, Blann AD. Angiopoietin-1 and angiopoietin-2 in diabetes mellitus: relationship to VEGF, glycaemic control, endothelial damage/dysfunction and atherosclerosis. *Atherosclerosis* (2005) 180(1):113–8. doi: 10.1016/j.atherosclerosis.2004.11.004

35. Rangasamy S, Srinivasan R, Maestas J, McGuire PG, Das A. A potential role for angiopoietin 2 in the regulation of the blood-retinal barrier in diabetic retinopathy. *Invest Ophthalmol Visual Sci* (2011) 52(6):3784–91. doi: 10.1167/iovs.10-6386

36. Friedrich N, Thuesen B, Jorgensen T, Juul A, Spielhagen C, Wallaschofksi H, et al. The association between IGF-I and insulin resistance: A general population study in Danish adults. *Diabetes Care* (2012) 35(4):768–73. doi: 10.2337/dc11-1833

37. van der Klaauw AA, Biermasz NR, Feskens EJ, Bos MB, Smit JW, Roelfsema F, et al. The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. *Eur J Endocrinol* (2007) 156(4):455–62. doi: 10.1530/EJE-06-0699

38. Melmed S. Medical progress: Acromegaly. *New Engl J Med* (2006) 355(24):2558–73. doi: 10.1056/NEJMra062453

39. Emmerson E, Campbell L, Davies FC, Ross NL, Ashcroft GS, Krust A, et al. Insulin-like growth factor-1 promotes wound healing in estrogen-deprived mice: New insights into cutaneous IGF-1R/ERalpha cross talk. *J Invest Dermatol* (2012) 132 (12):2838–48. doi: 10.1038/jid.2012.228

40. Achar RA, Silva TC, Achar E, Martines RB, Machado JL. Use of insulin-like growth factor in the healing of open wounds in diabetic and non-diabetic rats. *Acta cirurgica Bras* (2014) 29(2):125–31. doi: 10.1590/S0102-86502014000200009

41. Celorrio M, Abellanas MA, Rhodes J, Goodwin V, Moritz J, Vadivelu S, et al. Gut microbial dysbiosis after traumatic brain injury modulates the immune response and impairs neurogenesis. *Acta Neuropathologica Commun* (2021) 9(1):40. doi: 10.1186/s40478-021-01137-2

42. Valido E, Bertolo A, Fränkl GP, Itodo OA, Pinheiro T, Pannek J, et al. Systematic review of the changes in the microbiome following spinal cord injury: animal and human evidence. *Spinal Cord* (2022) 60(4):288–300. doi: 10.1038/s41393-021-00737-y

43. Madison A, Kiecolt-Glaser JK. Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Curr Opin Behav Sci* (2019) 28:105–10. doi: 10.1016/j.cobeha.2019.01.011

44. Sharma S, Tripathi P. Gut microbiome and type 2 diabetes: where we are and where to go? *J Nutr Biochem* (2019) 63:101–8. doi: 10.1016/j.jnutbio.2018.10.003

45. Sanz Y, Rastmanesh R, Agostoni C. Understanding the role of gut microbes and probiotics in obesity: How far are we? *Pharmacol Res* (2013) 69(1):144–55. doi: 10.1016/j.phrs.2012.10.021

46. Han H, Li Y, Fang J, Liu G, Yin J, Li T, et al. Gut microbiota and type 1 diabetes. *Int J Mol Sci* (2018) 19(4). doi: 10.3390/ijms19040995

47. Roesch LF, Lorca GL, Casella G, Giongo A, Naranjo A, Pionzio AM, et al. Culture-independent identification of gut bacteria correlated with the onset of diabetes in a rat model. *Isme J* (2009) 3(5):536–48. doi: 10.1038/ismej.2009.5

48. Mithieux G. Does akkermansia muciniphila play a role in type 1 diabetes? *Gut* (2018) 67(8):1373–4. doi: 10.1136/gutjnl-2017-315732

49. Wu W, Lv L, Shi D, Ye J, Fang D, Guo F, et al. Protective effect of akkermansia muciniphila against immune-mediated liver injury in a mouse model. *Front Microbiol* (2017) 8:1804. doi: 10.3389/fmicb.2017.01804

50. Dingemanse C, Belzer C, van Hijum SA, Gunthel M, Salvatori D, den Dunnen JT, et al. Akkermansia muciniphila and helicobacter typhlonius modulate intestinal tumor development in mice. *Carcinogenesis* (2015) 36(11):1388–96. doi: 10.1093/carcin/bgv120

51. Larsen N, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, et al. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLos One* (2010) 5(2):e9085. doi: 10.1371/journal.pone.0009085

52. Dellepiane S, Leventhal JS, Cravedi P. T Cells and acute kidney injury: A two-way relationship. *Front Immunol* (2020) 11. doi: 10.3389/fimmu.2020.01546

53. Boothby IC, Cohen JN, Rosenblum MD. Regulatory T cells in skin injury: At the crossroads of tolerance and tissue repair. *Sci Immunol* (2020) 5(47). doi: 10.1126/sciimmunol.aaz9631

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The gut microbiota correlate with the disease characteristics and immune status of patients with untreated diffuse large B-cell lymphoma

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Background: Gut microbiota characteristics in patients with diffuse large B-cell lymphoma (DLBCL) are reportedly different when compared with the healthy population and it remains unclear if the gut microbiota affects host immunity and clinical disease features. This research investigated the gut microbiota in patients with untreated DLBCL and analyzed its correlation with patient clinical characteristics, humoral, and cell immune status.

Methods: Thirty-five patients with untreated DLBCL and 20 healthy controls (HCs) were recruited to this study and microbiota differences in stool samples were analyzed by 16S rDNA sequencing. Absolute ratios of immune cell subset counts in peripheral blood were detected by flow cytometry and peripheral blood cytokine levels were detected by enzyme-linked immunosorbent assay. Relationships between changes in patient microbiomes and clinical characteristics, such as clinical stage, international prognostic index (IPI) risk stratification, cell origin, organ involved and treatment responses were investigated and correlations between differential microbiota and host immune indices were analyzed.

Results: The alpha-diversity index of intestinal microecology in DLBCL patients was not significantly different when compared with HCs (P>0.05), nonetheless beta-diversity was significantly decreased (P=0.001). *p_Proteobacteria* were dominant in DLBCL, while *p_Bacteroidetes* abundance was significantly decreased when compared with HCs (P<0.05). Gut microbiota characteristics were identified that were associated with clinical features, such as tumor load, risk stratification and cell origin, and correlation analyses were performed between differential flora abundance associated with these clinical features and host immune status. The *p_Firmicutes* was positively correlated with absolute lymphocyte values, *g_Prevotella_2* and *s_un_g_Prevotella_2* were

negatively correlated with absolute lymphocyte values, T cell counts and CD4 cell counts, while *g_Pyramidobacter*, *s_un_g_Pyramidobacter*, and *f_Peptostreptococcaceae* were negatively correlated with IgA.

Conclusions: Dominant gut microbiota, abundance, diversity, and structure in DLBCL were influenced by the disease, correlated with patient immune status and this suggested that the microecology-immune axis may be involved in regulating lymphoma development. In the future, it may be possible to improve immune function in patients with DLBCL by regulating the gut microbiota, improve treatment response rates and increase patient survival rates.

KEYWORDS

diffuse large B-cell lymphoma, gut microbiota, 16s rDNA sequencing, lymphocyte subsets, cytokines

1 Introduction

Diffuse large B-cell lymphoma (DLBCL) is the largest non-Hodgkin's lymphoma (NHL) subtype, is highly invasive and accounts for about 30% of NHL cases (1). It is a highly heterogeneous tumor in terms of biological features and clinical prognosis, is divided into germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subgroups based on cell origin and depends on different oncogenic pathways with different clinical courses (2). International prognostic (IPI) and age-adjusted IPI (AaIPI) indices can help predict prognosis outcomes (3). As an aggressive NHL, the natural course of DLBCL is relatively short, with immunochemotherapy, based on the CD20 monoclonal antibody such as rituximab substantially improving complete remission (CR) and survival rates in DLBCL patients. However, 30 to 40% of patients will relapse and endure refractory or drug-resistance problems which pose significant challenges for prognoses (4).

With approximately 100 trillion microorganisms in the human gut representing one to three percent of the body's weight, the gut microbiota is considered the body's other major organ and affects health *via* an ancient evolutionary symbiotic relationship (5). Many studies have shown that the gut microbiota is linked with diabetes, obesity, cardiovascular disease, inflammatory bowel disease, irritable bowel syndrome and tumors (6–9). The gut microbiota both promotes and inhibits cancer and affects antitumor therapy efficacy. Its mechanism of action toward malignant tumor development may involve inflammation, immune responses, material metabolites and genetic material alteration (10, 11). The microbiota also improves host metabolic capacity and immunoglobulin levels inside and outside the intestinal tract and helps regulate intestinal mucosal immunity (12). Intestinal microecology is not only involved in host digestion, metabolism and energy conversion, but also provides the host with benefits, including strengthening intestinal integrity, regulating intestinal epithelial function, resisting pathogens, supporting lipid metabolism and angiogenesis (13, 14). Intestinal microecology also influences tumor development by interfering with the immune system, inducing genetic mutations, causing chronic inflammation and disrupting the balance between cell proliferation and apoptosis (15).

Although many studies have confirmed that intestinal microecology influences hematological tumor progression and patient prognoses, in DLBCL patients, differences in gut microecological structures between untreated DLBCL onset and healthy populations have been reported (16), while the relationship between intestinal microecology and the immune system and its role in DLBCL development remain poorly understood. More in-depth studies investigating correlations between intestinal microecology, disease characteristics and immune functions in DLBCL patients may provide a basis for disease prevention, early diagnosis, prognostic marker development, and the gut microbiota as a new therapeutic target for DLBCL.

This research used *16S rDNA* sequencing to clarify differences in gut microbiota between DLBCL patients and HCs, by dividing DLBCL into early and advanced-stage groups, high-risk and not-high-risk groups with IPI scores, GCB and non-GCB groups, DLBCL with gastrointestinal involvement (GI) and non-gastrointestinal involvement (NGI) groups, complete remission (CR) and non-complete remission(NCR) groups to compare gut microbiota differences between groups. Correlations between immune indicators and the gut microbiota, which affected patient clinical characteristics, were specifically examined to possibly elucidate microecology-immune axis functions in DLBCL development and develop novel prognostic markers and intervention strategies.

2 Materials and methods

2.1 Participant characteristics

From September 2018 to November 2021, 35 patients with untreated DLBCL at the Ningbo First Hospital, and 20 matched healthy controls (HCs), were recruited. Patients were required to be

free of antibiotics, chemotherapy drugs and other medications that affect the gut microbiota four weeks before stool collection. Exclusion criteria included active gastrointestinal disease, chronic diarrhea or constipation, history of tumor or autoimmune disease. Participants signed an informed consent form. The study was approved by the Ethics Committee of Ningbo First Hospital and registered at the China Clinical Trials Registry (registration number: ChiCTR2100054354).

Based on the World Health Organization (2016) morphological criteria (17), all 35 DLBCL patients were pathologically consistent with DLBCL and patients were immunohistochemically classified into GCB and non-GCB types according to Han's classification (18). The staging was performed according to disease staging using the Ann Arbor staging system (19) and patient physical status and prognosis information were also assessed using current physical status according to the Eastern Cooperative Oncology Group and IPI scoring systems. Patients were treated with an R-CHOP regimen (rituximab d0, cyclophosphamide d1, doxorubicin d1,vindesine d1, and prednisone d1-5) (20) and chemotherapy dose and frequency schedules were adjusted according to specific conditions. Patients were reviewed by imaging to assess lesions after four cycles of treatment and clinical efficacy was classified as CR and NCR according to international NHL efficacy evaluation standards (21).

2.2 Fecal collection and 16S rDNA analysis

Fecal specimens were collected from patients at onset and also from HCs and stored at -80°C in a sterile preservation tube containing an anti-DNA degradation solution.

Following the manufacturer's instructions, DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, CA, USA). Detection of the isolated DNAs was determined by spectrophotometry (Multiskan™ GO, Thermo Fisher Scientific, USA). The DNA extracts also were checked by 1.5% agarose gel electrophoresis in 1× Tris-Acetate-EDTA buffer. Reaction volumes for polymerase chain reaction (PCR)in a total of 20 µL consisted of 10 µL KAPA HiFi Hot Start Ready Mix (KAPA Biosystems, MA, USA), 2 µL DNA of approximately 30 ng/µL and 1 µL forward and reverse primers of 10 µM. The *16S rDNA* primer sequences were forward 5'-GTGCCAGCMGCCGCGGTAA-3'; reverse 5′-GGACTACNVGGGTWTCTAAT-3′. PCR reaction conditions were configured as required. Products were analyzed using the Qubit 3.0 (Thermo Fisher Scientific, MA, USA) to determine concentrations and then mixed in equal amounts to create a sequencing library. Insert fragments and library molar concentrations were detected and quantified using a QSEP100 (Bioptic, Taiwan) and an ABI7300 fluorescence quantitative PCR instrument (Thermo Fisher Scientific, MA, USA) was used to generate a qualified library for sequencing on the MiniSeq Illumina platform (Illumina, CA, USA). Public access to the original datasets is accessible. This data can be found at https://www.ncbi.nlm.nih.gov/sra/PRJNA906033.

The raw reads were assembled with Flash (22). Primers were removed and clean tags were generated by deleting lower reads with the use of cutadapt (23). The sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using UCHIME in reference mode (24). Representative OTU sequences were aligned in the Silva_132_97_16S database (25) for taxonomic classification using the RDP Classifier (26).

2.3 Microbial community composition and differential abundance - statistical analyses

Alpha-diversity estimates included Shannon, Simpson, ACE, and Chao1 by R vegan package. Sample beta-diversity was assessed by weighted and unweighted UniFrac distances and visualized using Principal Coordinate Analysis (PCoA) plots. These analyses were performed in R v3.4.1. Linear discriminant analysis effect size (LEfSe) analysis was performed on the Galaxy platform (www.huttenhower.sph.harvard.edu/galaxy/).

2.4 Serum cytokine and immunoglobulin measurements

From participants, 5 mL fasting peripheral venous blood was drawn and interleukin 2 (IL-2), IL-4, IL-6, IL-10, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) were measured by enzyme-linked immunosorbent assay (ELISA) (Beckman, CA, USA). Immunoglobulin A (IgA), IgG, and IgM were quantified using an IMMAGE-800 automatic immunochemistry system (Beckman, CA, USA) and accompanying test kits.

2.5 Flow cytometry

A two ml peripheral venous blood sample was collected from each participant. Then, 100 µl was mixed with an antibody solution containing20 µl of each of the following antibodies; CD3-fluorescein isothiocyanate (FITC), CD4-allophycocyanin (APC), CD8-phycoerythrin (PE), CD45-peridinin-chlorophyll protein (PerCP), CD16+56-PE, CD19-APC, CD127-PE and CD25- PE-Cy7 according to manufacturer's instructions. Antibodies were purchased from BD Biosciences (San Jose, CA, USA). Data from 10,000 cells were collected using FACSCanto™ flow cytometry and analyzed using Flowjo 7.6 software.

2.6 Statistical methods

Statistical analysis was performed using SPSS23 software. Patient clinical characteristics in groups were compared using the x^2 test for categorical variables or one-way analysis of variance tests for continuous variables. The t-tests were used for comparisons between groups. Correlations between intestinal microecology and immune indices were analyzed by Spearman's correlation analysis. Differences were considered statistically significant at $P<0.05$.

3 Results

3.1 Baseline characteristics of the study population

Thirty-five patients with DLBCL and 20 HCs were included in this study. In the DLBCL group, there were 21 males and 14 females with a median age of 64 years; nine patients had stage I–II and 26 had stage III–IV disease; 14 cases were GCB and 21 cases were non-GCB type; lesions involved the gastrointestinal tract in nine cases and other extra-nodal organs in 17 cases; lactate dehydrogenase levels were increased in 13 cases and normal in 22 cases; IPI scores were low-intermediate risk in 19 cases and medium-high risk in 16 cases. After four cycles of chemotherapy, 10(29.4%) of 34 evaluable patients achieved CR, 20 (58.8%) achieved partial remission (PR) and 4 (11.7%) had disease progression. The proportion of CR obtained in the mid-term evaluation of this study is slightly lower than the literature data (27), which may be related to the high proportion of patients (74.29%) with high tumor load in stage III-IV among the enrolled patients.

In the HCs group, there were seven males and 13 females with a median age of 57.5 years. There was no statistical difference between these groups in terms of gender and age (Table 1).

TABLE 1 Clinical Characteristics of DLBCL patients and healthy controls (HCs).

Clinical Characteristics	DLBCL (n=35)	HCs(n=20)
Number of patients	35	20
Age (years)	34-86(64)	26-78 (57.5)
Patient's sex (male: female)	21:14	7:13
Clinical staging	–	–
Stage I-II	9	–
Stage III-IV	26	–
Pathological subtype	–	–
GCB	14	–
non-GCB	21	–
Involvement site	–	–
Involvement of the gastrointestinal tract	9	–
Involvement of other extra-nodal organs	17	–
Elevated LDH (≥250u/l)	13	–
IPI Score	–	–
IPI <3 points	19	–
IPI ≥3 points	16	–
Ki-67≥80%	21	–
Bcl-2 positive (≥50%)	27	–
Bcl-6 positive (≥50%)	32	–
c-Myc positive (≥30%)	31	–
CD5-positive	9	–
Efficacy assessment	–	–
CR	10	–
NCR	24	–

LDH, lactate dehydrogenase; GCB, germinal center B-cell-like; CR, complete remission; NCR, non-complete remission; IPI, international prognostic index.

3.2 Altered intestinal microecology in patients with untreated DLBCL

A total of 55 fecal specimens were analyzed by 16SrDNA gene sequencing. 2,360,952 reads were obtained from all samples, and 25,220 reads were obtained from per sample. OUT analysis showed that the DLBCL group had 588 unique OTUs,the HCs group had 177 unique OTUs, and both groups had 1098 identical OTUs (Figure 1A).

The alpha-diversity index of a single-sample can reflect the fecal microbial richness, (ACE and Chao1) and diversity (Shannon and Simpson). Chao1, ACE and Shannon indices were higher in the HCs group when compared with the DLBCL group, but the Simpson index was higher in the DLBCL group, with no statistical differences (P>0.05) (Figure 1B). Beta-diversity analysis showed that intestinal microbial communities of HCs group were richer than that of the DLBCL group (P=0.001) (Figure 1C).

The LEfSe analysis found that compared with the HCs group, *p_Proteobacteria*, *c_Gammaproteobacteria*, *o_Enterobacteriales*, *f_Enterobacteriaceae*, and *g_ Escherichia-Shigella* were higher in relative abundance in the DLBCL group, and *p_Bacteroidetes* was lower in relative abundance in the HCs group when the Linear discriminant analysis (LDA) score cutoff was set to 4.0 (Figures 1D, E).

At the phylum level, *p_Proteobacteria* (P<0.001), *p_Verrucomicrobia* (P=0.04), and *p_Synergistetes* (P=0.03) abundance was significantly higher in the DLBCL group when compared with the HCs group, while *p_Bacteroidetes* (P<0.001) was significantly increased in the HCs group. At the genus level, *g_Escherichia-Shigella* (P<0.001), *g_Veillonella* (P< 0.001), *g_Roseburia* (P=0.004), *g_Lachnoclostridium* (P<0.001), and *g_Alistipes* (P=0.002) were significantly more abundant in the DLBCL group when compared with the HCs group, while the opposite was true for *g_Bacteroides* (P=0.001), *g_Prevotella_9* (P=0.019), and *g_Megamonas* (P=0.026) (Figure 1F).

3.3 The relationship between gut microbiota at DLBCL onset and clinical disease characteristics

Using *Ann Arbor* clinical staging criteria, DLBCL cases were divided into early (I–II) and advanced stage groups (III–IV); no significant differences in alpha- and beta-diversity indices were observed between groups. When differences between two groups were compared at each flora level, it was observed that *p_Firmicutes* (P=0.029), *p_Verrucomicrobia* (P=0.045), *c_Bacilli* (P=0.036), *c_Verrucomicrobiae* (P=0.045), *c_Clostridia* (P=0.032), *o_Clostridiales* (P=0.032), *o_Lactobacillales* (P=0.036), *o_Pasteurellales* (P=0.001),

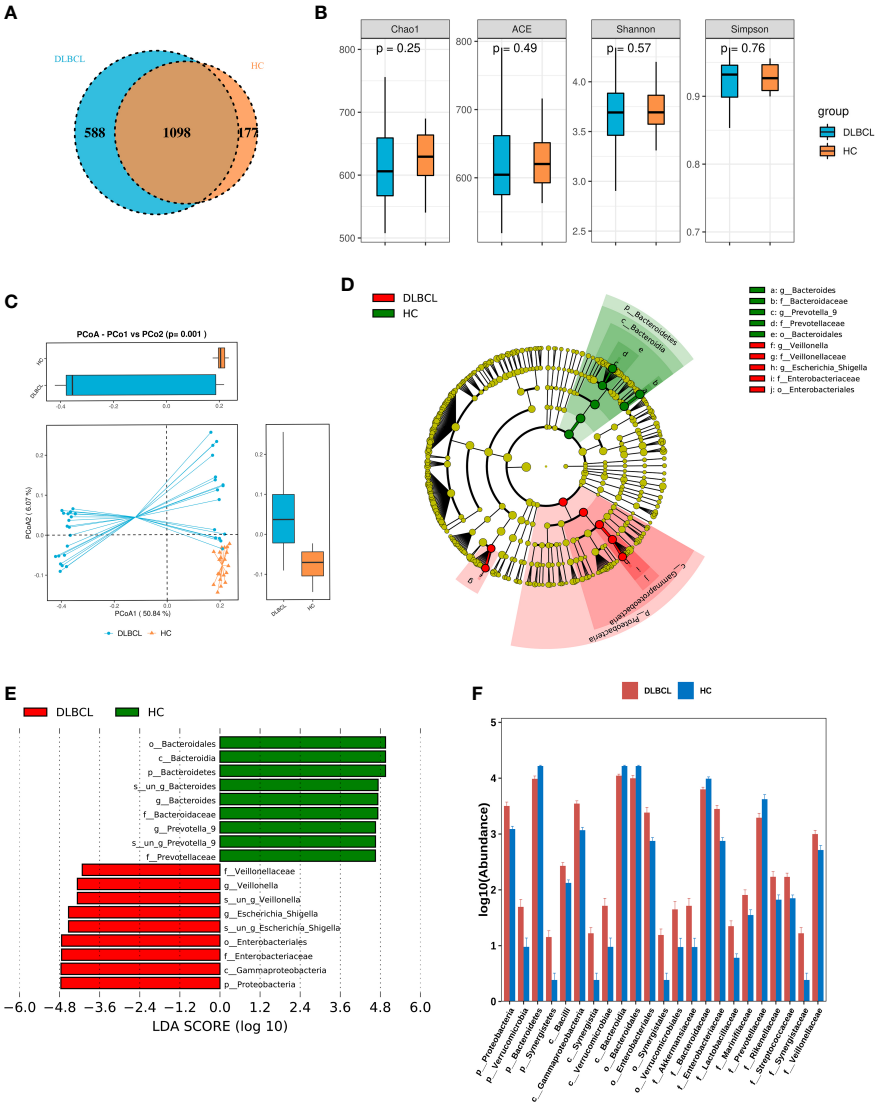

FIGURE 1
Comparing the gut microbiota between patients with untreated diffuse large B-cell lymphoma (DLBCL) patients and healthy controls (HCs). **(A)** Similarity analysis between DLBCL patients and HCs gut microbiota OTUs. **(B)** Alpha-diversity analysis of DLBCL patients and HCs. **(C)** Beta-diversity analysis of DLBCL patients and HCs. **(D)** Evolutionary relationship diagram: circles arranged radially from inside to outside indicate taxonomic levels from phylum to genus. Yellow nodes indicate taxonomic characters not clearly distinguishable between DLBCL patients and HCs, red nodes indicate richer taxonomic types in the DLBCL group, and green nodes represent richer taxonomic types in the HCs. **(E)** LDA displays species with significant differences in abundance between DLBCL patients and HCs. Red bars indicate taxa enriched in the DLBCL group; green bars indicate taxa enriched in the HCs. **(F)** Relative abundance of DLBCL patients and HCs at the phylum level, order, phylum and family levels.

o_Verrucomicrobiales (*P*=0.045), *g_Acidaminococcus* (*P*=0.037), *g_Akkermansia* (*P*=0.045), *g_Haemophilus* (*P*=0.001), *g_Prevotella_2* (*P*=0.048), *g_Pyramidobacter* (*P*=0.019), *g_Ruminococcus_1* (*P*=0.026), and *g_Streptococcus* (*P*=0.008) were all elevated in patients with advanced lymphoma (Figure 2A).

The DLBCL cases were divided into IPI < 3 and IPI ≥ 3 subgroups according to IPI scores and there were no significant differences in alpha- and beta-diversity indices between group IPI < 3 and group IPI ≥ 3. When differences in flora between two groups were compared, *c_Negativicutes* (*P*<0.001), *g_Agathobacter* (*P*<0.001), *g_Lachnospira* (*P*=0.013), *g_Lachnospiraceae_UCG-008* (*P*=0.030), *g_Prevotellaceae_NK3B31_group* (*P*=0.009), *g_Roseburia* (*P*=0.005) and *s_un_g_Roseburia* (*P*=0.005) were elevated in abundance in IPI < 3 individuals (Figure 2B).

From Han typing, DLBCL cases were divided into GCB and non-GCB types and the differences in alpha- and beta-diversity indices between groups were not significant. By comparing differences in flora levels between groups, *f_Enterococcaceae* abundance was elevated in the non-GCB group (*P*=0.04) (Figure 2C).

Based on GI involvement or not, DLBCL cases were divided into GI and NGI groups. No significant differences in alpha- and beta-diversity were identified between groups. By comparing differences between flora levels, we showed that *g_Faecalibacterium* (*P*=0.04), *g_ Hungatella* (*P*=0.019), and *g_*

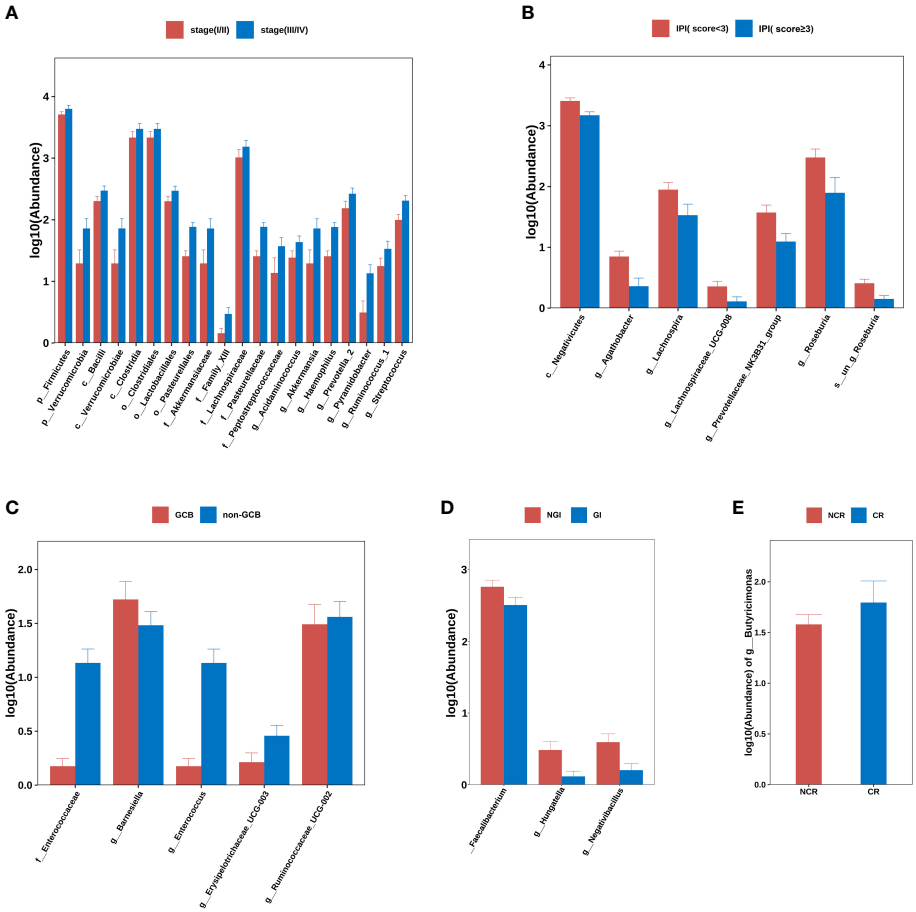

FIGURE 2
Group differences at phylum, order, family, genus, and species levels. **(A)** stage (I/II) group vs. stage (III/IV) group. **(B)** IPI (score<3) group vs. IPI (score≥3) group. **(C)** GCB group vs. non-GCB group. **(D)** NGI group vs. GI group. **(E)** NCR group vs. CR group. Note: GCB, germinal center B-cell-like. NGI, non-gastrointestinal involvement. GI, gastrointestinal involvement.CR, complete remission. NCR, non-complete remission. IPI, international prognostic index.

Negativibacillus (P=0.010) abundance levels were elevated in the GI group (Figure 2D).

By comparing the differences in gut microbiota between the CR and NCR groups before chemotherapy, there were no significant differences in alpha- and beta-diversity indices between two groups, but it was found that there were significant differences in *g_Butyricimonas* abundance in the CR group when compared with the NCR group (P=0.048) (Figure 2E).

3.4 Correlation analyses between gut microbiota and immune levels

The correlation between these differential intestinal microflora closely related to the clinical characteristics of DLBCL and the host immune levelwasinvestigated. It was found that *p_Firmicutes* was positively correlated with absolute lymphocyte values, *g: Prevotella_2 and s:un_g_Prevotella_2* were negatively correlated with absolute lymphocyte values, T cell, and CD4 cell counts, while *g_Pyramidobacter*, *s_un_g_Pyramidobacter*, and *f_Peptostreptococcaceae* were negatively correlated with IgA levels (Figure 3).

4 Discussion

A lot of recent research has revealed a strong relationship between the development of lymphomas and intestinal microecology (28, 29). Using *16S rDNA* gene sequencing of fecal specimens, this study investigated whether intestinal microecology in DLBCL patients was altered. From alpha- and beta-diversity analyses, while gut microbiota alpha-diversity in untreated DLBCL patients was not significantly different when compared with HCs, beta-diversity analysis showed a richer gut microbiota in the HCs group when compared with the DLBCL group. This demonstrated a significant change in intestinal microecology in DLBCL patients.

In the gut microbiota of untreated DLBCL patients, not only was *p_Proteobacteria* abundance significantly higher when compared with HCs, but *c_Gammaproteobacteria*, *o_Enterobacteriales*, *f_Escherobacteriaceae*, and *f_Escherobacteria-Shigella*, which have a continuous evolutionary relationship with

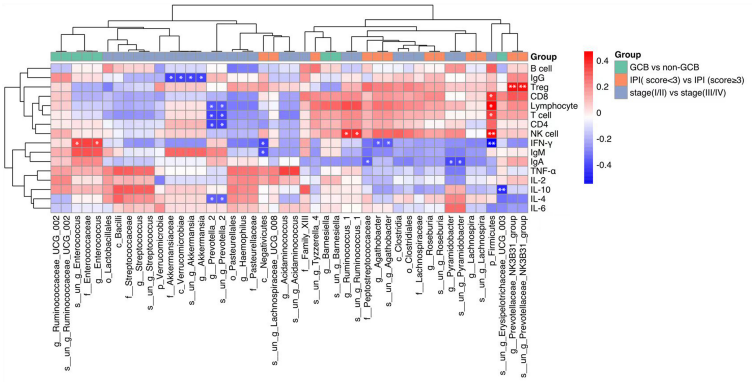

FIGURE 3
Heat map showing Spearman's correlations between differential gut microbiota groups and immune indicators. Red indicates a positive correlation and blue indicates a negative correlation. The darker the color, the larger the correlation coefficient. *P<0.05, **P< 0.01. GCB, germinal center B-cell-like; CR, complete remission; NCR, non-complete remission; IPI, international prognostic index.

p_Proteobacteria, were significantly higher when compared with HCs. *f_*Enterobacteriaceae and *g_Escherichia-Shigella* were significantly higher when compared with the HCs group. These microbiotas produce corresponding proteins to affect cell functions, so 75 proteins were specific to *c_Gammaproteobacteria* or *o_Enterobacteriales*, while four including b0354, b1132, b1179, and b3033 were present only in *c_Gammaproteobacteria.* The functions of these specific proteins may help explain the common physiological or biochemical characteristics of these bacteria and future studies may use the molecular characterization of microorganisms to establish gut microbiota markers in DLBCL patients and lead to earlier disease detection.

The *p_Bacteroidetes* abundance was significantly higher in the HCs group when compared with the untreated DLBCL group. *p_Bacteroidetes*, *c_Bacteroidia*, *o_Bacteroidales*, *f_Bacteroidaceae*, *g_Bacteroides*, and *s_un_g_Bacteroides* also demonstrated a continuous evolutionary link at six levels (phylum, class, order, family, genus, and species). It was previously shown that increased *p_Bacteroidetes* levels were closely associated with higher butyrate production (30). Butyrate is a short-chain fatty acid (SCFA) that is produced in the intestine by the bacterial fermentation of dietary fiber (31) and these SCFAs regulate intestinal inflammation by modulating intestinal Treg cell numbers and functions (32, 33). Butyric acid has also been associated with anti-cancer activity in several human tumor cell lines and has acted as a histone deacetylase (HDAC) inhibitor preventing colorectal cancer development (34). A previous study investigated the effects of high-fiber diets on lymphoma and found that butyrate enhanced histone acetylation and pro-apoptotic gene expression, inhibiting lymphoma cell proliferation and apoptosis (35). Additionally, *g_Bacteroides*, as an intestinal commensal bacteria, protects the intestine from pathogens by producing mucin-type O-glycans (36), so it is suggested that increased *p_Bacteroidetes* abundance helps protect the gastrointestinal tract, reduce inflammation and combats lymphoma.

Clinical characteristics such as disease stage, IPI score, and cell origin at disease onset are closely related to disease prognosis (2–4), so the relationships between intestinal microecological alterations at disease onset and clinical features were analyzed to identify associations between gut microbiota, disease onset, disease characteristics and prognosis.

In terms of disease staging, although no significant differences in alpha- and beta-diversity indices were identified between early and advanced groups, among the groups with significantly higher abundance in DLBCL patients when compared with HCs were: *p_Verrucomicrobia*, *c_Bacilli*, *c_Verrucomicrobiae*, *o_Verrucomicrobiales*, *g_Acidaminococcus*, *g_Akkermansia*, *g_Pyramidobacter*, and *g_Streptococcus*; all were elevated in patients with advanced lymphoma, suggesting these floraemay predict a more aggressive clinical course and a worse prognosis for lymphoma.

The organism *Akkermansia muciniphila* is a highly-important and representative human intestinal *p_Verrucomicrobia*, which improves host metabolism and modulates immune responses and is considered a highly promising novel probiotic agent (37). Much evidence now suggests that *A. muciniphila* reductions are associated with several diseases, including obesity, non-alcoholic fatty liver disease, type 2 diabetes, and cardiovascular diseases (38, 39). This bacterium is also believed to enhance antitumor activity and its increased abundance is putatively associated with the reduced incidence of some cancers and the improved efficacy of immune checkpoint inhibitors (40, 41). *A. muciniphila* appears to exert anti-inflammatory effects by suppressing HDACs *via* SCFA production, down-regulating toll-like receptor 4 (TLR4) expression *via* secreted vesicles which regulate the nuclear factor-κB (NF-κB) pathway, and thus inhibiting inflammatory factor release (42). However, *A. muciniphila* itself increases TLR4 expression and elevates cytokines such as IL-6 (42, 43) and was positively associated with IL-6 in a gut microbiota study in elderly debilitated individuals (44). Critically, the IL-6 signaling pathway is a negative predictor in aggressive DLBCL (45), so it is hypothesized that *g_Akkermansia* may increase IL-6 levels and predict a greater tumor load in DLBCL patients, and in this study, the bacterium was elevated in DLBCL patients, especially in those with advanced DLBCL, however it is not possible to infer a causal relationship between the two. The relationship between *Akkermansia* and lymphoma requires more animal studies and clinical trials.

The bacterium *g_Streptococcus* is another that was elevated in this study in patients with advanced lymphoma, and is closely related to colon cancer (46, 47), but its pathogenic mechanisms are unclear. In a study exploring colon cancer and *Streptococcus bovis*, it was found that *S. bovis* promoted proliferation and IL-8 production and promoted precancerous lesion progression in human colorectal adenocarcinoma epithelial cells (48). An *S. bovis* abundance in the intestine of patients with chronic kidney disease may also suggest that renal damage is associated with streptococcal-mediated immune disorders (49). Although *g_Streptococcus* was more abundant in patients with advanced disease when compared with patients with early disease in this study, it remains unclear if the bacterium is involved in DLBCL development and progression, thus further studies are needed.

No significant difference in alpha- and beta-diversity indices was observed between *IPI scores in* not-high-risk and high-risk groups, while comparisons between groups at each taxonomic rank showed that *c_Negativicutes*, *g_Agathobacter*, *g_Lachnospira*, *g_Lachnospiraceae_UCG-008*, *g_Prevotellaceae_ NK3B31_group*, *g_Roseburia*, and *s_un_g_Roseburia* were elevated in IPI< 3individuals. Studies have confirmed that *g_Roseburia* was more closely associated with controlling intestinal inflammation, reducing atherosclerosis, and promoting immune system maturation due to major SCFA production, of which butyrate has a major mediating role (50). *f_Lachnospiraceae* hydrolyzes starch and other sugars to produce butyrate and other SCFAs, thereby promoting immune system maturation (51). Martini et al. (52) extensively analyzed the fecal microbiota in patients with advanced malignancy and found that those with high *Agathobacter* abundance had better progression-free survival, which may be associated with butyrate production. In this study, *g_Roseburia, f_Lachnospiraceae*, and *Agathobacter* flora were elevated in non-high-risk individuals with IPI; these bacteria produce butyrate, therefore it is speculated that they may inhibit lymphoma progression *via* SCFA production.

By comparing differences between GCB and non-GCB groups at all flora levels, *f_Enterococcaceae* abundance was significantly higher in patients with non-GCB. Recently, it was reported that *Enterococcus faecalis* translocated endotoxin to the liver and increased the expression of liver proliferation genes, which are dependent on TLR4-MYD88 signaling, promoting hepato-carcinogenesis (53)The MYD88 activates NF-κB signaling in response to TLR stimulation and also IL-1 and IL-18 receptors and MYD88 mutations are believed to be closely associated with non-GCB type DLBCL development (54). It is therefore hypothesized that *f_Enterococcaceae* may be involved in lymphoma development by acting on MYD88 and activating NF-κB signaling.

Microorganisms are closely associated with the pathogenesis of malignant lymphomas of the gastrointestinal tract. The relationship between *Helicobacter pylori* infection and gastric mucosa-associated lymphoid tissue lymphoma has been established (55), and altered microbiota composition in patients with gastrointestinal follicular lymphoma has also been demonstrated (56). In the present study, there was no significant difference in microbiota composition was observed between the GI and NGI DLBCL patient groups as assessed by diversity indicators. The most well-known species of *g_ Faecalibacterium* is *Faecalibacterium prausnitzii*, which is one of the most important commensal bacteria in the human intestine and one of the main producers of butyrate (57). *F. prausnitzii* and its metabolites can exert an anti-inflammatory effect against colitis in mice, improve intestinal flora dysbiosis, and have the potential to treat inflammatory bowel disease (58, 59). Colorectal cancer patients have fewer butyrate-producing bacteria, including *F. prausnitzii*, and it has been suggested that due to lower butyrate production, the epithelial cell may be more susceptible to being damaged, which may increase the risk of developing cancer (57, 58). In the present study, the abundance of this bacterium was reduced in DLBCL patients with GI, suggesting that the reduction of this bacteria may cause a decrease in intestinal antitumor effects as well as gastrointestinal involvement of lymphoma. However, our study had some limitations due to the small sample size, so further validation in other studies is required.

Several studies have shown the prognostic value of interim [(18)F]fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) and can guide subsequent treatment decisions to some extent (27, 60), so patients with PET-CT were evaluated after four cycles of chemotherapy and DLBCL cases divided into CR and NCR group. By comparing the differences in gut microbiota between the two groups before chemotherapy, it was found that higher *g_Butyricimonas* abundance was putatively associated with DLBCL remission after chemotherapy, so it is speculated that the abundance of *g_Butyricimonas* in untreated DLBCL patients could potentially be used as a surrogate predictor of interim outcome and prognosis. Previously, *g_Butyricimonas* occurred in higher abundance in NHL patients at low risk of bloodstream infection (61) and, as SCFA-producing genera, it may influence patient prognoses by modulating the immune system and inflammatory responses (62) and this observation concurred with these findings.

From these analyses, gut microbiota characteristics were identified which were associated with key clinical features such as tumor load, disease risk stratification and cell origin, but it is unclear if these species, with significant effects, affected patient immune functions and disease progression. Several host-microbial interaction studies have shown that relationships between the gut microbiota and the body are mutual and symbiotic. The relationship improves host metabolic capacity, immunoglobulin levels in and outside the gut, and helps regulate intestinal mucosal immunity to some extent. A wide variety of microorganisms in the gut produce antitumor substances that remove malignant proliferating cells before they form tumors (63). The gut microbiota can also influence the activity of the cytotoxic T lymphocyte-associated antigen-4 or programmed death (PD)-1/PD ligand (PD-L) 1 axis by affecting anti-cancer immuno-surveillance and thus immuno-checkpoint inhibitor therapy efficacy (64). Sivan et al. reported that the combination of oral *Bifidobacterium* with PD-L1 virtually eliminated tumor growth. Previous studies have shown that microbial immune interactions may affect tumor occurrence, development, and prognosis, but in patients with lymphoma, it is unclear if disease related intestinal microecological variations are closely related to host immunity, so correlations between differential flora and host immune indices were investigated. Patients with

advanced-stage DLBCL tended to have a higher abundance of *g_Prevotella_2* and *s_un_g_Prevotella_2*. This abundance is often related to lower absolute counts of total lymphocytes, CD3+T lymphocytes, and CD4+T cells, so it was hypothesized that *g_Prevotella_2* and *s_un_g_Prevotella_2* may contribute to lymphoma development by reducing CD4+ Th cell and lymphocyte numbers, weakening the host's anti-lymphoma immunity. *Firmicutes* abundance was positively correlated with absolute lymphocyte counts and it was previously reported that absolute lymphocyte counts in lymphoma patients were closely related to patient prognosis (65). However, this study showed that high *Firmicutes* abundance correlated with disease progression, which suggested that *Firmicutes*, as a pro-inflammatory gut microbiota, did not produce effective anti-lymphoma immune responses although it increased lymphocyte numbers, so *Firmicutes* may be involved in immune depletion associated with chronic inflammation.

The most abundant type of antibody, IgA, serves as the first line of defense for the immune system on mucosal surfaces. Recent studies suggested that changes in microbiota diversity could modulate the IgA-microbiota axis and affect antitumor immunity by altering cancer development risks and modulating responses to immunotherapy. IgA exerts antitumor or pro-tumor effects on different tumor types and may be influenced by tumor type, environmental, and host factors (66). However, the role of IgA in lymphoma is unclear. In this study, *f_Peptostreptococcaceae*, *g_Pyramidobacter* and *s_un_g_Pyramidobacter* showed negative correlations with IgA, while high *g_Pyramidobacter* abundance was associated with a more advanced disease stage, suggesting this bacterium may affect anti-tumor immunity *via* IgA. Studies should be conducted to disseminate its role in anti-lymphoma immunity.

5 Conclusions

This study described gut microbiota characteristics in *de novo* DLBCL patients for the first time and identified flora markers closely related to patient clinical characteristics. These markers included disease stage, cell source, IPI risk stratification, and immunochemotherapy responses. It also showed correlations between structural flora variations and host immunity, and provided new insights into the intestinal microecology immune axis during DLBCL development. However, further work is required to uncover relationships between intestinal microecology and DLBCL development. In future studies, the specific mechanisms of differential and dominant flora development in DLBCL must be characterized to determine new disease biomarkers and develop new therapeutic strategies.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA906033.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LS and YZ obtained funding. ZL and DM drafted the manuscript. ZL and CJ made the figures and tables. DM, JW, YZ and PZ analysed data. CJ, MZ, QG and YS collected and processed specimens. KX, YW, BL, HW collected clinical data. GO, HZ and LS designed the study. LS and GO revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1105293/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A) Alpha-diversity analysis of stage (I/II) and stage (III/IV). **(B)** Beta-diversity analysis of stage (I/II) and stage (III/IV).

SUPPLEMENTARY FIGURE 2
(A) Alpha-diversity analysis of IPI (score<3) and IPI (score≥3). **(B)** Beta-diversity analysis of IPI (score<3) and IPI (score≥3).

SUPPLEMENTARY FIGURE 3
(A) Alpha-diversity analysis of GCB and non-GCB. **(B)** Beta-diversity analysis of GCB and non-GCB.

SUPPLEMENTARY FIGURE 4
(A) Alpha-diversity analysis of NGI and GI. **(B)** Beta-diversity analysis of NGI and GI.

SUPPLEMENTARY FIGURE 5
(A) Alpha-diversity analysis of NCR and CR. **(B)** Beta-diversity analysis of NCR and CR.

References

1. Zelenetz AD, Gordon LI, Abramson JS, Advani RH, Bartlett NL, Caimi PF, et al. NCCN guidelines insights: B-cell lymphomas, version 3.2019. *J Natl Compr Canc Netw* (2019) 17(6):650–61. doi: 10.6004/jnccn.2019.0029

2. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, et al. Molecular subtypes of diffuse large b cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* (2018) 24(5):679–90. doi: 10.1038/s41591-018-0016-8

3. Ziepert M, Hasenclever D, Kuhnt E, Glass B, Schmitz N, Pfreundschuh M, et al. Standard international prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ b-cell lymphoma in the rituximab era. *J Clin oncol: Off J Am Soc Clin Oncol* (2010) 28(14):2373–80. doi: 10.1200/JCO.2009.26.2493

4. Susanibar-Adaniya S, Barta SK. Update on diffuse large b cell lymphoma: A review of current data and potential applications on risk stratification and management. *Am J Hematol* (2021) 96(5):617–29. doi: 10.1002/ajh.26151

5. Moeller AH. Loyal gut microbes. *Sci (New York NY).* (2022) 377(6612):1263–4. doi: 10.1126/science.ade2879

6. Lee M, Chang EB. Inflammatory bowel diseases (IBD) and the microbiome-searching the crime scene for clues. *Gastroenterology* (2021) 160(2):524–37. doi: 10.1053/j.gastro.2020.09.056

7. Wu H, Tremaroli V, Schmidt C, Lundqvist A, Olsson LM, Krämer M, et al. The gut microbiota in prediabetes and diabetes: A population-based cross-sectional study. *Cell Metab* (2020) 32(3):379–90.e3. doi: 10.1016/j.cmet.2020.06.011

8. Rodriguez J, Hiel S, Neyrinck AM, Le Roy T, Pötgens SA, Leyrolle Q, et al. Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients. *Gut* (2020) 69(11):1975–87. doi: 10.1136/gutjnl-2019-319726

9. Ramírez-Macías I, Orenes-Piñero E, Camelo-Castillo A, Rivera-Caravaca JM, López-García C, Marín F. Novel insights in the relationship of gut microbiota and coronary artery diseases. *Crit Rev Food Sci Nutr* (2022) 62(14):3738–50. doi: 10.1080/10408398.2020.1868397

10. Liu X, Chen Y, Zhang S, Dong L. Gut microbiota-mediated immunomodulation in tumor. *J Exp Clin Cancer Res* (2021) 40(1):221. doi: 10.1186/s13046-021-01983-x

11. Park EM, Chelvanambi M, Bhutiani N, Kroemer G, Zitvogel L, Wargo JA. Targeting the gut and tumor microbiota in cancer. *Nat Med* (2022) 28(4):690–703. doi: 10.1038/s41591-022-01779-2

12. Wang L, Zhu L, Qin S. Gut microbiota modulation on intestinal mucosal adaptive immunity. *J Immunol Res* (2019) 2019:4735040. doi: 10.1155/2019/4735040

13. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. *Nature* (2016) 535(7610):85–93. doi: 10.1038/nature18849

14. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Sci (New York NY).* (2001) 291(5505):881–4. doi: 10.1126/science.291.5505.881

15. Lu H, Xu X, Fu D, Gu Y, Fan R, Yi H, et al. Butyrate-producing eubacterium rectale suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF-κB axis. *Cell Host Microbe* (2022) 30(8):1139–50.e7. doi: 10.1016/j.chom.2022.07.003

16. Yuan L, Wang W, Zhang W, Zhang Y, Wei C, Li J, et al. Gut microbiota in untreated diffuse Large b cell lymphoma patients. *Front Microbiol* (2021) 12:646361. doi: 10.3389/fmicb.2021.646361

17. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the world health organization classification of lymphoid neoplasms. *Blood* (2016) 127(20):2375–90. doi: 10.1182/blood-2016-01-643569

18. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large b-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* (2004) 103(1):275–82. doi: 10.1182/blood-2003-05-1545

19. Lister TA, Crowther D, Sutcliffe SB, Glatstein E, Canellos GP, Young RC, et al. Report of a committee convened to discuss the evaluation and staging of patients with hodgkin's disease: Cotswolds meeting. *J Clin Oncol* (1989) 7(11):1630–6. doi: 10.1200/JCO.1989.7.11.1630

20. He MY, Kridel R. Treatment resistance in diffuse large b-cell lymphoma. *Leukemia* (2021) 35(8):2151–65. doi: 10.1038/s41375-021-01285-3

21. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the lugano classification. *J Clin Oncol* (2014) 32(27):3059–68. doi: 10.1200/JCO.2013.54.8800

22. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* (2011) 27(21):2957–63. doi: 10.1093/bioinformatics/btr507

23. Kechin A, Boyarskikh U, Kel A, Filipenko M. cutPrimers: A new tool for accurate cutting of primers from reads of targeted next generation sequencing. *J Comput Biol* (2017) 24(11):1138–43. doi: 10.1089/cmb.2017.0096

24. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* (2010) 26(19):2460–1. doi: 10.1093/bioinformatics/btq461

25. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* (2006) 72(7):5069–72. doi: 10.1128/AEM.03006-05

26. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* (2014) 42(Database issue):D633–42. doi: 10.1093/nar/gkt1244

27. Safar V, Dupuis J, Itti E, Jardin F, Fruchart C, Bardet S, et al. Interim [18F] fluorodeoxyglucose positron emission tomography scan in diffuse large b-cell lymphoma treated with anthracycline-based chemotherapy plus rituximab. *J Clin oncol: Off J Am Soc Clin Oncol* (2012) 30(2):184–90. doi: 10.1200/JCO.2011.38.2648

28. Uribe-Herranz M, Klein-González N, Rodríguez-Lobato LG, Juan M, de Larrea CF. Gut microbiota influence in hematological malignancies: From genesis to cure. *Int J Mol Sci* (2021) 22(3). doi: 10.3390/ijms22031026

29. Diefenbach CS, Peters BA, Li H, Raphael B, Moskovits T, Hymes K, et al. Microbial dysbiosis is associated with aggressive histology and adverse clinical outcome in b-cell non-Hodgkin lymphoma. *Blood Adv* (2021) 5(5):1194–8. doi: 10.1182/bloodadvances.2020003129

30. Effenberger M, Reider S, Waschina S, Bronowski C, Enrich B, Adolph TE, et al. Microbial butyrate synthesis indicates therapeutic efficacy of azathioprine in IBD patients. *J Crohn's colitis.* (2021) 15(1):88–98. doi: 10.1093/ecco-jcc/jjaa152

31. Martin-Gallausiaux C, Marinelli L, Blottière HM, Larraufie P, Lapaque N. SCFA: mechanisms and functional importance in the gut. *Proc Nutr Soc* (2021) 80(1):37–49. doi: 10.1017/S0029665120006916

32. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic treg cell homeostasis. *Sci (New York NY).* (2013) 341(6145):569–73. doi: 10.1126/science.1241165

33. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, et al. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat Commun* (2020) 11(1):4457. doi: 10.1038/s41467-020-18262-6

34. Mirzaei R, Afaghi A, Babakhani S, Sohrabi MR, Hosseini-Fard SR, Babolhavaeji K, et al. Role of microbiota-derived short-chain fatty acids in cancer development and prevention. *Biomed pharmacother* (2021) 139:111619. doi: 10.1016/j.biopha.2021.111619

35. Wei W, Sun W, Yu S, Yang Y, Ai L. Butyrate production from high-fiber diet protects against lymphoma tumor. *Leuk Lymphoma.* (2016) 57(10):2401–8. doi: 10.3109/10428194.2016.1144879

36. Zafar H, Saier MHJr. Gut bacteroides species in health and disease. *Gut Microbes* (2021) 13(1):1–20. doi: 10.1080/19490976.2020.1848158

37. Cani PD, Depommier C, Derrien M, Everard A, de Vos WM. Akkermansia muciniphila: paradigm for next-generation beneficial microorganisms. *Nat Rev Gastroenterol hepatol* (2022) 19(10):625–37. doi: 10.1038/s41575-022-00631-9

38. Rodrigues VF, Elias-Oliveira J, Pereira ÍS, Pereira JA, Barbosa SC, Machado MSG, et al. Akkermansia muciniphila and gut immune system: A good friendship that attenuates inflammatory bowel disease, obesity, and diabetes. *Front Immunol* (2022) 13:934695. doi: 10.3389/fimmu.2022.934695

39. Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat Med* (2019) 25(7):1096–103. doi: 10.1038/s41591-019-0495-2

40. Wang L, Tang L, Feng Y, Zhao S, Han M, Zhang C, et al. A purified membrane protein from akkermansia muciniphila or the pasteurised bacterium blunts colitis

associated tumourigenesis by modulation of CD8(+) T cells in mice. *Gut* (2020) 69 (11):1988–97. doi: 10.1136/gutjnl-2019-320105

41. Li L, McAllister F. Too much water drowned the miller: Akkermansia determines immunotherapy responses. *Cell Rep Med* (2022) 3(5):100642. doi: 10.1016/j.xcrm.2022.100642

42. Ashrafian F, Behrouzi A, Shahriary A, Ahmadi Badi S, Davari M, Khatami S, et al. Comparative study of effect of akkermansia muciniphila and its extracellular vesicles on toll-like receptors and tight junction. *Gastroenterol Hepatol bed to bench.* (2019) 12(2):163–8.

43. Ottman N, Reunanen J, Meijerink M, Pietilä TE, Kainulainen V, Klievink J, et al. Pili-like proteins of akkermansia muciniphila modulate host immune responses and gut barrier function. *PloS One* (2017) 12(3):e0173004. doi: 10.1371/journal.pone.0173004

44. Xu Y, Wang Y, Li H, Dai Y, Chen D, Wang M, et al. Altered fecal microbiota composition in older adults with frailty. *Front Cell infect Microbiol* (2021) 11:696186. doi: 10.3389/fcimb.2021.696186

45. Hashwah H, Bertram K, Stirm K, Stelling A, Wu CT, Kasser S, et al. The IL-6 signaling complex is a critical driver, negative prognostic factor, and therapeutic target in diffuse large b-cell lymphoma. *EMBO Mol Med* (2019) 11(10):e10576. doi: 10.15252/emmm.201910576

46. Zhou CB, Pan SY, Jin P, Deng JW, Xue JH, Ma XY, et al. Fecal signatures of streptococcus anginosus and streptococcus constellatus for noninvasive screening and early warning of gastric cancer. *Gastroenterology* (2022) 162(7):1933–47.e18. doi: 10.1053/j.gastro.2022.02.015

47. Yang Y, Du L, Shi D, Kong C, Liu J, Liu G, et al. Dysbiosis of human gut microbiome in young-onset colorectal cancer. *Nat Commun* (2021) 12(1):6757. doi: 10.1038/s41467-021-27112-y

48. Ellmerich S, Schöller M, Duranton B, Gossé F, Galluser M, Klein JP, et al. Promotion of intestinal carcinogenesis by streptococcus bovis. *Carcinogenesis* (2000) 21 (4):753–6. doi: 10.1093/carcin/21.4.753

49. Felizardo RJF, de Almeida DC, Pereira RL, Watanabe IKM, Doimo NTS, Ribeiro WR, et al. Gut microbial metabolite butyrate protects against proteinuric kidney disease through epigenetic- and GPR109a-mediated mechanisms. *FASEB J* (2019) 33 (11):11894–908. doi: 10.1096/fj.201901080R

50. Nie K, Ma K, Luo W, Shen Z, Yang Z, Xiao M, et al. Roseburia intestinalis: A beneficial gut organism from the discoveries in genus and species. *Front Cell infect Microbiol* (2021) 11:757718. doi: 10.3389/fcimb.2021.757718

51. Wong J, Piceno YM, DeSantis TZ, Pahl M, Andersen GL, Vaziri ND. Expansion of urease- and uricase-containing, indole- and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am J nephrol* (2014) 39 (3):230–7. doi: 10.1159/000360010

52. Martini G, Ciardiello D, Dallio M, Famiglietti V, Esposito L, Corte CMD, et al. Gut microbiota correlates with antitumor activity in patients with mCRC and NSCLC treated with cetuximab plus avelumab. *Int J Cancer.* (2022) 151(3):473–80. doi: 10.1002/ijc.34033

53. Ray KE. Faecalis promotes liver carcinogenesis. *Nat Rev Gastroenterol hepatol* (2021) 18(12):832–3. doi: 10.1038/s41575-021-00540-3

54. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature* (2011) 470(7332):115–9. doi: 10.1038/nature09671

55. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary b-cell gastric lymphoma. *Lancet (London England).* (1991) 338(8776):1175–6. doi: 10.1016/0140-6736(91)92035-Z

56. Zeze K, Hirano A, Torisu T, Esaki M, Shibata H, Moriyama T, et al. Mucosal dysbiosis in patients with gastrointestinal follicular lymphoma. *Hematol Oncol* (2020) 38(2):181–8. doi: 10.1002/hon.2717

57. Balamurugan R, Rajendiran E, George S, Samuel GV, Ramakrishna BS. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, desulfovibrio and enterococcus faecalis in the feces of patients with colorectal cancer. *J Gastroenterol Hepatol* (2008) 23(8 Pt 1)::1298–303. doi: 10.1111/j.1440-1746.2008.05490.x

58. Lopez-Siles M, Martinez-Medina M, Surís-Valls R, Aldeguer X, Sabat-Mir M, Duncan SH, et al. Changes in the abundance of faecalibacterium prausnitzii phylogroups I and II in the intestinal mucosa of inflammatory bowel disease and patients with colorectal cancer. *Inflamm bowel dis* (2016) 22(1):28–41. doi: 10.1097/MIB.0000000000000590

59. Martín R, Miquel S, Chain F, Natividad JM, Jury J, Lu J, et al. Faecalibacterium prausnitzii prevents physiological damages in a chronic low-grade inflammation murine model. *BMC Microbiol* (2015) 15:67. doi: 10.1186/s12866-015-0400-1

60. Yang DH, Min JJ, Song HC, Jeong YY, Chung WK, Bae SY, et al. Prognostic significance of interim ^{18}F-FDG PET/CT after three or four cycles of r-CHOP chemotherapy in the treatment of diffuse large b-cell lymphoma. *Eur J Cancer.* (2011) 47(9):1312–8. doi: 10.1016/j.ejca.2010.12.027

61. Montassier E, Al-Ghalith GA, Ward T, Corvec S, Gastinne T, Potel G, et al. Pretreatment gut microbiome predicts chemotherapy-related bloodstream infection. *Genome Med* (2016) 8(1):49. doi: 10.1186/s13073-016-0301-4

62. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* (2016) 7(3):189–200. doi: 10.1080/19490976.2015.1134082

63. Zhou YJ, Zhao DD, Liu H, Chen HT, Li JJ, Mu XQ, et al. Cancer killers in the human gut microbiota: diverse phylogeny and broad spectra. *Oncotarget* (2017) 8 (30):49574–91. doi: 10.18632/oncotarget.17319

64. Routy B, Gopalakrishnan V, Daillère R, Zitvogel L, Wargo JA, Kroemer G. The gut microbiota influences anticancer immunosurveillance and general health. *Nat Rev Clin Oncol* (2018) 15(6):382–96. doi: 10.1038/s41571-018-0006-2

65. Mohsen A, Taalab M, Abousamra N, Mabed M. Prognostic significance of absolute lymphocyte count, absolute monocyte count, and absolute lymphocyte count to absolute monocyte count ratio in follicular non-Hodgkin lymphoma. *Clin lymphoma myeloma leuk* (2020) 20(9):e606–e15. doi: 10.1016/j.clml.2020.03.007

66. Zhong Z, Nan K, Weng M, Yue Y, Zhou W, Wang Z, et al. Pro- and anti- effects of immunoglobulin a- producing b cell in tumors and its triggers. *Front Immunol* (2021) 12:765044. doi: 10.3389/fimmu.2021.765044

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Gut microbiota composition in patients with advanced malignancies experiencing immune-related adverse events

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Introduction: The gut microbiota is implicated in the occurrence and severity of immune-related adverse events (irAEs), but the role it plays as well as its causal relationship with irAEs has yet to be established.

Methods: From May 2020 to August 2021, 93 fecal samples were prospectively collected from 37 patients with advanced thoracic cancers treated with anti-PD-1 therapy, and 61 samples were collected from 33 patients with various cancers developing different irAEs. 16S rDNA amplicon sequencing was performed. Antibiotic-treated mice underwent fecal microbiota transplantation (FMT) with samples from patients with and without colitic irAEs.

Results: Microbiota composition was significantly different in patients with and without irAEs (P=0.001) and with and without colitic-type irAEs (*P*=0.003). *Bifidobacterium*, *Faecalibacterium*, and *Agathobacter* were less abundant and *Erysipelatoclostridium* more abundant in irAE patients, while *Bacteroides* and *Bifidobacterium* were less abundant and *Enterococcus* more abundant in colitis-type irAE patients. Major butyrate-producing bacteria were also less abundant in patients with irAEs than those without (P=0.007) and in colitic vs. non-colitic irAE patients (*P*=0.018). An irAE prediction model had an AUC of 86.4% in training and 91.7% in testing. Immune-related colitis was more common in colitic-irAE-FMT (3/9) than non-irAE-FMT mice (0/9).

Conclusions: The gut microbiota is important in dictating irAE occurrence and type, especially for immune-related colitis, possibly by modulating metabolic pathways.

KEYWORDS

gut microbiome, immune-related adverse events, immune-related colitis, metabolic pathways, anti-PD-1 therapy

1 Introduction

Immune checkpoint inhibitors (ICIs) have become the "fourth pillar" of cancer management. ICIs have improved survival outcomes for patients with various types of cancer, including advanced lung cancer (1), which is the leading cause of cancer death worldwide (2). However, some patients taking immunotherapy develop immune-related adverse events (irAEs), ultimately limiting the full clinical application and potential of immunotherapy (3, 4). IrAEs represent a special class of toxicity caused by immune system overactivation induced by ICIs, leading to their temporary or permanent discontinuation and consequent life-threating tumor progression. There are still no sufficiently specific nor sensitive biomarkers to predict irAE occurrence.

Recently, the gut microbiota has been shown to play a role in shaping ICI efficacy. Baseline microbiota composition has been reported to be different in responders and non-responders to ICIs in various cancers, and fecal microbiota transplantation (FMT) from ICI responders improves ICI efficacy both preclinically (5–7) and clinically (8, 9). A recent clinical trial reported increased response rates to ICIs in metastatic renal carcinoma patients after taking bifidogenic probiotics (10). The microbiota may also influence irAE occurrence, and a few studies have now explored baseline gut microbiota differences in patients with and without colitic irAEs. Indeed, some specific intestinal bacteria appear to distinguish patients without or with mild irAEs from those with severe irAEs (11–15). Moreover, Wang et al. reported two cases of refractory colitic irAE successfully treated with FMT from healthy donors (16). However, most of these studies remain limited to the study of colitic irAEs in melanoma patients.

To broaden our knowledge of the relationship between the gut microbiota, ICI response, and irAE occurrence, here we studied the gut microbiota of patients experiencing a spectrum of irAEs in different cancers, especially lung cancer. We first assessed changes in microbiota composition before and after anti-PD-1 therapy and irAE treatment. Then, we analyzed differences in microbiota composition in patients: (i) with and without irAEs; (ii) with irAEs of different severity; and (iii) with colitic and non-colitic irAEs. To explore the underlying mechanisms, we investigated differences in predicted microbiota function and butyrate production in different patient subgroups. Furthermore, we performed patient-to-mouse FMT experiments to explore the causal relationship between microbiota composition and immune-related colitis.

2 Methods

2.1 Patient enrollment

Fifty patients were consecutively enrolled into the anti-PD-1-treated lung cancer cohort between May 2020 and August 2021 at the Lung Cancer Center, Peking Union Medical College Hospital, Beijing, China according to the following inclusion criteria: (i) aged 18-75 years; (ii) advanced thoracic cancers; and (iii) initially treated with anti-PD-1 therapy. Thirteen patients were excluded due to unblinding of placebo recipients or for other reasons. Patients with irAEs were simultaneously consecutively enrolled according to the inclusion criteria: (i) aged 18-75 years; (ii) diagnosed with an advanced malignant tumor; and (iii) developed irAEs after anti-PD-1 therapy. For both groups, exclusion criteria were: (i) patients with unstable vital signs; and (ii) patients exposed to antibiotics and/or probiotics within four weeks of enrolment.

The Ethical Committee of Peking Union Medical College Hospital approved the study protocol (No. ZS-3037). Written informed consent was obtained from all patients. This clinical study was registered on the Chinese Clinical Trial Register (ChiCTR-2000032088).

2.2 Clinical and demographic information collection

Demographic and clinical information including gender, age, tumor type, tumor stage, previous treatment, evaluation of treatment efficacy, progression-free survival (PFS), irAE type, and irAE grade were collected. Treatment efficacy was assessed according to RECIST version 1.1 criteria (17). PFS was defined as time from initiation of ICI therapy to first clinical and/or radiographically confirmed progression. IrAEs were monitored from the initiation of ICI therapy to August 31, 2021. IrAEs were diagnosed according to National Comprehensive Cancer Network (NCCN) guidelines on ICI-related toxicities without restrictions as to the involved organs (18). All clinical data were collected by experienced physicians.

2.3 Intestinal microbiota analysis

For patients started on anti-PD-1 therapy, fresh fecal samples were prospectively collected before and after ICI therapy. For patients with irAEs, fresh fecal samples were collected before irAE treatment, after irAE treatment, and after resuming ICIs. All fecal samples were stored in the Clinical Biobank, Medical Research Center, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences.

Fecal samples were subjected to microbiota analysis by 16S rDNA amplicon sequencing on the Illumina MiSeq (PE300) sequencing platform (Illumina, San Diego, CA) (19). Operational taxonomic units (OTUs) were detected with QIIME2 (20) and grouped according to phylum and genus. Differences in microbiota composition were compared according to relative abundance of these two levels. α-diversity was assessed according to the observed species, Shannon index, and Chao1 index, while β-diversity was assessed by Bray-Curtis and weighted UniFrac distances. Bray-Curtis distances were also used for ordination by principal coordinate analysis (PCoA), and differences in composition structure were assessed by Adonis and analysis of molecular variance (AMOVA) (21). The multiple response permutation procedure (MRPP) (22) was based on OTUs. Species with statistically significant differences between groups were evaluated by linear discriminant analysis effect size (LEfSe) (23). Functional prediction of microbiota differences was performed using Tax4fun (24) and STAMP (Statistical Analysis of Metagenomic Profiles) (25) using functional inferences from the Kyoto Encyclopedia of Gene and Genomes (KEGG) database. The abundances of the main butyrate-producing bacteria

(*Faecalibacterium*, *Agathobacter*, *Roseburia*, *Subdoligranulum*, *Ruminococcus_gnavus_group*, *Megasphaera*, *Phascolarctobacterium*, *Flavonifractor*, *Eubacterium_ruminantium_group*, *Coprococcus*, *Eubacterium_hallii_group*, *Oscillibacter*, *Butyricicoccus*, *Butyricimonas*, *Anaerostipes*, *Odoribacter*, *Porphyromonas*, *Eubacterium_ventriosum_group*, *Oscillospira*, and *Butyrivibrio*) were compared.

2.4 FMT experiments in mice

2.4.1 Mice and interventions

Six- to 8-week-old male C57BL/6 mice were fed under specific pathogen–free conditions. Mice were treated with a cocktail of antibiotics (ANVM: 4 mg/mL ampicillin, 2 mg/mL neomycin, 4 mg/mL metronidazole, 2 mg/mL vancomycin) 250 μL each day by oral gavage. 18 mice were randomly divided into two groups, colitic-irAE-FMT and non-irAE-FMT, receiving FMT from three patients with colitic irAE and two patients without irAE after anti-PD-1 therapy, respectively. The antibiotic pre-treated mice were first gavaged with 150 μL of fecal suspension every other day for 14 days, then 250 μg anti-PD-1 and 100 μg anti-CTLA-4 monoclonal antibodies were injected intraperitoneally, with FMT continued every other day for 10 days. All mice were euthanized at the last day of injection or on the verge of death. The Experimental Animal Ethics Committee of Peking Union Medical College Hospital approved the study protocol (XHDW-2022-066).

2.4.2 Colitis evaluation and dynamic microbiota analysis

Body weight and colon length were recorded, and the disease activity index (DAI) (26) and colitis scores (by histopathological analysis) (27) were evaluated by an independent investigator.

Slides were incubated with primary antibodies targeting CD4 (#70437, Leica Biosystems, Wetzlar, Germany), CD8 (#70349, Leica Biosystems), and CD20 (#71902, Leica Biosystems) at 4°C overnight and then incubated with Polymer Detection System reagents (PV-9000, ZSGB BIO, Beijing, China), visualized with DAB (ZLI-9019, ZSGB BIO), and counterstained with hematoxylin. Positively stained cells were counted in ten randomized fields (40× objective) under a light microscope by two experienced pathologists. Immunohistochemistry (IHC) scores were calculated as the mean positive cell percentage and degree of staining.

Colon tissue was treated with TRIzol reagent (Invitrogen, Waltham, MA). Extracted RNA was then reverse transcribed into cDNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the S1000 PCR thermocycler (BioRad, Hercules, CA). The results were analyzed using Alpha Innotech 2000 software (ProteinSimple, Santa Clara, CA) and presented as the ratio of the relative absorbance of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) as a housekeeping gene using the $2^{-\Delta\Delta CT}$ method. Primer sequences are presented in Supplementary Table 1.

Feces of three representative mice in each group were collected at baseline, after antibiotic treatment, after FMT, and before euthanasia. The samples were also subjected to microbiota analysis by 16S rDNA amplicon sequencing as previously described, while amplicon sequence variants (ASVs) were detected with QIIME2 (20).

2.5 Statistical analyses

Statistical analysis was performed using R software v4.0.3 and GraphPad Prism v8.3.1 (GraphPad Software, La Jolla, CA). Continuous variables are expressed as means ± SD or medians and interquartile ranges (IQR), as appropriate. Categorical variables are expressed as numbers and percentages or frequencies. Demographic and clinical characteristics were compared with Pearson's chi-squared test, Fisher's exact test, or the Wilcoxon rank-sum test between two groups as appropriate. Comparisons of bacterial abundance, α-diversity, and β-diversity were performed with *t*-tests or Wilcoxon rank-sum tests between two groups, as appropriate. Survival was estimated by the Kaplan–Meier method, and differences in survival were evaluated with the log-rank test. Random forest (RF) analysis was performed based on genus abundance. The analytic data were randomly sampled as an 80/20 split into the training (80%) and test (20%) sets. We selected different numbers of genera to build a random forest (RF) model, screened the main genera by MeanDecreaseAccuracy and MeanDecreaseGin (28), cross-validated the model, and drew a receiver operating characteristic (ROC) curve to evaluate the optimal model describing the main differential genera between groups. A two-sided $P < 0.05$ was considered statistically significant.

3 Results

3.1 Patient demographics and clinical characteristics

Among the 37 patients taking anti-PD-1 therapy, three patients developed irAEs. An additional 33 patients with irAEs were included, four without pre-irAE treatment fecal samples. Thus, a total of 34 patients without irAEs (from patients with thoracic cancers taking anti-PD-1 therapies) and 32 patients with irAEs (from both cohorts) were included in the microbiota analysis (Figure 1 and Supplementary Figure S1).

Among patients without irAEs, 47.1% and 41.2% had histologically confirmed adenocarcinoma and squamous cell carcinoma of the lung, respectively. 97.1% of patients were treated with anti-PD-1 therapy combined with chemotherapy or targeted therapy. Anti-PD-1 therapy resulted in partial responses (PR) in 52.9% and stable disease (SD) in 41.2% of patients, with a median PFS of 160 days. During subsequent anti-PD-1 therapy, four patients progressed: one patient from PR to SD, two patients from SD to progressive disease (PD), and one patient from PR to PD (Supplementary Table 2 and Supplementary Figure S1).

Among patients with irAEs, 78.1% had lung cancer and 90.6% were treated with anti-PD-1 therapy combined with chemotherapy or targeted therapy. In this group, anti-PD-1 therapy was also mainly effective; PR in 56.3% and SD in 40.6%, with only one patient developing PD. The median PFS was 249.5 days. Grade 1-2 and grade 3-4 irAEs were present in 40.6% and 59.4% of patients, respectively, with 84.4% having one irAE and 15.6% multiple irAEs. There were 38 irAEs in total: colitis (8/38), pneumonitis (7/38), asymptomatic elevations in amylase/lipase

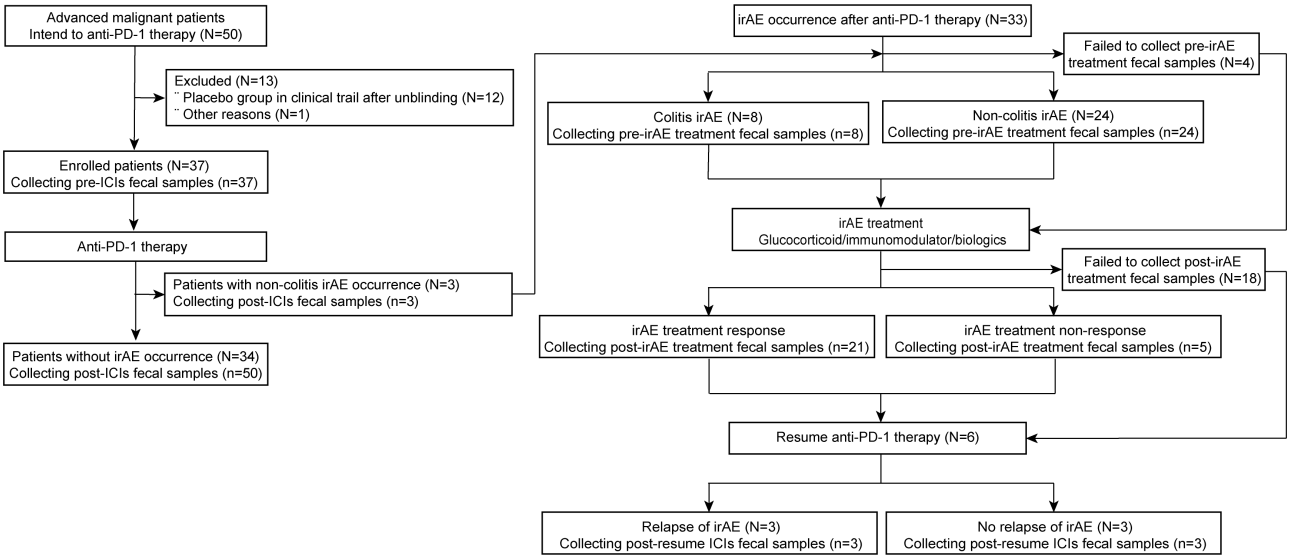

FIGURE 1
Flow diagram of participant enrollment and fecal sample collection in the anti-PD-1 therapy and irAE cohorts. N, number of patients; n, number of samples. PD-1, programmed death-1; irAE, immune-related adverse event; ICIs, immune checkpoint inhibitors.

or pancreatitis (6/38), transaminitis (5/38), rashes (4/38), myocarditis (3/38), acute kidney injury (3/38), myositis (1/38), and hyperglycemia-related diabetic ketoacidosis (1/38) (Supplementary Table 2).

After treatment with glucocorticoids, immunomodulators, or biologics, 21 samples were obtained from patients achieving irAE remission and 5 samples from patients who failed to respond. Among irAE remission patients, 6 patients resumed anti-PD-1 therapy but 3 relapsed with further irAEs (Supplementary Table 2; Figure 1 and Supplementary Figure S1).

3.2 Differences in the gut microbiota of patients with and without irAEs

We compared differences in microbiota composition between patients who did and did not develop irAEs at the timepoint of sample collection after anti-PD-1 therapy. Fifty samples from 34 patients without irAEs and 32 samples from 32 patients with irAEs were finally included in the analyses. The demographic and clinical characteristics of these patients were generally balanced between the two groups (Supplementary Table 2). There were 1664 common OTUs and 1638 and 1539 differential OTUs in patients who had taken ICIs and did and did not develop irAEs, respectively. A PCoA plot revealed significant differences in microbiota composition between patients who did and did not develop irAEs (Adonis: P=0.001) (Figure 2A). These differences in microbiota structure were also confirmed by MRPP (P=0.001) and AMOVA (P<0.001) analyses. These differences persisted when patients with colitic irAEs were excluded from the analysis (Adonis: P=0.002) (Figure 2B). The median α-diversity in the non-irAE group was higher than that of the irAE group, with a trend toward a significant difference in observed species (P=0.06) (Figure 2C). There were also differences in β-diversity as assessed by Bray-Curtis and weighted Unifrac distances (P<0.001) (Figure 2D).

LEfSe analysis revealed that patients who developed irAEs had a higher abundance of *Fusobacteriota* and a lower abundance of *Actinobacteriota* at the phylum level and a higher abundance of *Erysipelatoclostridium* and lower abundance of *Bifidobacterium*, *Faecalibacterium*, and *Agathobacter* at the genus level compared with those not developing irAEs (Figures 2E, F).

Finally, RF analysis established a predictive model for irAE occurrence with AUCs of 86.35% in the training set and 91.67% in the test set consisting of ten main differential genera led by *Gemella*, *Dubosiella*, and *Atopobium* (Figures 2G–I).

We further compared the 13 patients with grade 1-2 irAEs and 19 patients with grade 3-4 irAEs. There were no significant differences in demographic and clinical characteristics between groups, except for the gender (P=0.029) (Supplementary Table 3). There were 1122 common OTUs and 180 and 2000 differential OTUs in grade 1/2 irAE and grade 3/4 irAE patients, respectively (Supplementary Figure S2A). Histograms showed that the microbiota composition was different between grade 1/2 irAE and grade 3/4 irAE patients at the phylum and genus levels (Supplementary Figures S2B, C), as did the PCoA plot (Adonis: P=0.068; MRPP: P=0.058; AMOVA: P=0.065). The microbiota composition of grade 3/4 irAE patients was more dispersed (Supplementary Figure S2D), with the β-diversity calculated by Bray-Curtis and weighted Unifrac distances supporting these differences (P<0.001) (Supplementary Figure S2E). However, there was no significant difference in α-diversity between these two groups (P=0.69). LEfSe analysis revealed that grade 3/4 irAE patients had a higher abundance of *Streptococcus* and a lower abundance of *Agathobacter* at the genus level compared with grade 1/2 irAE patients (Supplementary Figure S2F).

Considering that there were differences between groups with respect to some demographic and clinical characteristics, we further compared microbiota differences in irAE patients according to gender, age, tumor type, and disease stage to exclude potential confounding effects of these clinical and pathological variables.

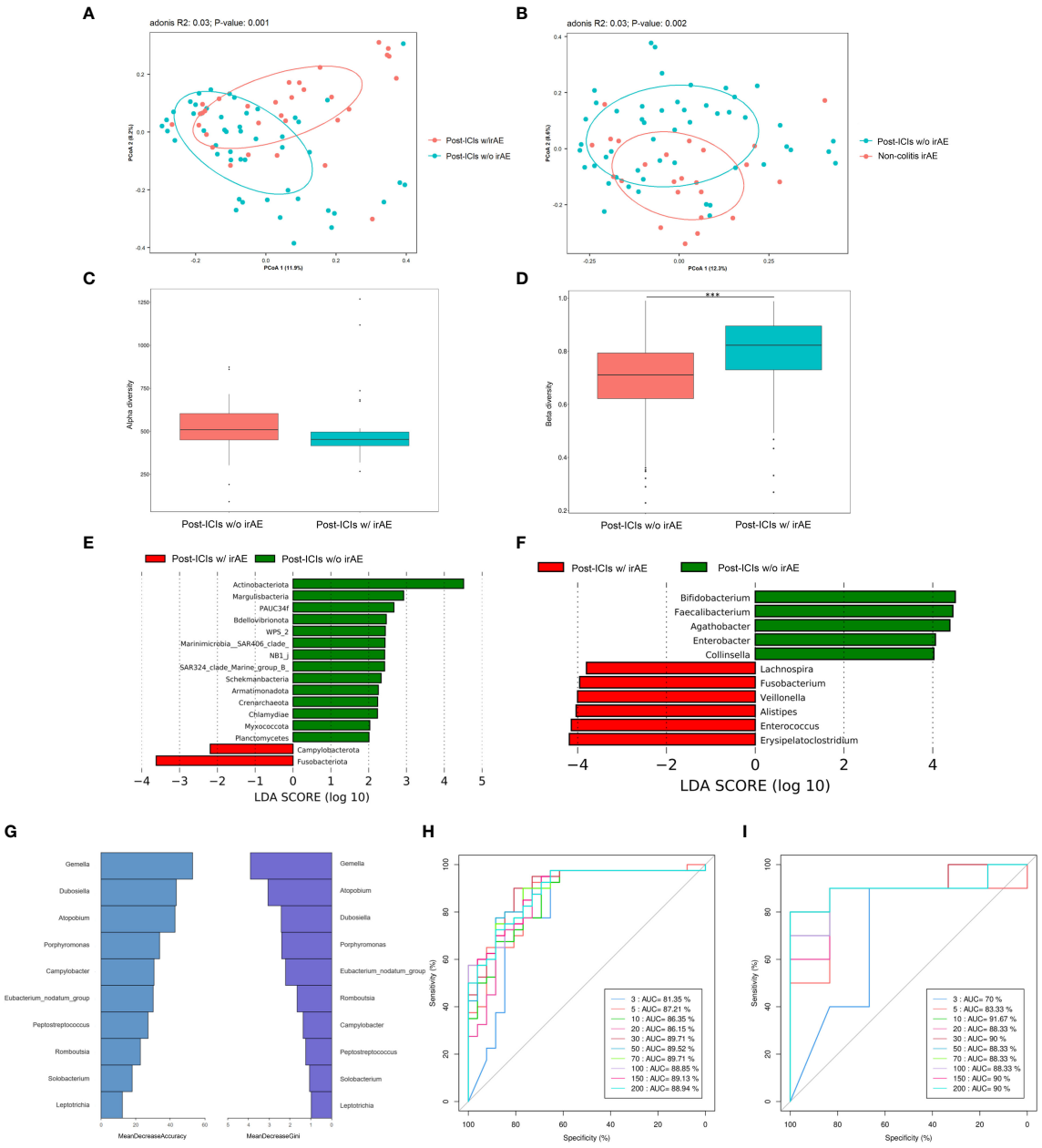

FIGURE 2
The microbiota of patients with and without irAEs. **(A)** PCoA plot of intestinal microbiota in patients with and without irAEs; **(B)** PCoA plot of intestinal microbiota in patients with non-colitic irAEs and patients without irAEs; **(C)** Box plot of α-diversity by observed species; **(D)** Box plot of β-diversity by Bray-Curtis distance of intestinal microbiota; LEfSe analysis of intestinal microbiota at the phylum level **(E)** and genus level **(F)**; Mean decrease in accuracy and mean decrease in Gini coefficient **(G)**, training set AUC **(H)**, and test set AUC **(I)** by random forest analysis at the genus level. AUC, area under the curve; ICIs, immune-checkpoint inhibitors; irAE, immune-related adverse event; LEfSe, LDA effect size; PCoA, principal coordinates analysis; w/, with; w/o, without. ***$P < 0.001$.

PCoA revealed no significant differences in microbiota composition with respect to gender, age, tumor type, and disease stage (Supplementary Figures S3A-D).

3.3 Differences in the gut microbiota of patients with colitic and non-colitic irAEs

IrAEs involved a variety of different organs. There were 162 common OTUs and 1281, 83, 87, 13, 55, 12, 26, 25, and 50 differential OTUs in patients with irAEs involving the gastrointestinal tract, liver, lung, muscle, pancreas, skin, endocrine, heart, and kidney, respectively. The PCoA plot also revealed that the gut microbiota of patients with irAEs involving the gastrointestinal tract was quite distinct from patients with irAEs involving other organs (Figure 3A).

We speculated that occurrence of colitic type irAEs was different to the other types and especially related to the gut microbiota. Thus, irAEs were further classified into colitic and non-colitic irAEs. There were no significant differences in the demographic and clinical characteristics of these two groups, except for the tumor type

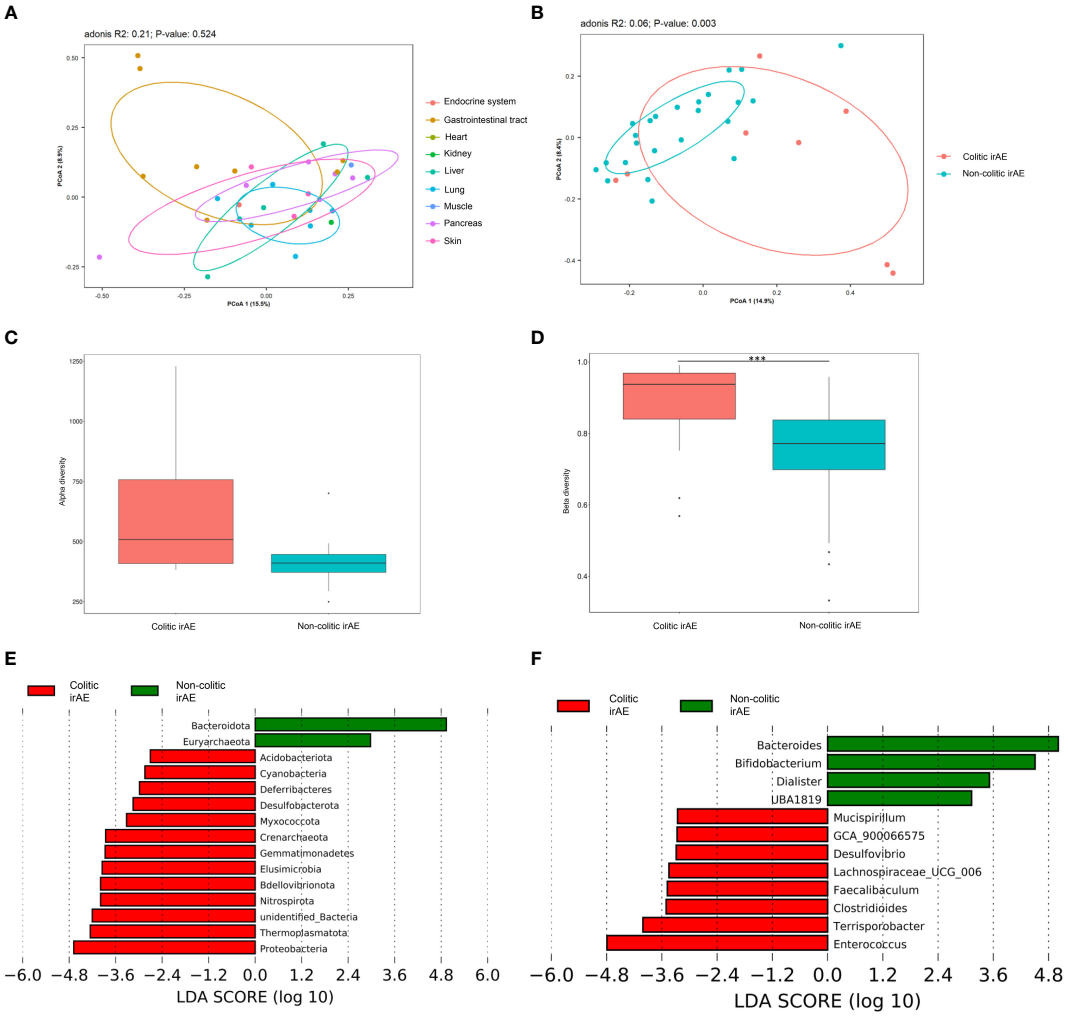

FIGURE 3
The gut microbiota of patients with colitic- and non-colitic-type irAEs. **(A)** PCoA plot of microbiota in patients with different kinds of irAEs; **(B)** PCoA plot of intestinal microbiota of patients with colitic and non-colitic irAEs; Box plots of α-diversity by observed species **(C)** and β-diversity by the Bray-Curtis method **(D)** of intestinal microbiota in patients with colitic and non-colitic irAEs; LEfSe analysis of intestinal microbiota at the phylum **(E)** and genus **(F)** levels in patients with colitic- and non-colitic irAEs. Abbreviations: irAE, immune-related adverse event; LEfSe, LDA effect size; PCoA, principal coordinates analysis. ***$P < 0.001$.

(P=0.02) (Supplementary Table 4). There were 1159 common OTUs and 1555 and 588 differential OTUs in colitic and non-colitic irAE patients, respectively. The PCoA plot also revealed a significant difference in gut microbiota composition between these two groups (Adonis: P=0.003; MRPP: P=0.006; AMOVA: P=0.002) (Figure 3B). The median α-diversity in colitic irAE patients was higher than that of non-colitic irAE patients, with a non-significant trend in differences in observed species (P=0.10) (Figure 3C). The β-diversity was significantly different between groups by both Bray-Curtis and weighted Unifrac distances (P<0.001) (Figure 3D). LEfSe analysis of the main differences detected a lower abundance of *Bacteroidota* and a higher abundance of *Proteobacteria* at the phylum level and a lower abundance of *Bacteroides* and *Bifidobacterium* and higher abundance of *Enterococcus* at the genus level in colitic irAE compared with non-colitic irAE (Figures 3E, F).

3.4 The causal role of the gut microbiota in immune-related colitis development in a humanized microbiota mouse model

Although the above analysis revealed an association between the gut microbiota and irAEs, the causal relationship between the microbiota and irAEs or vice versa remained uncertain. Therefore, we performed FMT experiments using human donor feces in mice to explore causality.

Three of nine colitic-irAE-FMT mice developed fatal severe colitis, while all nine non-irAE-FMT mice survived without colitis (P=0.052) (Figure 4A). Weight loss was more rapid and disease activity index (DAI) scores were higher in the three colitic mice than the other mice (Figures 4B, C). The irAE-FMT-Sac. mouse had a shorter colon (5.8 cm) than non-irAE-FMT mice (7.2 ± 0.7 cm) and

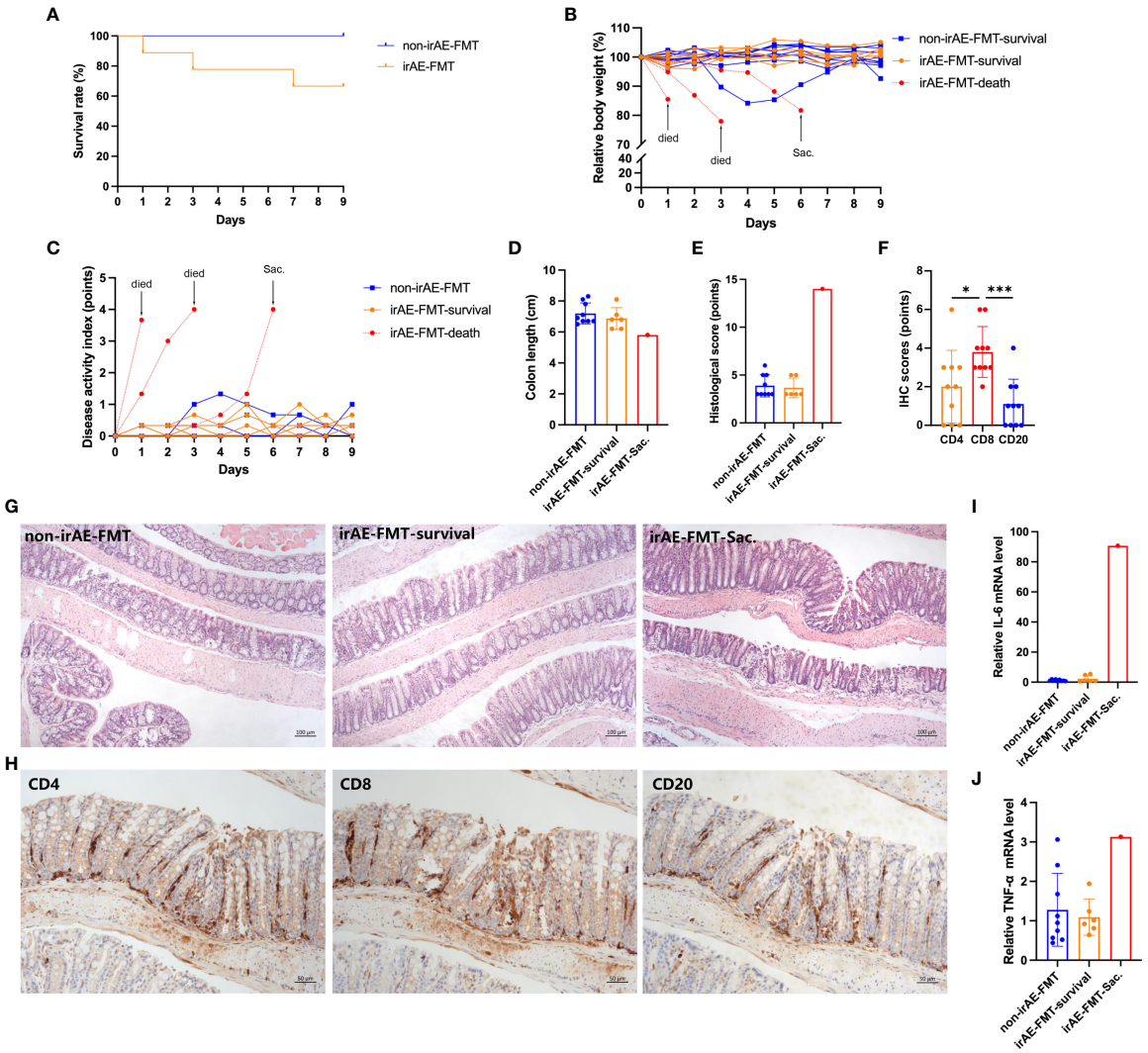

FIGURE 4
Comparison of colitis in colitic-irAE-FMT and non-irAE-FMT mice. **(A)** Survival curve; **(B)** Relative body weight change; **(C)** DAI scores; **(D)** Colon length; **(E)** Histological scoring of colonic tissue; **(F)** IHC scoring of CD4, CD8, and CD20 in colonic tissue from mice developing colitis; **(G)** HE-stained mouse colon tissue sections (100×); **(H)** CD4, CD8, and CD20 expression in mouse developing colitis by IHC (100×); **(I)** Colonic *Il6* mRNA levels; **(J)** Colonic *Tnf* mRNA levels by qRT-PCR with *Gapdh* as the internal reference. (Non-irAE-FMT n=9; irAE-FMT-survival n=6; irAE-FMT-death n=3; irAE-FMT-Sac. n=1). DAI, disease activity index; FMT, fecal microbiota transplantation; HE, hematoxylin-eosin; ICIs, immune checkpoint inhibitors; IHC, immunohistochemistry; *Il6*, interleukin-6; irAE, immune-related adverse event; *Tnf*, tumor necrosis factor. *$P < 0.05$, ***$P < 0.001$.

colitic-irAE-FMT-survival mice (6.9 ± 0.7 cm) (Figure 4D), and the histological score in the irAE-FMT-Sac. mouse (14 points) was higher than those in the non-irAE-FMT mice (3.89 ± 1.17 points) and colitic-irAE-FMT-survival mice (3.67 ± 1.03 points) (Figures 4E, G). The mean IHC scores in the irAE-FMT-Sac. mouse from high to low were CD8 3.8 ± 1.3 points, CD4 2.0 ± 1.9 points, and CD20 1.1 ± 1.3 points (Figures 4F, H). Relative colonic *Il6* and *Tnf* mRNA levels were also higher in the irAE-FMT-Sac. mouse (*Il6*: 90.68 and *Tnf*: 3.13) than the non-irAE-FMT mice (*Il6*: 1.12 ± 0.53 and *Tnf*: 1.28 ± 0.92) and colitic-irAE-FMT-survival mice (*Il6*: 2.26 ± 2.24 and *Tnf*: 1.09 ± 0.46) (Figures 4I, J).

Microbiota analyses showed the microbiota composition of donors with colitic irAE and without irAE were different at phylum and genus levels (Supplementary Figure S4A). Furthermore, longitudinal microbiota analysis revealed significant changes from baseline to after antibiotic treatment, although the microbiota was stable from FMT to euthanasia at phylum and genus levels in both colitic-irAE-FMT and non-irAE-FMT mice (Supplementary Figures S4B, C). After FMT, the microbiota differed between irAE-FMT mice and non-irAE-FMT mice. However, in irAE-FMT mice, there was no significant difference in microbiota composition between mice developing colitis (irAE-FMT-Sac.) or those not developing colitis (irAE-FMT-survival) (Supplementary Figure S4D). In colitic-irAE-FMT mice, there was a decrease in beneficial genera and increase in harmful genera with immune-related colitis development (Supplementary Figure S4E); the mean relative abundance of beneficial genera was lower and harmful genera higher in the irAE-FMT-Sac. mouse compared with irAE-FMT-survival and non-irAE FMT mice (Supplementary Table 5 and Supplementary Figure S4F).

3.5 IrAE patients express microbiomes representing different metabolism pathways

We next performed functional prediction analysis to explore the underlying mechanisms by which the microbiota influence irAE development. The main differential pathways between patients who did and did not develop irAEs included the two-component system (P=0.044), starch and sucrose metabolism (P=0.029), pyruvate metabolism (P<0.001), mitochondrial biogenesis (P=0.044), and glycolysis/gluconeogenesis (P=0.031). Other differential pathways were lipid, amino acid, and vitamin metabolism including vitamin B6 metabolism (P=0.044), biotin metabolism (P=0.042), nicotinate and nicotinamide metabolism (P<0.001), amino acid metabolism (P=0.034), fatty acid biosynthesis (P=0.014), and fatty acid degradation (P=0.049) (Figure 5A and Supplementary Figure S5).

Differential pathways between patients with colitic and non-colitic irAEs included the two-component system (P=0.011), amino acid-related enzymes (P<0.001), ABC transporters (P=0.041), and exosomes (P=0.013). In addition to sugar and fatty acid pathways, the pathways of several amino acids were also significantly different including alanine, aspartate, and glutamate metabolism (P=0.017), cysteine and methionine metabolism (P=0.028), and phenylalanine, tyrosine, and tryptophan biosynthesis metabolism (P=0.032) (Figure 5B and Supplementary Figure S6).

As mentioned above, both *Faecalibacterium* and *Agathobacter* genera are butyrate-producing bacteria. Thus, we compared the abundance of these main butyrate-producing bacteria and found that the total abundance of butyrate-producing bacteria (P=0.007), *Faecalibacterium* (P=0.002), and *Agathobacter* (P=0.002) was lower in patients who developed irAEs (Figure 5C). There was also a lower abundance of the total main butyrate-producing bacteria (P=0.018), *Roseburia* (P=0.039), and *Subdoligranulum* (P=0.046) in colitic irAE compared with non-colitic irAE (Figure 5D).

3.6 Gut microbiota remained stable after ICI and irAE treatment

Finally, we assessed the relationship between microbiota and ICI and irAE treatment. Thirty-four pre-ICI samples from 34 patients and 50 post-ICI samples from 34 patients without irAEs were included in the analyses to compare the microbiota of patients before and after ICI therapy. There were 2395 common OTUs and 456 and 808 differential OTUs in pre- and post-ICI patients, respectively (Supplementary Figure S7A). Histograms of microbiota composition at phylum and genus levels and the PCoA plot revealed no significant differences between pre- and post-ICI patients (Adonis: P=0.96) (Supplementary Figures S7B-D). Also, the α-diversity by observed species index (P=0.66) and β-diversity by weighted Unifrac distance (P=0.19) were not significantly different (Supplementary Figures S7E, F).

We also compared the microbiota composition of irAE patients before and after irAE treatment (32 and 26 samples, respectively) and found no significant differences (Adonis: P=0.77). However, the β-diversity was higher by Bray-Curtis distance after irAE treatment (P=0.02) (Supplementary Figure S8).

4 Discussion

Here we explored the association between the gut microbiota and the development of irAEs. We show that: (i) the gut microbiota differs significantly in patients who do and do not develop irAEs after ICI treatment; (ii) the gut microbiota differs significantly between patients with colitic and non-colitic irAEs; (iii) the gut microbiota partially determined the occurrence of immune-related colitis in the mouse model; and (iv) patients with irAEs express microbiomes with different environmental information processing, genetic information processing, and metabolism pathways.

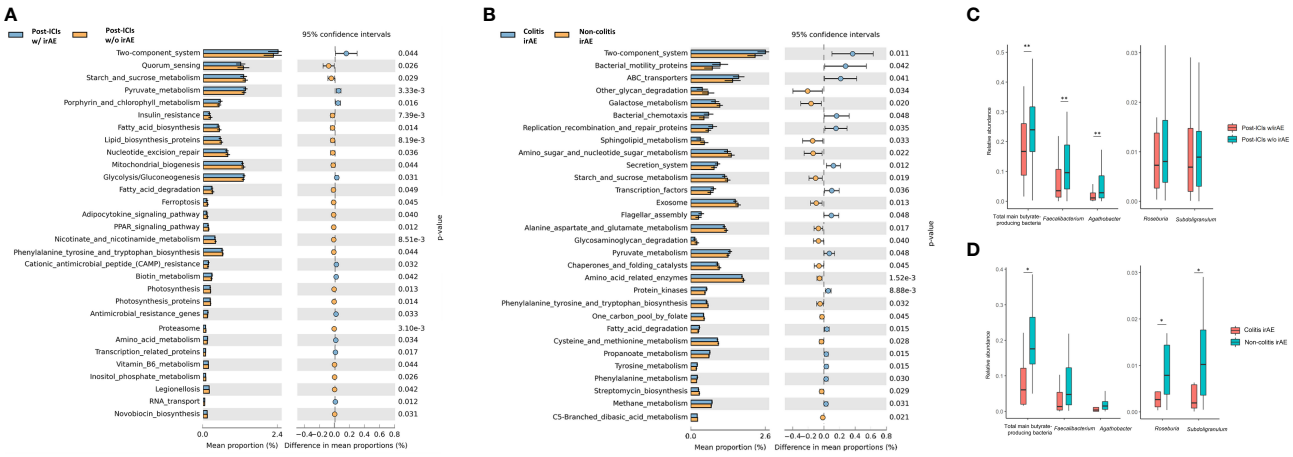

FIGURE 5
Functional predictions of the gut microbiota between different groups. Top 30 significantly different KEGG pathways with the highest abundance at level 3 between patients with and without irAEs **(A)** and between patients with colitic and non-colitic irAEs **(B)**; Box plot of main butyrate-producing bacteria abundance between patients with and without irAEs **(C)** and between patients with colitic- and non-colitic-type irAEs **(D)**. ICIs, immune checkpoint inhibitors; irAE, immune-related adverse event; KEGG, Kyoto Encyclopedia of Genes and Genome; w/, with; w/o, without. *P < 0.05, **P < 0.01.

There have been several studies on the role of the gut microbiota in irAE occurrence. The baseline microbiota has been widely reported to shape irAE/immune-related colitis occurrence and development, but mainly in the context of metastatic melanoma and NSCLC by 16S sequencing or shotgun metagenomics (11, 12, 14, 15, 29, 30). Our study provides new data in different cancer types and adds further weight to a causal relationship between the microbiota and colitis.

There was no significant difference in α-diversity (species richness in the gut microbiome within a single sample) in patients who did and did not develop irAEs. However, the β-diversity, which measures microbial composition heterogeneity between samples, was higher in patients who developed irAEs compared with those who did not, consistent with previous studies (11, 15). We therefore speculate that the occurrence of irAEs depends more on microbiota composition (i.e., presence or absence of certain organisms) rather than the species richness. Indeed, patients who did not develop irAEs had a higher abundance of *Bifidobacterium*, *Faecalibacterium*, and *Agathobacter*. As a probiotic, *Bifidobacterium* protects against immune-related colitis by modulating Treg cell metabolism through an IL-10/IL-10Rα self-stimulatory loop and reducing levels of inflammatory cytokines IL-6, CSF3, and KC (31, 32). *Bifidobacterium* also appears to influence the clinical efficacy of ICIs (10), perhaps by modulating the activation of dendritic cells (33), and similarly *Faecalibacterium* also favors ICI efficacy (5, 12). Here we found that organisms in the *Faecalibacterium* genus tended to be more enriched in patients without irAEs, consistent with another study of patients receiving anti-PD-1 therapy (15). Conversely, Chaput et al. reported that abundant baseline *Faecalibacterium* was associated with colitis in patients receiving anti-CTLA-4 therapy (12). This discrepancy might be due to the context-dependent pro-inflammatory and anti-inflammatory effects of *Faecalibacterium* (34). *Agathobacter*, a butyrate-producing bacterium reported to be associated with ICI efficacy (13, 35), protected against irAEs in our study. We also compared the microbiota of patients with irAEs of different severity. Compared with those in mild-moderate irAE patients, the microbiota of severe irAE patients were more heterogeneous. Our results implicate the *Agathobacter* genus as protective against irAE occurrence and severity.

The microbiota of patients with colitic irAE was particularly different to those with irAEs affecting other organs. Patients with colitic irAE had a lower abundance of *Bacteroides* and *Bifidobacterium* genera. Usyk et al. recently reported that the presence of *Bacteroides vulgatus* and *Bacteroides dorei* predicts irAEs in patients with metastatic melanoma (14), and several studies have shown that higher representation of members of the *Bacteroidetes* phylum or its prominent genus *Bacteroides* is associated with protection from colitis (11, 12). This protective function might be related to the production of polysaccharide A, which might facilitate the development and function of Tregs (36). However, Andrews et al. found that *Bacteroides intestinalis* upregulated mucosal IL-1β and mediated immune-related colitis (37), suggesting that a more detailed species-level analysis is warranted.

It is worth considering whether microbiota differences resulted in immune-related colitis or whether the immune-related colitis disturbed the gut microbiota. This is, of course, impossible to establish in an observational study of clinical data. However, preclinical studies have provided some clues. For example, pre-treatment with vancomycin worsened immune-related colitis in mice, while administration of *Bifidobacterium* or *Lactobacillus reuteri* both alleviated the colitis (31, 38). These data suggest that the microbiota composition contributes to the development of immune-related colitis and that supplementation with probiotics to alter the gut microbiota effectively alleviates colitis. Even when colitic irAE patients in whom the microbiota may be influenced by the irAE were excluded, there were still significant differences between patients with and without irAEs. This evidence favors microbiota contributing to the occurrence of irAEs. Furthermore, our experiments in mice indicated that the gut microbiome caused immune-related colitis. After antibiotic treatment and establishment of humanized gut microbiota from colitic-irAE patients and non-irAE patients, colitis only occurred in the colitic-irAE-FMT mice after combined anti-PD-1 and anti-CTLA-4 treatment. The higher representation of CD8 compared with CD4 and CD20 cells by IHC further indicated that ICI-related colitis occurred in mice. Mouse microbiota analysis also supported the findings of microbiota differences in patients with and without irAEs. However, perhaps not surprisingly, not all colitic-irAE-FMT mice developed colitis, since other immune factors are likely to be involved in the pathogenesis of microbiota-related colitis.

Our functional analyses of patients with and without irAEs revealed a set of differentially expressed pathways mainly involved in environmental information processing, genetic information processing, and metabolism, e.g., sugar, lipid, amino acid, and purine and vitamin metabolism. Further analysis revealed that these differences were particularly significant in colitic irAE, suggesting that the pathogenesis of immune-related colitis may be highly related to gut microbiota-mediated mechanisms. While Mager et al. found that the microbiome could modulate ICI efficacy through the actions of the bacteria-derived metabolite inosine (39), there are still very few studies on the mechanism of action of microbiota and irAEs. Microbiota-derived metabolites such as short-chain fatty acids (SCFAs), bacterial tryptophan catabolites, branched chain amino acids (BCAAs), and vitamins play a role in immune-mediated inflammatory diseases including autoimmune disease and IBD (40), providing clues about the role of metabolism in irAEs and immune-related colitis. Butyrate is one of the most important SCFAs and is widely reported to be associated with anti-tumor efficacy (41) and immune-related diseases, and Chen et al. reported that butyrate can alleviate anti-PD-1/PD-L1-related cardiotoxicity (42). We also found decreased abundance of butyrate-producing bacteria in patients with irAEs, especially colitic irAE. With respect to other metabolites, amino acids can undergo complex processing to produce toxic compounds such as amines, phenols/indoles, and sulphurous compounds (43). Furthermore, pyruvate has also been reported to enhance immune responses by inducing dendrite protrusion from intestinal $CX3CR1^{+}$ cells (44). We also observed differences in B vitamin pathways (vitamin B6, biotin, and niacin) in irAE patients, which are reported to regulate host immunity (45, 46).

There is still relatively little information on genetic information processing pathways in irAE patients, although several studies on breast cancer and IBD have reported similar findings (47, 48). The two-component system pathway was also more abundant in irAE patients, especially those with colitic irAE, perhaps reflecting bacterial responses to external stimuli. We speculate that these pathways might mediate irAEs by affecting bacterial growth, replication, and signaling, thereby affecting bacterial functions such as metabolism.

We also performed random forest modelling to distinguish patients with and without irAEs, and the resultant model achieved good performance for predicting irAEs. Of the three leading genera in

the model, *Atopobium parvulum* is a key network hub of H_2S producers that induces colitis (49), and it has previously been implicated in the development of intramucosal carcinomas (50). *Gemella* (51) and *Dubosiella* (52), involved in the synthesis of short-chain fatty acids (SCFAs), may also be related to protection from colitis. However, as random forests are nonlinear, it is difficult to explain the functional contribution of the individual biomarkers based on previous studies or putative mechanisms. Although there was no obvious change in the gut microbiota before and after ICI treatment, whether a predictive model based on post-ICI microbiota is the best choice of sample for predictive biomarkers still needs to be verified. Furthermore, the small sample size might lead to model overfitting, so extensive external validation is also required.

Interestingly, we found that the gut microbiota did not significantly change after anti-PD-1 therapy or irAE treatment, further suggesting that the baseline microbiota may shape ICI efficacy and irAE occurrence, and the microbiota remained stable after therapy initiation (12, 14, 30). Two recent clinical studies showed that FMT from ICI responders to primary non-responders partially reversed ICI efficacy in melanoma patients (8, 9). All these data suggest that altering the baseline microbiota may contribute to improving the ICI efficacy and avoiding irAEs.

There are several limitations to our study. The sample size was relatively small and the incidence of irAEs generally low (53). Therefore, subgroup analyses may have lacked statistical power or were not possible, for instance for comparing the microbiota of patients with different types of irAE. Furthermore, the efficacy of anti-PD-1 therapy combined with chemotherapy can be as high as 84.6% in advanced NSCLC patients (54), so we could not analyze associations between microbiota composition and ICI efficacy. We prospectively collected fecal samples from patients initially treated with ICIs, and only 3/37 patients developed irAEs, so baseline fecal samples before ICI treatment were generally lacking. Finally, functional analyses were performed based on 16S rDNA amplicon sequencing rather than the newer, more granular metagenomic sequencing and metabolomics analyses.

In conclusion, we detected significant differences in the gut microbiota of patients with and without irAEs and between patients with colitic and non-colitic irAE. FMT experiments from humans to mice indicated a causal link between the gut microbiota and immune-related colitis. We also built a predictive irAE model to identify patients at high risk of irAEs, who may particularly benefit from strategies to prevent irAEs such as by altering the gut microbiota. IrAEs, especially immune-related colitis, seem to be driven by metabolic mechanisms. The roles of the gut microbiota in irAEs and immune-related colitis require further verification in larger studies, and the predictive model for irAEs needs validating in external, independent patient cohorts. Metabolomics research is now needed to better identify and understand the protective protagonists and potential underlying protective mechanisms. Supplementation with probiotics to prevent and attenuate irAEs should also be considered given that they are generally safe and easy to administer.

Data availability statement

The data that support the findings of this study are openly available in figshare at http://doi.org/10.6084/m9.figshare.21431871.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Peking Union Medical College Hospital (No. ZS-3037). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Experimental Animal Ethics Committee of Peking Union Medical College Hospital (XHDW-2022-066).

Author contributions

Analyzed and interpreted data, drafted the manuscript: XL. Animal experiments and analyzed data: HT, QZ. Analyzed microbiota data: YZ, DC, YL. Enrolled patients and collected samples: MC, JZ, YX. Supervised and supported the study: MW, JQ. Designed and performed the study, critically revised the manuscript, funding support: BT. The work reported in the paper was performed by the authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1109281/full#supplementary-material

References

1. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of fda-approved immune checkpoint inhibitors. *Int Immunopharmacol* (2018) 62:29–39. doi: 10.1016/j.intimp.2018.06.001

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

3. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-Ctla-4 antibodies: Systematic review and meta-analysis. *BMC Med* (2015) 13:211. doi: 10.1186/s12916-015-0455-8

4. Wang Y, Zhou S, Yang F, Qi X, Wang X, Guan X, et al. Treatment-related adverse events of pd-1 and pd-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *JAMA Oncol* (2019) 5(7):1008–19. doi: 10.1001/jamaoncol.2019.0393

5. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-Pd-1 immunotherapy in melanoma patients. *Science* (2018) 359(6371):97–103. doi: 10.1126/science.aan4236

6. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The commensal microbiome is associated with anti-Pd-1 efficacy in metastatic melanoma patients. *Science* (2018) 359(6371):104–8. doi: 10.1126/science.aao3290

7. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of pd-1-Based immunotherapy against epithelial tumors. *Science* (2018) 359(6371):91–7. doi: 10.1126/science.aan3706

8. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-Pd-1 therapy in melanoma patients. *Science* (2021) 371(6529):595–602. doi: 10.1126/science.abf3363

9. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science* (2021) 371(6529):602–9. doi: 10.1126/science.abb5920

10. Dizman N, Meza L, Bergerot P, Alcantara M, Dorff T, Lyou Y, et al. Nivolumab plus ipilimumab with or without live bacterial supplementation in metastatic renal cell carcinoma: A randomized phase 1 trial. *Nat Med* (2022) 28(4):704–12. doi: 10.1038/s41591-022-01694-6

11. Dubin K, Callahan MK, Ren B, Khanin R, Viale A, Ling L, et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-Blockade-Induced colitis. *Nat Commun* (2016) 7:10391. doi: 10.1038/ncomms10391

12. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol* (2017) 28(6):1368–79. doi: 10.1093/annonc/mdx108

13. Hakozaki T, Richard C, Elkrief A, Hosomi Y, Benlaïfaoui M, Mimpen I, et al. The gut microbiome associates with immune checkpoint inhibition outcomes in patients with advanced non-small cell lung cancer. *Cancer Immunol Res* (2020) 8(10):1243–50. doi: 10.1158/2326-6066.Cir-20-0196

14. Usyk M, Pandey A, Hayes RB, Moran U, Pavlick A, Osman I, et al. Bacteroides vulgatus and bacteroides dorei predict immune-related adverse events in immune checkpoint blockade treatment of metastatic melanoma. *Genome Med* (2021) 13(1):160. doi: 10.1186/s13073-021-00974-z

15. Liu W, Ma F, Sun B, Liu Y, Tang H, Luo J, et al. Intestinal microbiome associated with immune-related adverse events for patients treated with anti-Pd-1 inhibitors, a real-world study. *Front Immunol* (2021) 12:756872. doi: 10.3389/fimmu.2021.756872

16. Wang Y, Wiesnoski DH, Helmink BA, Gopalakrishnan V, Choi K, DuPont HL, et al. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med* (2018) 24(12):1804–8. doi: 10.1038/s41591-018-0238-9

17. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: Revised recist guideline (Version 1.1). *Eur J Cancer* (2009) 45(2):228–47. doi: 10.1016/j.ejca.2008.10.026

18. Thompson JA, Schneider BJ, Brahmer J, Andrews S, Armand P, Bhatia S, et al. Nccn guidelines insights: Management of immunotherapy-related toxicities, version 1.2020. *J Natl Compr Canc Netw* (2020) 18(3):230–41. doi: 10.6004/jnccn.2020.0012

19. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-High-Throughput microbial community analysis on the illumina hiseq and miseq platforms. *Isme J* (2012) 6(8):1621–4. doi: 10.1038/ismej.2012.8

20. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nat Biotechnol* (2019) 37(8):852–7. doi: 10.1038/s41587-019-0209-9

21. Anderson M. A new method for non-parametric multivariate analysis of variance. *Austral Ecol* (2001) 26:32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

22. Mielke PW. 34 meteorological applications of permutation techniques based on distance functions. In: *Handbook of statistics*, vol. 4. Elsevier (1984). p. 813–30. doi: 10.1016/S0169-7161(84)04036-0

23. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* (2011) 12(6):R60. doi: 10.1186/gb-2011-12-6-r60

24. Aßhauer KP, Wemheuer B, Daniel R, Meinicke P. Tax4fun: Predicting functional profiles from metagenomic 16s rrna data. *Bioinformatics* (2015) 31(17):2882–4. doi: 10.1093/bioinformatics/btv287

25. Parks DH, Tyson GW, Hugenholtz P, Beiko RG. Stamp: Statistical analysis of taxonomic and functional profiles. *Bioinformatics* (2014) 30(21):3123–4. doi: 10.1093/bioinformatics/btu494

26. Murano M, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, et al. Therapeutic effect of intracolonically administered nuclear factor kappa b (P65) antisense oligonucleotide on mouse dextran sulphate sodium (Dss)-induced colitis. *Clin Exp Immunol* (2000) 120(1):51–8. doi: 10.1046/j.1365-2249.2000.01183.x

27. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG, et al. Chronic experimental colitis induced by dextran sulphate sodium (Dss) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* (1998) 114(3):385–91. doi: 10.1046/j.1365-2249.1998.00728.x

28. Breiman L. Random forests. *Mach Learn* (2001) 45(1):5–32. doi: 10.1023/A:1010933404324

29. Sakai K, Sakurai T, De Velasco MA, Nagai T, Chikugo T, Ueshima K, et al. Intestinal microbiota and gene expression reveal similarity and dissimilarity between immune-mediated colitis and ulcerative colitis. *Front Oncol* (2021) 11:763468. doi: 10.3389/fonc.2021.763468

30. McCulloch JA, Davar D, Rodrigues RR, Badger JH, Fang JR, Cole AM, et al. Intestinal microbiota signatures of clinical response and immune-related adverse events in melanoma patients treated with anti-Pd-1. *Nat Med* (2022) 28(3):545–56. doi: 10.1038/s41591-022-01698-2

31. Wang F, Yin Q, Chen L, Davis MM. Bifidobacterium can mitigate intestinal immunopathology in the context of ctla-4 blockade. *Proc Natl Acad Sci U S A* (2018) 115(1):157–61. doi: 10.1073/pnas.1712901115

32. Sun S, Luo L, Liang W, Yin Q, Guo J, Rush AM, et al. Bifidobacterium alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc Natl Acad Sci U S A* (2020) 117(44):27509–15. doi: 10.1073/pnas.1921223117

33. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal bifidobacterium promotes antitumor immunity and facilitates anti-Pd-L1 efficacy. *Science* (2015) 350(6264):1084–9. doi: 10.1126/science.aac4255

34. Routy B, Gopalakrishnan V, Daillère R, Zitvogel L, Wargo JA, Kroemer G. The gut microbiota influences anticancer immunosurveillance and general health. *Nat Rev Clin Oncol* (2018) 15(6):382–96. doi: 10.1038/s41571-018-0006-2

35. Martini G, Ciardiello D, Dallio M, Famiglietti V, Esposito L, Della Corte CM, et al. Gut microbiota correlates with antitumor activity in patients with mcrc and nsclc treated with cetuximab plus avelumab. *Int J Cancer* (2022) 151(3):473–80. doi: 10.1002/ijc.34033

36. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* (2010) 107(27):12204–9. doi: 10.1073/pnas.0909122107

37. Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen WS, Derosa L, et al. Gut microbiota signatures are associated with toxicity to combined ctla-4 and pd-1 blockade. *Nat Med* (2021) 27(8):1432–41. doi: 10.1038/s41591-021-01406-6

38. Wang T, Zheng N, Luo Q, Jiang L, He B, Yuan X, et al. Probiotics lactobacillus reuteri abrogates immune checkpoint blockade-associated colitis by inhibiting group 3 innate lymphoid cells. *Front Immunol* (2019) 10:1235. doi: 10.3389/fimmu.2019.01235

39. Mager LF, Burkhard R, Pett N, Cooke NCA, Brown K, Ramay H, et al. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* (2020) 369(6510):1481–9. doi: 10.1126/science.abc3421

40. Yang W, Cong Y. Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cell Mol Immunol* (2021) 18(4):866–77. doi: 10.1038/s41423-021-00661-4

41. He Y, Fu L, Li Y, Wang W, Gong M, Zhang J, et al. Gut microbial metabolites facilitate anticancer therapy efficacy by modulating cytotoxic Cd8(+) T cell immunity. *Cell Metab* (2021) 33(5):988–1000.e7. doi: 10.1016/j.cmet.2021.03.002

42. Chen Y, Liu Y, Wang Y, Chen X, Wang C, Chen X, et al. Prevotellaceae produces butyrate to alleviate pd-1/Pd-L1 inhibitor-related cardiotoxicity *Via* pparα-Cyp4x1 axis in colonic macrophages. *J Exp Clin Cancer Res* (2022) 41(1):1. doi: 10.1186/s13046-021-02201-4

43. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. *Microbiome* (2019) 7(1):91. doi: 10.1186/s40168-019-0704-8

44. Morita N, Umemoto E, Fujita S, Hayashi A, Kikuta J, Kimura I, et al. Gpr31-dependent dendrite protrusion of intestinal Cx3cr1(+) cells by bacterial metabolites. *Nature* (2019) 566(7742):110–4. doi: 10.1038/s41586-019-0884-1

45. Yoshii K, Hosomi K, Sawane K, Kunisawa J. Metabolism of dietary and microbial vitamin b family in the regulation of host immunity. *Front Nutr* (2019) 6:48. doi: 10.3389/fnut.2019.00048

46. Peterson CT, Rodionov DA, Osterman AL, Peterson SN. B vitamins and their role in immune regulation and cancer. *Nutrients* (2020) 12(11):3380. doi: 10.3390/nu12113380

47. Goedert JJ, Hua X, Bielecka A, Okayasu I, Milne GL, Jones GS, et al. Postmenopausal breast cancer and oestrogen associations with the iga-coated and iga-noncoated faecal microbiota. *Br J Cancer* (2018) 118(4):471–9. doi: 10.1038/bjc.2017.435

48. Qi Y, Zang SQ, Wei J, Yu HC, Yang Z, Wu HM, et al. High-throughput sequencing provides insights into oral microbiota dysbiosis in association with inflammatory bowel disease. *Genomics* (2021) 113(1 Pt 2):664–76. doi: 10.1016/j.ygeno.2020.09.063

49. Mottawea W, Chiang CK, Mühlbauer M, Starr AE, Butcher J, Abujamel T, et al. Altered intestinal microbiota-host mitochondria crosstalk in new onset crohn's disease. *Nat Commun* (2016) 7:13419. doi: 10.1038/ncomms13419

50. Yachida S, Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, et al. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* (2019) 25(6):968–76. doi: 10.1038/s41591-019-0458-7

51. Ma N, Wu Y, Xie F, Du K, Wang Y, Shi L, et al. Dimethyl fumarate reduces the risk of mycotoxins *Via* improving intestinal barrier and microbiota. *Oncotarget* (2017) 8 (27):44625–38. doi: 10.18632/oncotarget.17886

52. Yuan Y, Lu L, Bo N, Chaoyue Y, Haiyang Y. Allicin ameliorates intestinal barrier damage *Via* microbiota-regulated short-chain fatty acids-Tlr4/Myd88/Nf-Kb cascade response in acrylamide-induced rats. *J Agric Food Chem* (2021) 69(43):12837–52. doi: 10.1021/acs.jafc.1c05014

53. Baxi S, Yang A, Gennarelli RL, Khan N, Wang Z, Boyce L, et al. Immune-related adverse events for anti-Pd-1 and anti-Pd-L1 drugs: Systematic review and meta-analysis. *BMJ* (2018) 360:k793. doi: 10.1136/bmj.k793

54. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-Small-Cell lung cancer. *N Engl J Med* (2018) 378(22):2078–92. doi: 10.1056/NEJMoa1801005

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Mechanismbased role of the intestinal microbiota in gestational diabetes mellitus: A systematic review and meta-analysis

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Background: Metabolic disorders caused by intestinal microbial dysregulation are considered to be important causes of gestational diabetes mellitus (GDM). Increasing evidence suggests that the diversity and composition of gut microbes are altered in disease states, yet the critical microbes and mechanisms of disease regulation remain unidentified.

Methods: PubMed® (National Library of Medicine, Bethesda, MD, USA), Embase® (Elsevier, Amsterdam, the Netherlands), the Web of Science™ (Clarivate™, Philadelphia, PA, USA), and the Cochrane Library databases were searched to identify articles published between 7 July 2012 and 7 July 2022 reporting on case–control and controlled studies that analyzed differences in enterobacteria between patients with GDM and healthy individuals. Information on the relative abundance of enterobacteria was collected for comparative diversity comparison, and enterobacterial differences were analyzed using random effects to calculate standardized mean differences at a *p*-value of 5%.

Results: A total of 22 studies were included in this review, involving a total of 965 GDM patients and 1,508 healthy control participants. Alpha diversity did not differ between the participant groups, but beta diversity was significantly different. *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* were the dominant bacteria, but there was no significant difference between the two groups. Qualitative analysis showed differences between the groups in the *Firmicutes*/*Bacteroidetes* ratio, *Blautia*, and *Collinsella*, but these differences were not statistically different.

Conclusion: Enterobacterial profiles were significantly different between the GDM and non-GDM populations. Alpha diversity in patients with GDM is similar to that in healthy people, but beta diversity is significantly different. *Firmicutes*/

Bacteroidetes ratios were significantly increased in GDM, and this, as well as changes in the abundance of species of *Blautia* and *Collinsella*, may be responsible for changes in microbiota diversity. Although the results of our meta-analysis are encouraging, more well-conducted studies are needed to clarify the role of the gut microbiome in GDM. The systematic review was registered with PROSPERO (https://www.crd.york.ac.uk/prospero/) as CRD42022357391.

KEYWORDS

gestational diabetes mellitus, gut microbiota, meta-analysis, systematic review, insulin resistance

1 Introduction

Gestational diabetes mellitus (GDM) is characterized by insufficient insulin secretion and impaired glucose intolerance during pregnancy (1). It has been estimated that over the past 20 years the worldwide prevalence of GDM has been up to 14% of pregnancies, though estimates may vary among regions depending on diagnostic criteria (2). GDM has been associated with adverse pregnancy outcomes among pregnant women including dystocia, macrosomia, neonatal hypoglycemia, and birth injuries (3). In addition, it has been reported that, in the long run, GDM is associated with type 2 diabetes mellitus (T2DM), metabolic syndrome, and cardiovascular disease, making it more potential of developing T2DM than ordinary pregnancies. It is generally believed that GDM develops when pancreatic beta cells fail to produce sufficient insulin to meet the demands of the relevant tissues for blood glucose regulation (4, 5). Therefore, GDM can be considered a manifestation of prediabetes in the form of impaired glucose tolerance in non-pregnant individuals (1). Undoubtedly, diagnosis and treatment can not only reduce the risk of perinatal complications but can also reduce the economic burden on both patients and countries. The human gut microbiota is considered to have a profound influence on host metabolism (6), and alteration of the homeostasis of the intestinal microbiota can have far-reaching consequences. For instance, a reduced number of bacteria such as bifidobacteria and *Bacteroides* affects lipid metabolism, whereas an increased number of enterobacteria can lead to insulin resistance (6–9). Although previous studies have explored different populations, most have focused on the components of and changes in the gut microbiota in GDM during different pregnancy periods. Few studies have explored differences in the microbiota in patients with GDM and healthy controls. In addition, the results have been inconsistent and findings cannot be reproduced. Specific and meticulous pathogenesis remains to be identified.

Various mechanisms explaining the link between the gut microbiota and GDM have been proposed. A lack of SCFAs (short-chain fatty acids) is one reason that has been suggested. This is particularly common among those consuming Western-style diets, which are known to be low in fiber and digestible carbohydrates, which may contribute to a reduction in microbial diversity and cause microbial dysbiosis. Such diets could also change the profile of gut microbiota, so as to impair the integrity of the wall of the intestine and cause gut permeability. Thus, one effect of insufficient SCFAs may be translocation of toxins from the gut lumen to the systemic circulation (10).

Another proposed mechanism is associated with some other foods, such as fish and red meat. The intestinal flora can produce trimethylamine *N*-oxide (TMAO) by metabolizing those meat, thus affecting the immune system. In addition, activation of the intracellular thioredoxin-interacting protein (TXNIP) leads to an in increase in the expression of the NLRP3 gene [NOD-like receptor (NLR) family pyrin domain containing 3] and increased inflammatory markers in blood, especially tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), interleukin 18 (IL-18), and interleukin-1β (IL-1β) (11–13). Dietary TMAO then further increases fasting insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR) by inducing adipose tissue inflammation (14), exacerbating impaired glucose tolerance. The mechanisms affected by the gut microbiota may indicate possible treatments for diabetes mellitus.

The aim of this review was to collect evidence from cohort and case–control studies to provide a theoretical basis for the diagnosis, intervention, and treatment of diseases linked to the gut microbiota by analyzing differences in enterobacteria and exploring inflammation and possible immune mechanisms associated with disease pathophysiology.

2 Materials and methods

The scheme of the metareview has been registered with PROPSERO (as CRD42022357391) and searches were conducted in accordance with the updated 2008 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (15) and checklist.

2.1 Data sources and search strategy

A systematic literature search of four databases [PubMed® (National Library of Medicine, Bethesda, MD, USA), the Cochrane Library, Embase® (Elsevier, Amsterdam, the Netherlands), and Web of Science™ (Clarivate™, Philadelphia, PA, USA)] was conducted by combining medical subject headings (MeSH) words with free words. We selected articles in English published between 7 July 2012 and 7 July 2022 and focused on human studies.

The search strategy was built by combining the following MeSH words with free words: ((("Diet"[Mesh]) OR ("Life Style"[Mesh]) OR ("Exercise"[Mesh]) OR ("Motor Activity"[Mesh]) OR ("Probiotics"[Mesh]) AND (("Cohort Studies"[Mesh]) OR (Cohort studies [Title/Abstract])) AND ((16S rRNA [Title/Abstract])) AND (("Diabetes, Gestational"[Mesh])). Various free words were added to improve the search results and identify articles that might otherwise have been missed. The search strings are provided in Supplementary Table 1.

2.2 Eligibility criteria

Titles and abstracts were screened by two investigators. Any disagreements between the two researchers were resolved by a third one. Before the formal literature selection, the three investigators were trained to ensure that they had consistent screening standards. Studies were included if they met the following criteria (1): they were cohort studies or case studies among pregnant women with GDM (2); metagenomics sequencing or 16S rRNA sequence analysis was carried out (3); they reported maternal outcomes such as HbA1c, fasting blood glucose level, and gestational weight gain (4); they were published from 7 July 2012 to 7 July 2022 (5); they were published in English; and (6) the population studied was aged >18 years.

Articles were excluded if they met any of the following criteria (1): they reported on trials that were not carried out in humans (2); they were non-randomized controlled studies (3); they analyzed probiotics in conjunction with other GDM therapies in the same intervention group (4); they were abstracts, case reports, expert opinions, reviews, letters, or editorials; or (5) they lacked sufficient data or did not meet the inclusion criteria.

2.3 Data extraction and synthesis

Articles on pregnant women under 45 years old who had been diagnosed with GDM at a specific time point were chosen. The diagnosis of GDM was confirmed by a national or international standard. A further requirement was that the control groups should be healthy and the GDM group should not also have other metabolic diseases.

All articles were screened, and those deemed ineligible by the two researchers were removed; any disagreements were resolved by the third researcher. Detailed information was then recorded in an Excel spreadsheet. This included basic information such as authors, publication year, district, and study types, as well as maternal outcomes such as HbA_{1c} and fasting blood glucose levels and details of the gut microbiome, including diversities in richness, evenness, and similarity between communities.

2.4 Quality assessment and risk of bias

We used the Newcastle–Ottawa Scale (NOS), which was developed for the assessment of cohort studies and case studies, to score the included studies (16). For the purpose of reaching accurate methodological quality, the NOS was evaluated through three dimensions (1): selection (2), comparability, and (3) outcome. A quality score ranging from 0 to 9 was obtained through a rating algorithm, with a score of 0–5 meaning poor quality, a score of 6–7 meaning moderate quality, and a score of 8–9 meaning high quality. The specific scores of each article according to the NOS are shown in Figure 1 and Supplementary Table 2.

2.5 Statistical analysis

Standardized mean differences (SDMs) were used to summarize and study differences among the studies and relevant measures, and 95% confidence intervals (CI) were used to estimate the differences in gut microbiota diversity between the GDM and the non-GDM groups. Operational taxonomic unit (OTU) data for each study were analyzed using RevMan 5.3 software, provided by the Cochrane Collaboration Network. Meta-analysis was performed using a random-effects model or a fixed-effects model. Sensitivity

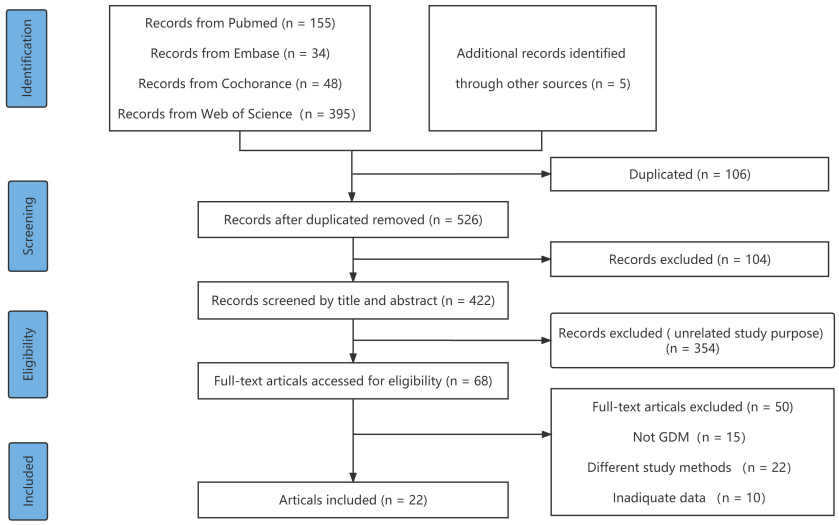

FIGURE 1
The PRISMA flow diagram of study identification.

analyses were performed using the Egger test. Forest plots were created to visualize differences in microbial community structure between samples using a random-effects (RE) model and a fixed-effects (FE) model according to the reported I^2-value. An I^2-value > 50% and a p-value > 0.05 were considered statistically significant (17).

Qualitative data, such as the relative abundance of specific genera, were recorded for analysis. Subgroup analyses for confounding factors, such as diet, were performed. Because qualitative beta diversity was not provided, descriptive analyses could not be performed. We therefore chose to perform semiquantitative analysis. If two or more articles reported consistent study results, it was considered that the results were related to the disease and were worthy of further exploration and explanation.

3 Results

3.1 Search results and study eligibility

A total of 632 articles identified from four databases (PubMed, 155; Embase, 34; the Cochrane Library, 48; and Web of Science, 395) were retrieved. Of these, 106 articles were duplicates and were removed, 354 articles were excluded because they reported on different study purposes, and 20 were excluded because they described other study designs. After excluding publications that did not meet the inclusion criteria, 22 (8, 18–27) studies remained and were subjected to full-text scanning for this systematic review. The flow chart illustrating the selection process is provided in Figure 1.

Among all the selected studies, 18 studies were conducted in Asia (16 from China, one from Malaysia, and one from Japan). Nine were cohort studies, while 13 were case studies. The age of the participants ranged from 28 to 45 years. The information on participants that was recorded included findings related to blood biochemistry, chronic inflammation, and biomarkers. Most of these studies used the International Association of Diabetes Pregnancy Group's criteria; only one study, from Thailand, chose its own criteria (22). Owing to different methodologies of analysis, the storage temperature of the stool sample varied (–20°C in one study and –80°C in 19 studies). We focused on the differences emerging when comparing GDM patients with healthy people. One article divided participants into four groups according to their blood pressure and lipid measurement (20). Therefore, we took only the GDM group and the healthy group into consideration. One article did not exclude those who had probiotics or antibiotic treatment during pregnancy (23). Only two articles included the sample size calculation, which makes these studies more reliable than the others (18, 28) (Supplementary Table 4).

3.2 Quality of included studies

Quality was assessed using the NOS. Each study was evaluated based on three criteria, namely selection of the participants, comparability of groups (i.e., absence of confounder bias), and exposure (measurement of outcome influencing). Ten articles were assessed as being of fair quality and 12 as being of good quality, no studies were of poor quality The detailed scores for every study are shown in Figure 2.

FIGURE 2
Quality score of included articles calculated using the NOS. The overall article quality score and risk of publication bias are obtained by calculating three indicators.

3.3 GDM detection and study criteria

In general, GDM is diagnosed at 24–28 gestational weeks, using established criteria from the International Association of Diabetes and Pregnancy Study Groups (IADPSG) based on the results of a standard 2-h, 75-g oral glucose tolerance test (OGTT) (29). Pregnant women are diagnosed with GDM if one or more glucose levels is elevated, as follows: fasting ≥ 5.1 mmol/L, 1 h ≥ 10.0 mmol/L, and 2 h ≥ 8.5 mmol/L.

The basic demographics and clinical characteristics of the participants are shown in Supplementary Table 3. The included studies involved 965 participants with GDM and 1,508 healthy people. Only four studies recorded and accounted for pre-study body mass index (BMI) in order to obtain more objective results (18, 19, 28, 30). Participants with other metabolic diseases or who had been treated with antibiotics or probiotics were excluded.

3.4 Methodological characteristics of selected articles about stool sample

Stool samples were generally stored at −80°C, except in one study, in which the samples were stored at −20°C (18). Studies used a wide range of DNA extraction kits. Only the QIAamp Fast DNA StoolMini Kit (Portsmouth, NH, USA) was used more than twice. Target DNA sequencing in the V3–V4 region was the most common technique, used in 15 studies, whereas four studies (20, 21, 31, 32) sequenced in the V4 region, one (23) sequenced in the V1–V2 region, and one (26) sequenced in the V6–V8 region. There were four criteria for defining clustering of OTUs, namely 95% OTUs (1/22), 97% OTUs (13/22), 99% OTUs (5/22), and 100% OTUs (1/22). Taxonomy annotation was conducted mostly with an RDP (Ribosomal Database Project) classifier trained on the SILVA (6/20), RDP (3/22), and Greengenes (5/22) databases. Specific information is shown in Supplementary Table 4.

3.5 Alpha diversity and beta diversity

Diversities are commonly known as alpha diversity and beta diversity. They are used to describe the species composition of the gut microbiota, which may be considered a significant factor influencing outcomes.

Alpha diversity can predict both the number of the species and individual distribution, known as richness and evenness, and can be measured by the ACE (abundance-based coverage estimator), Chao1, Simpson, and Shannon indexes. Among the included studies, four (18, 19, 33, 34) compared the ACE in GDM and non-GDM patients [SMD −0.28 (95% CI −1.98 to 1.42), $p = 0.75$, $I^2 = 98\%$]. Six studies (18, 19, 24, 33–35) reported the Chao1 index [SMD 0.48 (95% CI 0.23 to 0.73), $p < 0.05$, $I^2 = 67\%$] for quality assessment. The Shannon index [SMD −0.06 (95% CI −0.67, 0.94), $p = 0.84$, $I^2 = 88\%$] was provided in six studies (18, 19, 28, 33–35) and the Simpson index in seven studies (18, 19, 23, 24, 33–35) [SMD −0.44 (95% CI −1.30 to 0.41), $p = 0.31$, $I^2 = 95\%$]. The indexes were the same in each group (Figure 3).

The result for the heterogeneity assessment was not as good as we had anticipated. The I^2-value was >50%, indicating strong heterogeneity. The source of this heterogeneity should be further explored. Therefore, we further performed sensitivity analyses, omitting each study in turn, to ensure accuracy and stability of the results. If, after removing an article from the indexes, both the I^2-value and the p-value were stable, indicating that the article has stable sensitivity analysis results.

In addition, to test our hypothesis that the results were reliable, we assessed the risk of publication bias using Egger's tests, based on the symmetry of a funnel chart. The results indicated no evidence of publication bias, since the p-value of each index was above 0.05, showing that the conclusions of the meta-analysis were relatively robust. These results can be seen in Supplementary Figure 1.

As for beta diversity,17 articles reported beta diversity, of which 11 used principal component analysis (PCA) and 10 reported results using principal coordinate analysis (PCoA). We were unable to conduct a robust analysis of beta diversity because results were mostly provided in graphical form, rather than as specific data.

3.6 Subgroup analysis

Owing to the significant statistical heterogeneity encountered in the analysis, several subgroup analyses were conducted separately. Analyses of classifications of gut microbiome and dietary intakes were carried out provided these were reported in at least two articles.

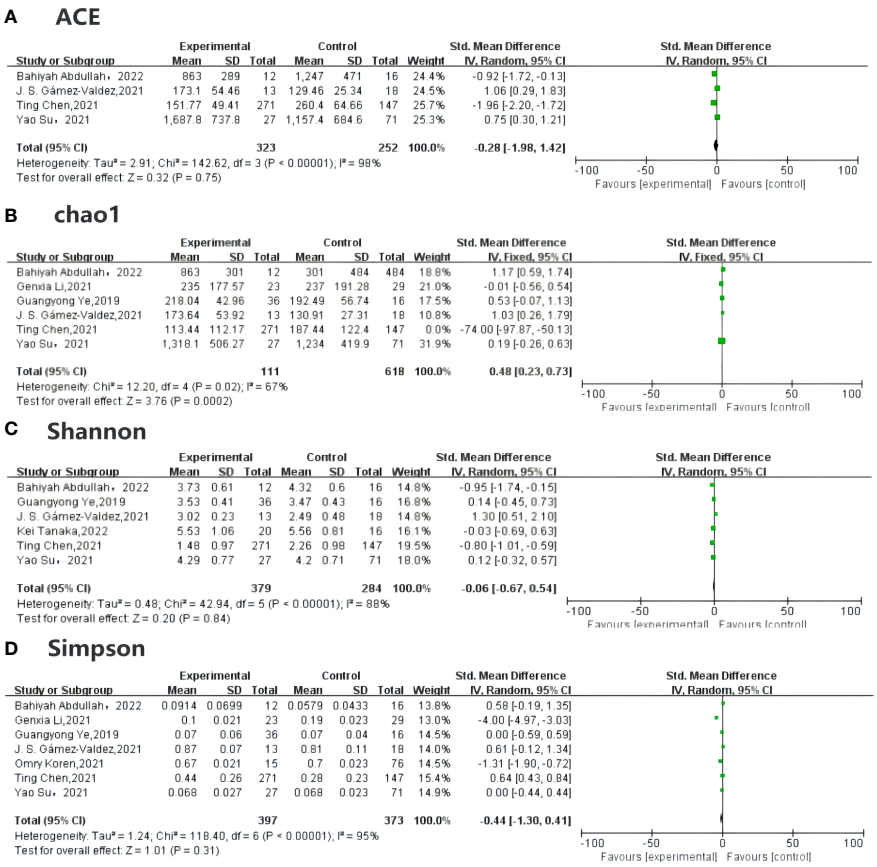

FIGURE 3
Forest plot of randomized controlled trials comparing the alpha diversity between GDM and NGDM. **(A)** plot means SDs of ACE, **(B)** plot described the Chao1between articles, and **(C)** plot indicates the result for Shannon index, **(D)** plot mentioned the Simpson index, which are all reported in studies. Differences between groups are presented as weights (percentages) and SMD (95% CI). CI, confidence interval; IV, inverse variance; SD, standard deviation; SMD, standardized mean difference.

Subgroup analysis of the gut microbiome at genus level showed that the abundances of *Blautia* [SMD 0.36 (95% CI 0.02 to 0.71), $p = 0.04$, $I^2 = 0\%$] and *Collinsella* [SMD −4.18 (95% CI −8.73 to 0.38), $p = 0.38$, $I^2 = 78.9\%$] were significantly higher in the GDM group than in the control group. There were no differences in-between groups in the abundances of *Clostridium* [SMD −0.47 (95% CI −0.92 to −0.01), $p = 0.187$, $I^2 = 42.4\%$] and *Faecalibacterium* [SMD −0.19 (95% CI −0.42 to 0.04), $p = 0.971$, $I^2 = 0\%$].

Similarly, subgroup analysis showed that high fiber intake, compared with lower fiber intake, protects against GDM [SMD −0.96 (95% CI −0.95 to −0.43), $p < 0.05$, $I^2 = 92\%$], whereas there were no differences between patients and control participants in energy intake [SMD −0.46 (95% CI −1.84 to 0.93), $p = 0.52$, $I^2 = 97\%$], cereal intake [SMD −0.31 (95% CI −0.92 to 0.29), $p = 0.31$, $I^2 = 86\%$], meat intake [SMD −0.08 (95% CI −0.31 to 0.15), $p = 0.15$, $I^2 = 93\%$], or milk intake [SMD −0.66 (95% CI −1.55 to 0.24), $p = 0.15$, $I^2 = 93\%$], although there remained considerable heterogeneity in all analyses. Specific results and data can be viewed in Table 1.

3.7 Differences in taxa abundance in the gut microbiota during pregnancy

The current study compared the gut microbiota in patients with GDM and healthy participants. Nine (18, 19, 21, 23, 24, 27, 30, 34, 36) articles analyzed the taxa at the phylum level. Three (18, 19, 24) articles mentioned *Firmicutes* and concluded that they were less abundant in GDM patients, whereas two (30, 36) articles reported the opposite results. *Bacteroidetes* are more abundant in GDM patients than in participants without DGM. Two articles (23, 34) reported that the abundance of *Actinobacteria* was lower in GDM patients than in those without GDM. Four studies (24, 34, 36, 37) reported that the abundance of *Proteobacteria* was higher in GDM patients than

TABLE 1 Subgroup analysis of effect of gut microbiota and dietary intake.

Subgroup	No. of studies	Effect (95% CI)	Coherence		I^2	Egger
			Q-value	*p*-value		
Blautia	3	0.36 (0.02 to 0.71)	2.72	0.040*	0%	0.332
Collinsella	2	−4.18 (−8.73 to 0.38)	2.08	0.038*	78.9%	–
Clostridium	2	−0.47 (−0.92 to −0.01)	1.31	0.187	42.4%	–
Faecalibacterium	2	−0.19 (−0.42 to 0.04)	0.97	0.332	0%	–
Diet						
Energy intake	4	−0.46 (−1.84 to 0.93)	5.80	0.52	97%	0.448
Cereal intake	4	−0.31(−0.92 to 0.29)	1.60	0.31	86%	0.515
Meat intake	3	−0.08 (−0.31 to 0.15)	0.83	0.48	0.0%	0.824
Milk intake	4	−0.66 (−1.55 to 0.24)	2.79	0.15	93%	0.511
Fiber intake	3	−0.96 (−0.95 to −0.43)	22.03	0.028*	92%	0.990

*Significant difference at a p-value of <0.05.

in healthy controls. Only one study (23) reported the opposite. Three studies (24, 27, 30) compared *Firmicutes*/*Bacteroides*, two of which (24, 30) found that their abundance was higher in the GDM group.

At the class level, one (21) study mentioned that *Rothia*, *Actinomyces*, *Bifidobacterium*, *Adlercreutzia*, and *Coriobacteriaceae* from *Actinobacteria* were reduced in the GDM population.

At the family level, there were slight differences between studies. Both *Veillonellaceae* (21, 37) and *Prevotella* group 9 (30, 37) were increased in GDM patients, whereas *Lachnospiraceae* and *Ruminococcaceae* (24) were decreased. Some studies drew controversial conclusions as regard *Streptococcaceae*, *Enterobacteriaceae*, *Lachnospira*, *Clostridiales*, *Clostridia*, and *Firmicutes*. Two (24, 36) studies found that, at the family level, members of the family *Clostridiales* were increased in GDM patients.

Studies reporting findings at the genus level were the most common. We found enrichment of *Ruminococcaceae*, *Lactococcus*, *Escherichia*, *Lachnospiraceae*, *Clostridia*, *Alistpes*, *Firmicutes*, and *Phascolarctobacterium*. Results regarding *Streptococcus* and *Bacteroidetes* varied. We also found enrichment of *Coprococcus*, *Staphylococcus*, *Oscillospira*, Burkholderiales, *Akkermansia*, *Prevotella* group 9, and *Faecalibacterium* in participants without GDM. Abundances of *Blautia*, *Staphylococcus*, *Sutterella*, *Oscillospira*, *Enterococcus*, and *Lactobacillus* also varied, and this needs further study.

We performed further random forest analysis of four dominant bacteria. *Proteobacteria* are relatively abundant in GDM [SMD −0.44 (95% CI −1.3 to 0.41), p = 0.31, I^2 = 95%], whereas the abundances of *Actinobacteria* [SMD −1.64 (95% CI −2.32 to −0.95), p < 0.05, I^2 = 94%], *Bacteroides* [SMD 7.96 (95% CI −7.77 to 23.69), p = 0.32, I^2 = 100%], *Firmicutes* [SMD 0.25 (95% CI −0.01 to 0.51), p = 0.06, I^2 = 49%], and *Proteobacteria* [SMD 1.10 (95% CI −1.59 to 3.80), p = 0.42, I^2 = 99%] did not differ significantly between the two groups.

3.8 Types of gut microbiota and their impacts on GDM

To further explore the potential correlations of key clinical indexes with altered gut microbiome in GDM, correlation analyses were performed using Spearman analysis. The results showed that phylum *Bacteroidetes* was positively associated with 1hPG, whereas *Proteobacteria*, *Verrucomicrobiota*, and *Actinobacteria* were all negatively associated with 1-hour plasma glucose level (1hPG) levels. Analysis at the genus level revealed a negative association between *Ruminococcaceae* UCG014 and 1hPG, but a positive association between *Ruminococcaceae* UCG014 and high-density lipoprotein (HDL) levels (36). The genus *Akkermansia* was negatively correlated with 1hPG and positive correlated with HDL levels. According to Chen et al., genera of *Clostridiales*, *Ruminococcaceae* and *Lachnospiraceae*, within the phylum *Firmicutes*, were significantly negatively correlated with at least one OGTT value (19). An unassigned genus of *Enterococcaceae* within the phylum *Firmicutes*, the genus *Atopobium* within the phylum *Actinobacteria* and the genus *Sutterella* within the phylum *Proteobacteria* were significantly positively associated with 1-h or 2-h OGTT values (Supplementary Table 5; Figure 4).

Indexes of inflammation, fecal calprotectin (FCALP), lipopolysaccharide (LPS), lipopolysaccharide-binding protein (LBP), and fecal LPS (FLPS), were reported in two (20, 26) studies. Levels of zonulin, FCALP, LPS, LBP, and FLPS were higher in GDM patients than in those without GDM, and the results were statistically significant. Cui et al. reported that

Enterococcus and *Vagococcus* are in direct proportion to FCALP and LPS, *Streptococcus* is inversely proportional to LBP, and *Staphylococcus* is directly proportional to FLPS (20). Women's diet, including total energy and fiber intake, remained unchanged between sampling times.

Four articles reported Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The conclusions drawn in each article vary widely. Chen et al. identified several microbial gene pathways including the glycan biosynthesis and vitamin metabolic pathways (19). Li et al. showed that the species of gut microbes found in normoglycemic pregnant women (NOR) and GDM are involved in cell wall/membrane/envelope biogenesis, organic ion transport and metabolism, post-translational modifications, protein turnover, chaperones, transcription, unknown function, intracellular transport, secretion, and vesicular transport (24). Su et al. found a positive relationship between *Bacteroides* species enriched in patients with GDM, and amino sugar and nucleotide sugar metabolism (34). Wang et al. found that the predicted metagenome of women who developed GDM was enriched in organisms involved in starch and sucrose metabolism, whereas those implicated in lysine biosynthesis and nitrogen metabolism were reduced (37).

4 Discussion

To the best of our knowledge, this is the first article to systematically analyze how enterobacterial differences affect metabolic health in GDM patients and healthy individuals. Consistent evidence has shown that the composition of the gut microbiota is specifically altered in GDM. We found differences between groups in the abundance of microorganisms. These suggested that an increase in *Blautia* and a reduction in *Clostridium* may make a huge contribution, and thus may provide bacterial targets to prevent or treat GDM by reconstructing the homeostasis of the gut microbiota. Future studies in this area are warranted. The primary purpose of this study was to determine the validations between the participant groups, which may provide insights into possible mechanistic links between the gut microbiota and GDM and the pathway leading to disease development. The available results suggested an association between microbial composition and disease. Despite differences in lateral suction patterns, diversity and composition results were generally consistent between studies. Alpha diversity is widely used for measuring the richness and evenness of the gut microbiome. Our meta-analysis demonstrated a significant association between the presence of GDM and reductions in diversity indexes, suggesting that species richness was reduced in the affected individuals. Although alpha diversity has been shown to be a marker of chronic diseases, such as T2DM, colorectal cancer, and non-alcoholic fatty liver disease (NAFLD), multiple studies of the gut microbiome in patients withT2DM have shown no statistically difference in alpha diversity among well-matched participants (38–42).

Some studies found that alpha diversity was slightly lower, but not significantly reduced, in T2DM patients than in healthy participants (41). Occasionally, richness (as measured by the Shannon and Simpson indexes), which has been associated with elevated insulin resistance, shows an overlapping trend in GDM patients and healthy individuals. However, our meta-analysis results showed that there were no significant differences in diversity or richness between GDM patients and healthy individuals, indicating that alpha diversity may not be a hallmark indicator distinguishing between individuals with and without GDM. In contrast to alpha diversity, beta diversity differed in the GDM group and the control group participants, highlighting the fact that the profile of gut microbiota was altered in GDM. Further studies at the phylum level identified the taxa of the gut microbiota whose abundance was altered by GDM and suggested that an increase in *Bacteroides* and *Proteobacteria* and a reduction in *Actinobacteria* may contribute to GDM.

The phyla identified after *in vitro* fecal fermentation were mainly *Firmicutes*, *Bacteroides*, *Actinobacteria*, and *Proteobacteria*. In addition, this review found that the *Firmicutes*/*Bacteroidetes* ratio was significantly higher in GDM patients than in healthy control participants. *Firmicutes* and *Bacteroides* are the two dominant bacterial groups in the gut. They can maintain energy balance in the host by participating in the metabolism of fats and bile acids. The *Firmicutes*/*Bacteroides* ratio is commonly used as a marker of low-grade systemic inflammation in obesity and insulin resistance and as an indicator of gut microbiota composition in different individuals (43–46). Similar to other findings (47, 48),the *Firmicutes*/*Bacteroides* ratio was higher in GDM patients, indicating that it is a sensitive indicator enabling patients with GDM to be distinguished from those who do not have GDM. Animal experiments have shown that colonization of the normal gastrointestinal tract, as shown in the cultivate experiments of *Bacteroides* species and meditated through toll-like receptors (TLRs) and other specific host–microbe interactions is a result of recognition and selection by the host immune system (49). In general, insulin resistance is associated with a higher *Firmicutes*/*Bacteroides* ratio and a reduction in the number of butyrate-producing bacteria, while *Bacteroidetes*, as the most stable component of the gastrointestinal microbiota in healthy adults, contains most genera that produce butyrate (50). Butyrate is considered a health-promoting molecule because it can increase insulin sensitivity, exert anti-inflammatory activity, regulate energy metabolism, and increase leptin gene expression (51–54). Propionate in the colon stimulates the release of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) from L-enteroendocrine cells, thereby suppressing appetite (55). It may also reach the portal circulation and get captured by the liver tissue, where it

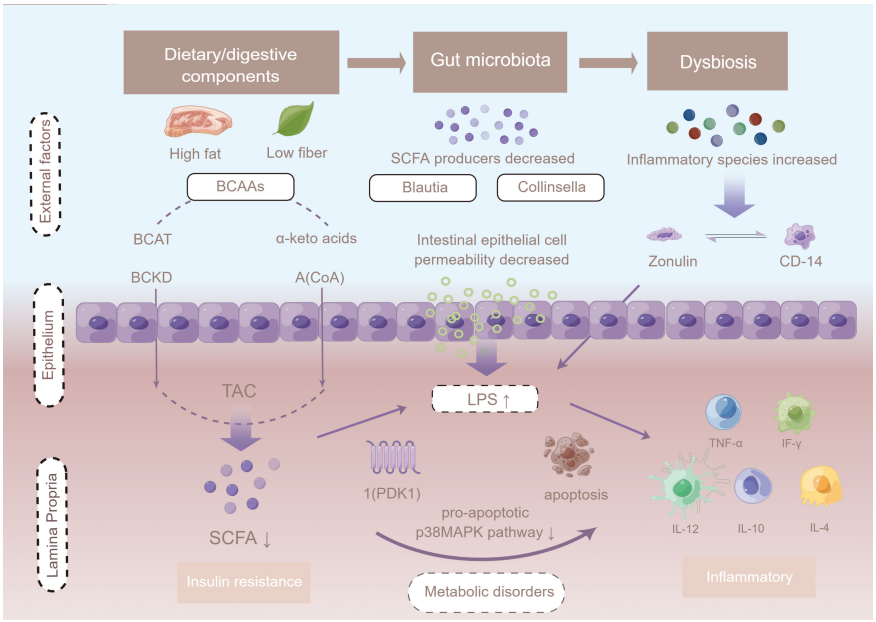

FIGURE 4
The potential mechanism linking gut dysbiosis and GDM. Intestinal permeability is regulated by dietary factors and the zonulin pathway. Cells in the basal layer of the intestinal epithelium secrete zonulin and bind to initiate complex intracellular signaling pathways, allowing phosphorylation of tight junctions. When LPS is ready to enter the advocate circulation, it increases absorption, and forms a complex with LBP that further binds CD14 released from monocytes, leading to the production of pro-inflammatory cytokines mediated by the MD2/TLR4 receptor complex, such as TNF-α, interleukin 1 (IL-1), and interleukin 6 (IL-6). LPS infiltrates peripheral adipose tissue and binds to TLRs, thus activating the adaptor proteins MyD-88, IRAK, TAK1, and TRAF6, and triggering macrophage infiltration and up-regulation of inflammatory pathways. Up-regulation of JNK/IKKβ/NF-κB may increase serine phosphorylation of IRS-1 + Ser307, resulting in PI3-K inhibition and Akt down-regulation of Ser473. Figure has been obtained for use of copyrighted material from Figuer (https://www.figdraw.com/, registered ID: 533420148).

participates in hepatic gluconeogenesis and reduces the expression of enzymes involved in fatty acid and cholesterol synthesis (56).

Blautia has shown a significant negative correlation with many diseases, including type 1 diabetes mellitus (T1DM), obesity, and Crohn's disease (57, 58). As a risk marker of adiposity and of cardiovascular and metabolic disease, *Blautia* abundance has been shown to be inversely associated with visceral fat tissue (58). This may be due to a potential anti-inflammatory effect that can reduce the ratio of TNF-α to IL-4 in T2DM patients, balance immunity with anti-inflammation, and help maintain glucose homeostasis, so as to regulate the transduction of insulin signaling, thereby regulating the conduction of insulin signals such as fasting blood glucose (FBG) level (59–61). The abundance of *Blautia* in patients with T1DM is consistent with HbA_{1c} and FBG results (57). This study did in fact find that *Blautia* abundance was slightly increased in GDM patients, which may be because disease was most often diagnosed in the first trimester (62). From the first to the third trimesters, *Blautia* levels gradually declined, resulting in a decrease in butyrate production, stimulating neutrophils and macrophages to release inflammatory factors (63). An increase in inflammatory factors is associated with low levels of fiber intake, which may lead to the metabolism, and thereby cause dysbiosis and aggravated inflammation (64).

The genus *Collinsella* from the family *Lachnospiraceae* is often described as a strictly anaerobic pathobiont that produces lactate, which is often correlated with SCFAs (65). The decreased abundance of this taxon is associated with the health status of patients suffering from T1DM and T2DM (41). This genus of bacteria seems to be stimulated by a low-fiber diet, which could be observed in GDM patients. Both *in vitro* and animal experiments have concluded that SCFAs modulate intestinal inflammation by improving transepithelial resistance, altering various signaling pathways, and inhibiting pro-inflammatory cytokines, while up-regulating anti-inflammatory cytokines (66, 67). SCFAs stimulate glucagon production and signal the hypothalamus as a mechanism of diabetes. Butyrate suppresses the production of pro-inflammatory cytokines, for example TNF-α, IL-12, and interferon γ (IF-γ), and up-regulates the production of anti-inflammatory IL-10 by monocytes, thereby producing anti-inflammatory effects (53). Butyrate has been shown to attenuate LPS-stimulated pro-inflammatory effects (68, 69). Intake of a high-fat and low-fiber diet may alter the normal composition of gut microbiota and dietary fermentation. Alterations in dietary fermentation may lead to excessive production of SCFAs and absorption of energy from the diet. The relative abundance of *Firmicutes* was elevated, while the abundances of *Actinobacteria* and *Bacteroidetes* were lower, in

women with GDM (70). This "gut microbiota signature" is similar to the phenotype of metabolic disorder, which is mainly due to the obese phenotype (71). Moreover, metabolic pathways mentioned in research are those linked with carbohydrate metabolism, such as glycolysis/gluconeogenesis, starch and sucrose metabolism, and galactose metabolism, which can be enriched in women with GDM (64). The results of our investigation of dietary status showed no difference in energy intake between the two groups of individuals (i.e., those with and without GDM), but that dietary fiber intake was lower in the GDM group in the non-GDM population, suggesting a possible mechanism of GDM. Butyrate-producing bacteria, such as *Faecalibacterium* and *Akkermansia* responses the same trend as on fiber intake in the context of the entire diet (72, 73). Thus, it is clear that dietary fiber components are enhanced with the microbiota.

Intestinal microbes also regulate the process of absorption of metabolites and endotoxins by affecting intestinal permeability. It has been shown that the development of GDM is associated with an increase in LPS in the intestine during late pregnancy as well as intestinal mucosal injury characterized by elevated levels of serum LPS and streptoglobulin. In this study, the level of inflammatory factors was significantly higher in GDM patients than in those without GDM, which is consistent with specific physiological changes. Intestinal permeability is regulated by dietary factors and the zonulin pathway. Cells in the basal layer of the intestinal epithelium secrete zonulin and bind to initiate complex intracellular signaling pathways, allowing phosphorylation of tight junctions, which in turn leads to an increased permeability (74). When LPS is ready to enter the advocate circulation, it would increase absorption, and form a complex with LBP that further binds CD14 from monocytes (75). This may lead to the production of pro-inflammatory cytokines mediated by the MD2/TLR4 receptor complex, such as TNF-α, IL-1, and IL-6 and LPS, which infiltrate peripheral adipose tissue and bind to TLRs, activating the adaptor proteins MyD-88, IRAK, TAK1, and TRAF6, and as a result triggering macrophage infiltration and up-regulation of inflammatory pathways. Up-regulation of JNK/IKKβ/NF-κB may increase serine phosphorylation of IRS-1 + Ser307, resulting in PI3-K inhibition and Akt down-regulation of Ser473. Reducing acetate Ser473 phosphorylation may impair insulin signaling and reduce glucose uptake in peripheral tissues, leading to hyperglycemia in women with GDM (74, 76).

Previous studies have shown that the efficiency of energy extraction from the diet is correlated with the enrichment of specific metabolic pathways, particularly those involved in carbohydrate transport and utilization (77). Amino acids are also insensitive to insulin action. Isoenzyme branched-chain amino acid aminotransferase (mitochondrial BCAT and cytosolic BCAT) catalyze the first reversible transamination/deamination of branched-chain amino acids (BCAAs) to their corresponding α-ketoacids. They are then combined in order to convert α-ketoglutarate into glutamate; leucine, isoleucine, and valine yield α-ketoisocaproate (KIC), α-keto-β-methylglutarate (KIM), and α-ketoisovalerate (KIV), respectively (78). The second step of BCAA catabolism is mainly regulated by the catalytic activity of the branched-chain ketoacid dehydrogenase (BCKD) complex (79). Branched-chain acyl-coenzyme A (CoA) species are produced from their cognate α-ketoacids (80). Complete metabolism of BCCA species generates cataplerotic metabolites that are subsequently used in the tricarboxylic acid (TCA) cycle for the generation of fuel (i.e., ATP), using lipogenic, ketogenic, or glucogenic substrates (81–83). In addition, down-regulated BCAA catabolic genes prominent in the adipose tissue of participants with elevated BCAA serum concentrations were correlated with high HOMA-IR values ($p < 0.05$) (84). It has been shown that bacteria of the genus *Lactobacillus* help maintain metabolic homeostasis by improving amino acid metabolic pathways in metabolically impaired mice to better compensate for impaired aryl hydrocarbon receptor (AhR) signaling by increasing the availability of intestinal metabolites capable of signaling through the AhR (85). It has been reported from experimental studies that the gut microbiota can directly utilize tryptophan and produce bacteria-derived indole (86, 87). Examples include indolyl sulfate and *p*-cresol sulfate, which stimulate GLP-1 and increase secretion insulin from pancreatic beta cells (83, 88, 89). Similarly, results from the KEGG enrichment analysis showed that by far the most-downregulated pathways were BCAA degradation pathways (74).

This review has several deficiencies. First, the small sample size means that the accuracy of the study is low. Second, most articles did not provide sufficient specific indicators of gut microbial diversity and composition, making accurate quantitative analysis impossible. As a result, the evidence provided by these studies is insufficient. Third, as a result of limited data, we were unable to stratify patients by sampling time, diet, obesity, Asian-European factors, etc. Mechanistic insight into the gut microbiota is still limited, so further study is needed to identify specific biomarkers and the mechanisms by which they cause disease.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

MY, XG and DH wrote the manuscript and researched the data. MY and RH researched data, contributed to discussion, and reviewed the manuscript. SC, ZL, RH, and DZ contributed

to the discussion and reviewed the manuscript. All authors read and approved the final version of the manuscript, and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1097853/full#supplementary-material

References

1. Szmuilowicz ED, Josefson JL, Metzger BE. Gestational diabetes mellitus. *Endocrinol Metab Clinics North America* (2019) 48(3):479–93. doi: 10.1016/j.ecl.2019.05.001

2. Siregar DAS, Rianda D, Irwinda R, Dwi Utami A, Hanifa H, Shankar AH, et al. Associations between diet quality, blood pressure, and glucose levels among pregnant women in the Asian megacity of Jakarta. *PloS One* (2020) 15(11): e0242150. doi: 10.1371/journal.pone.0242150

3. Shen Y, Li W, Leng J, Zhang S, Liu H, Li W, et al. High risk of metabolic syndrome after delivery in pregnancies complicated by gestational diabetes. *Diabetes Res Clin practice* (2019) 150:219–26. doi: 10.1016/j.diabres.2019.03.030

4. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nat Rev Endocrinol* (2012) 8(11):639–49. doi: 10.1038/nrendo.2012.96

5. Petersen JS, Dyrberg T, Damm P, Kühl C, Mølsted-Pedersen L, Buschard K. GAD65 autoantibodies in women with gestational or insulin dependent diabetes mellitus diagnosed during pregnancy. *Diabetologia* (1996) 39(11):1329–33. doi: 10.1007/s001250050578

6. Wang J, Zheng J, Shi W, Du N, Xu X, Zhang Y, et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* (2018) 67(9):1614–25. doi: 10.1136/gutjnl-2018-315988

7. Wickens K, Barthow C, Mitchell EA, Kang J, van Zyl N, Purdie G, et al. Effects of lactobacillus rhamnosus HN001 in early life on the cumulative prevalence of allergic disease to 11 years. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol* (2018) 29(8):808–14. doi: 10.1111/pai.12982

8. Kuang YS, Lu JH, Li SH, Li JH, Yuan MY, He JR, et al. Connections between the human gut microbiome and gestational diabetes mellitus. *GigaScience* (2017) 6 (8):1–12. doi: 10.1093/gigascience/gix058

9. Sanmiguel C, Gupta A, Mayer EA. Gut microbiome and obesity: A plausible explanation for obesity. *Curr Obes Rep* (2015) 4(2):250–61. doi: 10.1007/s13679-015-0152-0

10. Woldeamlak B, Yirdaw K, Biadgo B. Role of gut microbiota in type 2 diabetes mellitus and its complications: Novel insights and potential intervention strategies. *Korean J Gastroenterol = Taehan Sohwagi Hakhoe chi* (2019) 74(6):314–20. doi: 10.4166/kjg.2019.74.6.314

11. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* (2016) 165(1):111–24. doi: 10.1016/j.cell.2016.02.011

12. Sun X, Jiao X, Ma Y, Liu Y, Zhang L, He Y, et al. Trimethylamine n-oxide induces inflammation and endothelial dysfunction in human umbilical vein endothelial cells via activating ROS-TXNIP-NLRP3 inflammasome. *Biochem Biophys Res Commun* (2016) 481(1-2):63–70. doi: 10.1016/j.bbrc.2016.11.017

13. Al-Obaide MAI, Singh R, Datta P, Rewers-Felkins KA, Salguero MV, Al-Obaidi I, et al. Gut microbiota-dependent trimethylamine-n-oxide and serum biomarkers in patients with T2DM and advanced CKD. *J Clin Med* (2017) 6(9):86. doi: 10.3390/jcm6090086

14. Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine n-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. *J biosci bioengineering.* (2014) 118(4):476–81. doi: 10.1016/j.jbiosc.2014.03.001

15. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PloS Med* (2009) 6 (7):e1000097. doi: 10.1371/journal.pmed.1000097

16. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* (2010) 25(9):603–5. doi: 10.1007/s10654-010-9491-z

17. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ (Clinical Res ed)* (2003) 327(7414):557–60. doi: 10.1136/bmj.327.7414.557

18. Abdullah B, Daud S, Aazmi MS, Idorus MY, Mahamooth MIJ. Gut microbiota in pregnant Malaysian women: a comparison between trimesters, body mass index and gestational diabetes status. *BMC pregnancy childbirth* (2022) 22(1):152. doi: 10.1186/s12884-022-04472-x

19. Chen T, Zhang Y, Zhang Y, Shan C, Zhang Y, Fang K, et al. Relationships between gut microbiota, plasma glucose and gestational diabetes mellitus. *J Diabetes Invest* (2021) 12(4):641–50. doi: 10.1111/jdi.13373

20. Cui M, Qi C, Yang L, Zhang M, Wang H, She G, et al. A pregnancy complication-dependent change in SIgA-targeted microbiota during third trimester. *Food Funct* (2020) 11(2):1513–24. doi: 10.1039/C9FO02919B

21. Hu P, Chen X, Chu X, Fan M, Ye Y, Wang Y, et al. Association of gut microbiota during early pregnancy with risk of incident gestational diabetes mellitus. *J Clin Endocrinol Metab* (2021) 106(10):e4128–e41. doi: 10.1210/clinem/dgab346

22. Huang L, Sililas P, Thonusin C, Luewan S, Chattipakorn SC. Early gut dysbiosis could be an indicator of unsuccessful diet control in gestational diabetes mellitus. *J diabetes* (2021) 13(12):1054–8. doi: 10.1111/1753-0407.13225

23. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* (2012) 150(3):470–80. doi: 10.1016/j.cell.2012.07.008

24. Li G, Yin P, Chu S, Gao W, Cui S, Guo S, et al. Correlation analysis between GDM and gut microbial composition in late pregnancy. *J Diabetes Res* (2021) 2021:8892849. doi: 10.1155/2021/8892849

25. Ma S, You Y, Huang L, Long S, Zhang J, Guo C, et al. Alterations in gut microbiota of gestational diabetes patients during the first trimester of pregnancy. *Front Cell infect Microbiol* (2020) 10:58. doi: 10.3389/fcimb.2020.00058

26. Mullins TP, Tomsett KI, Gallo LA, Callaway LK, McIntyre HD, Dekker Nitert M, et al. Maternal gut microbiota displays minor changes in overweight and obese women with GDM. *Nutrition metabolism Cardiovasc Dis NMCD.* (2021) 31 (7):2131–9. doi: 10.1016/j.numecd.2021.03.029

27. Sililas P, Huang L, Thonusin C, Luewan S, Chattipakorn N, Chattipakorn S, et al. Association between gut microbiota and development of gestational diabetes mellitus. *Microorganisms* (2021) 9(8):1686. doi: 10.3390/microorganisms9081686

28. Tanaka K, Harata G, Miyazawa K, He F, Tanigaki S, Kobayashi Y. The gut microbiota of non-obese Japanese pregnant women with gestational diabetes mellitus. *Biosci Microbiota Food Health* (2022) 41(1):4–11. doi: 10.12938/bmfh.2021-025

29. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* (2010) 33(3):676–82. doi: 10.2337/dc10-0719

30. Zheng W, Xu Q, Huang W, Yan Q, Chen Y, Zhang L, et al. Gestational diabetes mellitus is associated with reduced dynamics of gut microbiota during the first half of pregnancy. *mSystems* (2020) 5(2):e00109-20. doi: 10.1128/mSystems.00109-20

31. Wei J, Qing Y, Zhou H, Liu J, Qi C, Gao J. 16S rRNA gene amplicon sequencing of gut microbiota in gestational diabetes mellitus and their correlation with disease risk factors. *J endocrinol Invest* (2022) 45(2):279–89. doi: 10.1007/s40618-021-01595-4

32. Wu Y, Bible PW, Long S, Ming WK, Ding W, Long Y, et al. Metagenomic analysis reveals gestational diabetes mellitus-related microbial regulators of glucose tolerance. *Acta diabetol* (2020) 57(5):569–81. doi: 10.1007/s00592-019-01434-2

33. Gámez-Valdez JS, García-Mazcorro JF, Montoya-Rincón AH, Rodríguez-Reyes DL, Jiménez-Blanco G, Rodríguez MTA, et al. Differential analysis of the bacterial community in colostrum samples from women with gestational diabetes mellitus and obesity. *Sci Rep* (2021) 11(1):24373. doi: 10.1038/s41598-021-03779-7

34. Su Y, Chen L, Zhang DY, Gan XP, Cao YN, Cheng DC, et al. The characteristics of intestinal flora in overweight pregnant women and the correlation with gestational diabetes mellitus. *Endocr connections* (2021) 10 (11):1366–76. doi: 10.1530/EC-21-0433

35. Ye G, Zhang L, Wang M, Chen Y, Gu S, Wang K, et al. The gut microbiota in women suffering from gestational diabetes mellitus with the failure of glycemic control by lifestyle modification. *J Diabetes Res* (2019) 2019:6081248. doi: 10.1155/2019/6081248

36. Su Y, Wang HK, Gan XP, Chen L, Cao YN, Cheng DC, et al. Alterations of gut microbiota in gestational diabetes patients during the second trimester of pregnancy in the shanghai han population. *J Trans Med* (2021) 19(1):366. doi: 10.1186/s12967-021-03040-9

37. Wang X, Liu H, Li Y, Huang S, Zhang L, Cao C, et al. Altered gut bacterial and metabolic signatures and their interaction in gestational diabetes mellitus. *Gut Microbes* (2020) 12(1):1–13. doi: 10.1080/19490976.2020.1840765

38. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* (2013) 500(7464):541–6. doi: 10.1038/nature12506

39. Ahn J, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, et al. Human gut microbiome and risk for colorectal cancer. *J Natl Cancer Instit* (2013) 105 (24):1907–11. doi: 10.1093/jnci/djt300

40. Aron-Wisnewsky J, Vigliotti C, Witjes J, Le P, Holleboom AG, Verheij J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol hepatol* (2020) 17(5):279–97. doi: 10.1038/s41575-020-0269-9

41. Lambeth SM, Carson T, Lowe J, Ramaraj T, Leff JW, Luo L, et al. Composition, diversity and abundance of gut microbiome in prediabetes and type 2 diabetes. *J Diabetes Obes* (2015) 2(3):1–7. doi: 10.15436/2376-0949.15.031

42. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, et al. Molecular characterisation of the faecal microbiota in patients with type II diabetes. *Curr Microbiol* (2010) 61(1):69–78. doi: 10.1007/s00284-010-9582-9

43. Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Curr Opin Pharmacol* (2019) 49:1–5. doi: 10.1016/j.coph.2019.03.011

44. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci United States America* (2015) 112(35):11060–5. doi: 10.1073/pnas.1502875112

45. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* (2017) 19(1):29–41. doi: 10.1111/1462-2920.13589

46. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer *via* NKT cells. *Sci (New York NY)* (2018) 360(6391):eaan5931. doi: 10.1126/science.aan5931

47. Fei N, Zhao L. An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice. *ISME J* (2013) 7(4):880–4. doi: 10.1038/ismej.2012.153

48. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* (2017) 5(1):14. doi: 10.1186/s40168-016-0222-x

49. Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev* (2014) 38(5):996–1047. doi: 10.1111/1574-6976.12075

50. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* (2004) 118(2):229–41. doi: 10.1016/j.cell.2004.07.002

51. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* (2013) 54(9):2325–40. doi: 10.1194/jlr.R036012

52. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* (2009) 58(7):1509–17. doi: 10.2337/db08-1637

53. Säemann MD, Böhmig GA, Osterreicher CH, Burtscher H, Parolini O, Diakos C, et al. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB J Off Publ Fed Am Societies Exp Biol* (2000) 14(15):2380–2. doi: 10.1096/fj.00-0359fje

54. Soliman MM, Ahmed MM, Salah-Eldin AE, Abdel-Aal AA. Butyrate regulates leptin expression through different signaling pathways in adipocytes. *J vet sci* (2011) 12(4):319–23. doi: 10.4142/jvs.2011.12.4.319

55. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SE, et al. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* (2015) 64(11):1744–54. doi: 10.1136/gutjnl-2014-307913

56. Demigné C, Morand C, Levrat MA, Besson C, Moundras C, Rémésy C. Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br J Nutr* (1995) 74(2):209–19. doi: 10.1079/BJN19950124

57. Liu X, Cheng YW, Shao L, Sun SH, Wu J, Song QH, et al. Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study. *World J gastroenterol* (2021) 27(19):2394–414. doi: 10.3748/wjg.v27.i19.2394

58. Ozato N, Saito S, Yamaguchi T, Katashima M, Tokuda I, Sawada K, et al. Blautia genus associated with visceral fat accumulation in adults 20-76 years of age. *NPJ biofilms microbiomes* (2019) 5(1):28. doi: 10.1038/s41522-019-0101-x

59. Osaki H, Jodai Y, Koyama K, Omori T, Horiguchi N, Kamano T, et al. Clinical response and changes in the fecal microbiota and metabolite levels after fecal microbiota transplantation in patients with inflammatory bowel disease and recurrent clostridioides difficile infection. *Fujita Med J* (2021) 7(3):87–98. doi: 10.20407/fmj.2020-021

60. Tong X, Xu J, Lian F, Yu X, Zhao Y, Xu L, et al. Structural alteration of gut microbiota during the amelioration of human type 2 diabetes with hyperlipidemia by metformin and a traditional Chinese herbal formula: a multicenter, randomized, open label clinical trial. *mBio* (2018) 9(3):e02392-17. doi: 10.1128/mBio.02392-17

61. Qi CJ, Zhang Q, Yu M, Xu JP, Zheng J, Wang T, et al. Imbalance of fecal microbiota at newly diagnosed type 1 diabetes in Chinese children. *Chin Med J* (2016) 129(11):1298–304. doi: 10.4103/0366-6999.182841

62. Borst SE. The role of TNF-alpha in insulin resistance. *Endocrine* (2004) 23 (2-3):177–82. doi: 10.1385/ENDO:23:2-3:177

63. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol* (2014) 121:91–119. doi: 10.1016/B978-0-12-800100-4.00003-9

64. Gomez-Arango LF, Barrett HL, Wilkinson SA, Callaway LK, McIntyre HD, Morrison M, et al. Low dietary fiber intake increases collinsella abundance in the gut microbiota of overweight and obese pregnant women. *Gut Microbes* (2018) 9 (3):189–201. doi: 10.1080/19490976.2017.1406584

65. Rajilić-Stojanović M, Heilig HG, Tims S, Zoetendal EG, de Vos WM. Long-term monitoring of the human intestinal microbiota composition. *Environ Microbiol* (2012). doi: 10.1111/1462-2920.12023

66. Hasain Z, Mokhtar NM, Kamaruddin NA, Mohamed Ismail NA, Razalli NH, Gnanou JV, et al. Gut microbiota and gestational diabetes mellitus: A review of host-gut microbiota interactions and their therapeutic potential. *Front Cell infect Microbiol* (2020) 10:188. doi: 10.3389/fcimb.2020.00188

67. Mukherjee A, Lordan C, Ross RP, Cotter PD. Gut microbes from the phylogenetically diverse genus eubacterium and their various contributions to gut health. *Gut Microbes* (2020) 12(1):1802866. doi: 10.1080/19490976.2020.1802866

68. Han YH, Onufer EJ, Huang LH, Sprung RW, Davidson WS, Czepielewski RS, et al. Enterically derived high-density lipoprotein restrains liver injury through the portal vein. *Sci (New York NY)* (2021) 373(6553):eabe6729. doi: 10.1126/science.abe6729

69. Maa MC, Chang MY, Hsieh MY, Chen YJ, Yang CJ, Chen ZC, et al. Butyrate reduced lipopolysaccharide-mediated macrophage migration by suppression of src enhancement and focal adhesion kinase activity. *J Nutr Biochem* (2010) 21 (12):1186–92. doi: 10.1016/j.jnutbio.2009.10.004

70. Ferrocino I, Ponzo V, Gambino R, Zarovska A, Leone F, Monzeglio C, et al. Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM). *Sci Rep* (2018) 8(1):12216. doi: 10.1038/s41598-018-30735-9

71. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, et al. Gut microbiota, obesity and diabetes. *Postgraduate Med J* (2016) 92(1087):286–300. doi: 10.1136/postgradmedj-2015-133285

72. Holscher HD, Bauer LL, Gourineni V, Pelkman CL, Fahey GCJr., Swanson KS. Agave inulin supplementation affects the fecal microbiota of healthy adults participating in a randomized, double-blind, placebo-controlled, crossover trial. *J Nutr* (2015) 145(9):2025–32. doi: 10.3945/jn.115.217331

73. Wang W, Uzzau S, Goldblum SE, Fasano A. Human zonulin, a potential modulator of intestinal tight junctions. *J Cell Sci* (2000) 113 Pt 24:4435–40. doi: 10.1242/jcs.113.24.4435

74. Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y, et al. Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Sci Rep* (2016) 6:24540. doi: 10.1038/srep24540

75. Tobias PS, Soldau K, Gegner JA, Mintz D, Ulevitch RJ. Lipopolysaccharide binding protein-mediated complexation of lipopolysaccharide with soluble CD14. *J Biol Chem* (1995) 270(18):10482–8. doi: 10.1074/jbc.270.18.10482

76. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine* (2008) 42(2):145–51. doi: 10.1016/j.cyto.2008.01.006

77. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature* (2009) 457 (7228):480–4. doi: 10.1038/nature07540

78. Zakaria NF, Hamid M, Khayat ME. Amino acid-induced impairment of insulin signaling and involvement of G-protein coupling receptor. *Nutrients* (2021) 13(7):2229. doi: 10.3390/nu13072229

79. Adeva MM, Calviño J, Souto G, Donapetry C. Insulin resistance and the metabolism of branched-chain amino acids in humans. *Amino Acids* (2012) 43 (1):171–81. doi: 10.1007/s00726-011-1088-7

80. Haufe S, Engeli S, Kaminski J, Witt H, Rein D, Kamlage B, et al. Branched-chain amino acid catabolism rather than amino acids plasma concentrations is associated with diet-induced changes in insulin resistance in overweight to obese individuals. *Nutrition metabolism Cardiovasc Dis NMCD.* (2017) 27(10):858–64. doi: 10.1016/j.numecd.2017.07.001

81. Gerber PA, Rutter GA. The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. *Antioxidants Redox Signaling* (2017) 26 (10):501–18. doi: 10.1089/ars.2016.6755

82. Schriever SC, Deutsch MJ, Adamski J, Roscher AA, Ensenauer R. Cellular signaling of amino acids towards mTORC1 activation in impaired human leucine catabolism. *J Nutr Biochem* (2013) 24(5):824–31. doi: 10.1016/j.jnutbio.2012.04.018

83. Mallmann NH, Lima ES, Lalwani P. Dysregulation of tryptophan catabolism in metabolic syndrome. *Metab syndrome related Disord* (2018) 16(3):135–42. doi: 10.1089/met.2017.0097

84. Natividad JM, Agus A, Planchais J, Lamas B, Jarry AC, Martin R, et al. Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. *Cell Metab* (2018) 28(5):737–49.e4. doi: 10.1016/j.cmet.2018.07.001

85. Su X, Gao Y, Yang R. Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells* (2022) 11(15):2296. doi: 10.3390/cells11152296

86. Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* (2018) 23(6):716–24. doi: 10.1016/j.chom.2018.05.003

87. Gao H, Liu S. Role of uremic toxin indoxyl sulfate in the progression of cardiovascular disease. *Life Sci* (2017) 185:23–9. doi: 10.1016/j.lfs.2017.07.027

88. Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM, Reimann F. Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine l cells. *Cell Rep* (2014) 9(4):1202–8. doi: 10.1016/j.celrep.2014.10.032

89. Croci S, D'Apolito LI, Gasperi V, Catani MV, Savini I. Dietary strategies for management of metabolic syndrome: Role of gut microbiota metabolites. *Nutrients* (2021) 13(5):1389. doi: 10.3390/nu13051389

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