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Article

Investigating the ileal microbiota biomechanical characteristics of patients with metabolic associated fatty liver disease (MAFLD)

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Abstract: Objective: To investigate the biomechanical characteristics of ileal microbiota in patients with metabolic associated fatty liver disease (MAFLD). **Methods:** 72 patients with MAFLD were recruited in our hospital between January 2024 and November 2024. They were divided into mild fatty liver (MF group, n = 36) and moderate to severe fatty liver (SF group, n = 36) based on CAP values, and a healthy control group (H, n = 36) was included. The ileal microbiota was sampled using endoscopy with newly designed accessories, followed by 16S rDNA sequencing to analyze the biomechanical properties of the microbial community. **Results:** Compared with the control group, Alpha diversity of ileal flora in MAFLD group elevated, a gradual decrease in the percentage of *Turicibacter*, a decrease in the abundance of *Lactobacillus* and *Veillonella*, but an increase in the abundance of *Prevotella*, *Leptotrichia*, *Porphyromonas*. **Conclusion:** The biomechanical diversity and abundance of ileal microbiota in MAFLD patients change, which is related to the severity of the disease. The reduction of *Turicibacter* and the migration of oral colonizing bacteria are characteristic features of the ileal microbiota in MAFLD patients, potentially influencing the biomechanical interactions within the gut environment.

Keywords: metabolic related fatty liver disease; biomechanics; endoscopic microbiota sampling; *Turicibacter* bacteria; oral flora migration

1. Introduction

The prevalence of metabolic associated fatty liver disease (MAFLD) has been increasing annually. It not only impairs the quality of life and life expectancy of patients, but also imposes a tremendous economic burden, emerging as a new challenge in the field of clinical medicine and a significant public health issue [1]. This rising prevalence is particularly alarming, as it signifies a shift in the health landscape, where lifestyle-related diseases are becoming more common and affecting broader populations. The implications of MAFLD extend beyond individual health, influencing healthcare systems and necessitating a reevaluation of public health strategies. The prevalence of MAFLD correlates with the epidemic trends of obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), and cardiovascular and cerebrovascular diseases [2–4]. These interrelated conditions not only share common risk factors but also contribute to a vicious cycle of worsening health outcomes. The overlapping pathophysiology of these diseases highlights the need for integrated approaches to prevention and treatment, focusing on lifestyle modifications and early interventions. Shared characteristics among these conditions include excessive body fat accumulation and insulin resistance. The interplay between these factors creates a vicious cycle, where each condition exacerbates the others, leading to a decline in

overall health and an increase in complications. Consequently, MAFLD notably shortens the life expectancy of high-risk groups and increases all-cause mortality, considerably higher than that of healthy populations [1]. This increased mortality risk underscores the urgency of addressing MAFLD as a critical public health concern, as it disproportionately affects vulnerable populations and exacerbates existing health disparities.

Studies have revealed a close association between the gut microbiota and MAFLD [5]. The gut microbiota, a complex community of microorganisms residing in the gastrointestinal tract, plays a crucial role in various physiological processes, including metabolism, immune function, and even mood regulation. The gut microbiota can interact with bile acids and regulate multiple signaling pathways that are closely related to MAFLD. This interaction is not merely passive; rather, it involves intricate biochemical processes that can influence the progression of liver disease. The composition of gut microbiota may affect the metabolism of bile acids, which in turn impacts liver function and health. Bile acids are mainly absorbed in the terminal ileum. The ileal mucosa expresses nuclear receptors involved in bile acid signaling, such as farnesoid X receptor (FXR) and G protein-coupled receptor TGR5, which can exert a significant impact on downstream metabolic pathways [6]. These receptors play pivotal roles in mediating the effects of bile acids, influencing not only liver metabolism but also systemic glucose and lipid metabolism. This highlights the ileum's critical function in maintaining metabolic homeostasis. The ileum is the primary site for the enterohepatic circulation of bile acids and the interaction between the microbiota and the host, and its special functions determine the characteristics of the microbial community in this region. The unique environment of the ileum, influenced by factors such as pH, bile acid composition, and nutrient availability, creates a niche that supports specific microbial populations. This niche is vital for the health of the host, as it can modulate inflammatory responses and metabolic processes. Previous studies on MAFLD typically utilized fecal samples. Nevertheless, there are significant differences between the microbiota in ileal and fecal samples. Fecal bacteria can only reflect approximately 40% of the low-abundance ileal bacterial genera, while the dominant microbiota and beta-diversity are completely different [7]. Therefore, the ileal microbiota plays a crucial role in the research of MAFLD. However, due to the special anatomical location and technical limitations, the data on the characteristics of the microbiota in the terminal ileum of humans are extremely scarce [8]. This scarcity of data poses a significant challenge for researchers aiming to understand the role of ileal microbiota in MAFLD and other metabolic disorders. The difficulty in obtaining ileal samples through standard clinical practices limits our ability to draw definitive conclusions about the microbial composition and its implications for health.

The development of digestive endoscopy has enabled targeted biopsy sampling of the intestinal mucosa. However, conventional endoscopy risks cross-contamination in biopsy channels. Therefore, based on the existing endoscopic sampling methods, this study innovatively designed an endoscopic working channel plug accessory to reduce the risk of cross-contamination. It aimed to analyze the characteristics of the ileal microbiota in patients with metabolic associated fatty liver disease (MAFLD) and

provide a foundation for subsequent research exploring microbiota-related mechanisms.

2. Materials and methods

2.1. Channel plug accessories and sampling brush

As shown in **Figure 1**, the accessory is fixed to the distal end of the endoscope. It is composed of a flexible silicone plug which is attached to a silicone ring. During the process of endoscope insertion, the silicone plug can efficiently seal the working forceps channel, thereby ensuring the sterility of the channel. The silicone ring, on the other hand, is fastened to the endoscope body. Its primary function is to enable the removal of the entire accessory along with the endoscope once the sampling procedure is concluded. In this study, a disposable endoscope cleaning brush was employed to collect the bacterial flora samples present on the surface of the ileal mucosa.

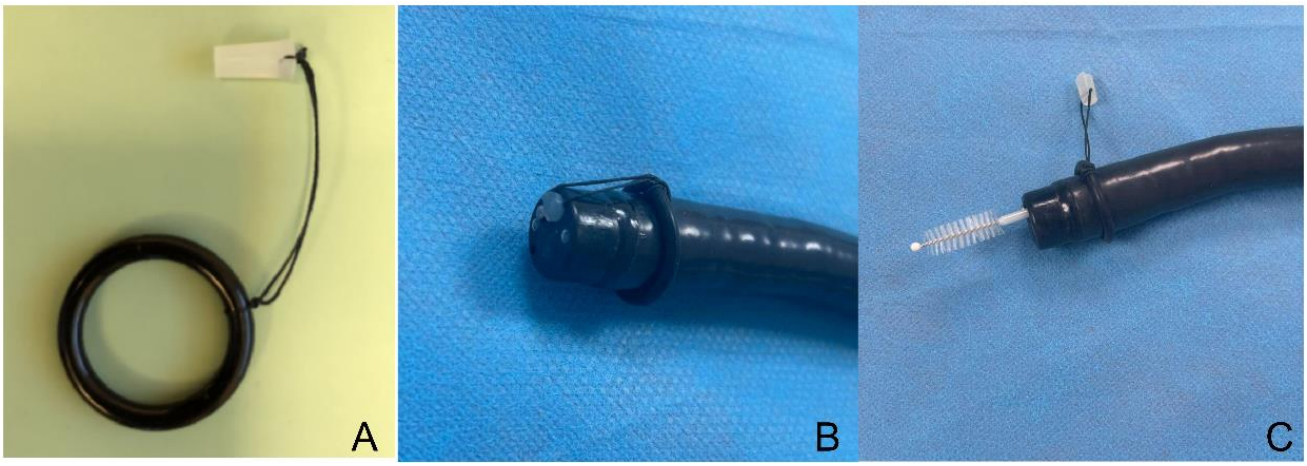

Figure 1. (A) Channel plug accessory; **(B)** the accessory is fixed to the front end of the endoscope; **(C)** biopsy brush sampling through the channel.

2.2. Equipment sterilization

In this study, a gastroscope (EC38-iL Pentax Japan) was utilized. All of the endoscopes, associated accessories, and the silicone forceps channel plug were subjected to sterilization with ethylene oxide. The sterilization conditions were precisely configured as follows: a relative humidity level of 50% and an ethylene oxide concentration of 600 mg/L, with a sterilization duration of 5 h. Subsequently, a 12-hour aeration process was implemented to guarantee the complete removal of any residual ethylene oxide gas. The transportation, assembly, and sampling operations related to the endoscope were all performed by medical staff, who strictly complied with aseptic manipulation techniques throughout the entire process.

2.3. Patient recruitment and sample collection

72 patients with MAFLD were recruited in this study. According to the Controlled Attenuation Parameter (CAP) value, MAFLD was classified into mild (238 dB/m $\leq$ CAP $\leq$ 258 dB/m) and moderate-to-severe (CAP > 258 dB/m). Patients with moderate-to-severe fatty liver were included in the SF group (n = 36), and those with

mild fatty liver were included in the MF group (n = 36). Additionally, healthy individuals who underwent physical examinations during the same period were selected as the control group (H group, n = 36). This study was approved by the Ethics Committee of Wuxi Hospital of Traditional Chinese Medicine [SWJW2022062701]. and written informed consent was obtained.

After routine preparations, the patients underwent blood tests and endoscopic examinations. The anesthesiologist administered intravenous general anesthesia with propofol. The procedure is performed under monitoring. Before inserting the endoscope, the silicone plug was installed at the front end of the endoscope, and the suction tube was disconnected to ensure the sterility of the working forceps channel. During the operation, the endoscopist directly inserted the endoscope into the terminal ileum and collected ileal contents with the nurse assisting. All collected samples are incubated at room temperature for 30 min and then stored in a −80 °C freezer for subsequent analysis.

2.4. 16S rRNA extraction and amplification

The samples were thawed under room temperature conditions. Subsequently, the total bacterial DNA was isolated from 0.5 g of the samples by means of the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek). Once the purity and concentration of the DNA had been examined, PCR amplification was initiated. The specific primers employed for PCR amplification were targeted at the V3–V4 region of 16S, where the upstream primer 338F was ACTCCTACGGGAGGCAGCA and the downstream primer 806R was GGACTACHVGGGTWTCTAAT. The PCR products from three replicates were combined and then purified. Library construction was carried out using the NEXTFLEX Rapid DNA-Seq Kit, followed by sequencing on the Miseq PE300/NovaSeq PE250 platform provided by Illumina. With the utilization of UPARSE software (version 7.1), OTU clustering and analysis were executed on the sequences with a 97% similarity threshold.

2.5. Microbiota analysis

The analysis approaches encompassed: (1) Alpha diversity analysis: Alpha diversity indices like Chao1, Simpson, and Shannon were utilized to assess the microbiota, and inter-group diversity tests were also conducted; (2) Beta diversity analysis: PLS-DA analysis was applied to exhibit the disparities in microbiota composition among samples. The compositional differences were gauged by visually inspecting the aggregation and separation of samples; (3) Community structure analysis: Venn diagrams and bar charts of species composition were utilized to showcase the dominant bacteria possessing high abundance rankings at the genus level; (4) Inter-group species difference analysis.

3. Results

3.1. Comparison of patient serological indicators

Significant differences were observed in the levels of ALT, AST, GGT, TC, TG, and LDL among the groups (P < 0.001), while there was no significant difference in

the level of HDL, as shown in **Table 1**.

Table 1. Comparison of patient baseline data.

	SF (*n* = 36)	MF (*n* = 36)	H (*n* = 36)	Statistic	*P*
Gender, *n* (%)				X^2 = 4.85	0.089
Male	23 (63.8)	17 (47.2)	12 (33.3)		
Female	13 (36.2)	19 (52.8)	24 (66.7)		
TC (mmol/L)	4.9 ± 1.3[a]	4.0 ± 1.1[b]	3.7 ± 1.3[b]	*F* = 7.14	0.001
LDL (mmol/L)	3.6 ± 0.8[a]	2.8 ± 0.9[b]	2.5 ± 1.0[b]	*F* = 11.27	<0.001
ALT (U/L)	60.6 (50.3, 75.5)[a]	22.8 (15.6, 37.1)[b]	16.2 (10.8, 20.9)[b]	*Z* = 49.44	<0.001
AST (U/L)	49.4 (42.5, 58.6)[ab]	22.75 (18.4, 40.4)[a]	19.8 (14.9, 24.2)[b]	*Z* = 33.95	<0.001
GGT (U/L)	79.0 (38.9, 116.1)[a]	34.5 (20.1, 50.5)[b]	14.0 (12.7, 16.4)[c]	*Z* = 29.92	<0.001
TG (mmol/L)	2.40 (1.6, 3.4)[a]	1.5 (1.2, 2.3)[b]	1.0 (0.7, 1.3)[c]	*Z* = 30.16	<0.001
HDL (mmol/L)	1.1 (0.9, 1.3)	1.0 (0.9, 1.2)	1.2 (0.9, 1.6)	*Z* = 2.56	0.278

TC: Total Cholesterol, LDL: Low-Density Lipoprotein, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, GGT: Gamma-Glutamyl Transferase, TG: Triglyceride. F: ANOVA, Z: Kruskal-wailis test, χ^2: Chi-square test. SD: standard deviation, M: Median, Q_1: 1st Quartile, Q_3: 3st Quartile. Different superscript letters (a, b, c,) indicate statistically significant differences between groups ($P < 0.05$).

3.2. Analysis of alpha and beta diversity

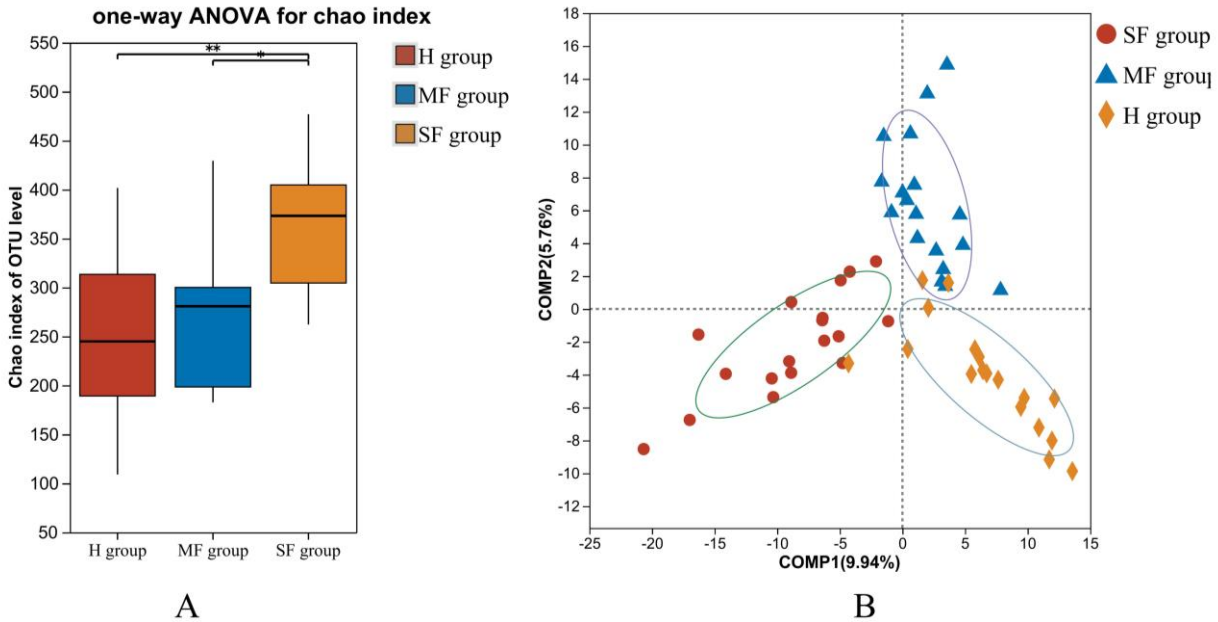

Figure 2. (A) Alpha diversity: one-way ANOVA for chao index; **(B)** beta diversity diversity: PLS-DA analysis on the OTU level.

Compared with the healthy control group, the Alpha diversity of the ileal microbiota in the fatty liver group was elevated (**Figure 2A**). Specifically, the Chao1 index increased, with significant differences observed ($P < 0.05$, $P < 0.001$), while there was no obvious difference in the Shannon index and Simpson index. This suggests that the richness of the ileal microbiota in the fatty liver group was enhanced

and might be related to the severity of the disease, although the diversity did not change significantly.

On the OTU level, PLS-DA analysis was employed to display the Beta diversity analysis results of the microbiota composition among samples (**Figure 2B**). In this figure, the explained variance of the first principal coordinate was 9.14%, and that of the second principal coordinate was 5.5%. The points representing each group did not completely overlap, indicating differences in clustering among the groups.

3.3. Analysis of species composition

Figure 3A is a Venn diagram showing the composition of the microbiota in each group at the genus level. **Figure 3B** is a community bar chart presenting the top 10 dominant microbiota in terms of relative abundance, mainly including *Streptococcus*, *Fusobacterium*, *Achromobacter*, *Veillonella*, *Rothia*, and *Bacteroides*. The proportion of *Turicibacter* is relatively high in the healthy patient group, but it gradually decreases in patients with fatty liver, while the proportion of *Bacteroides* increases.

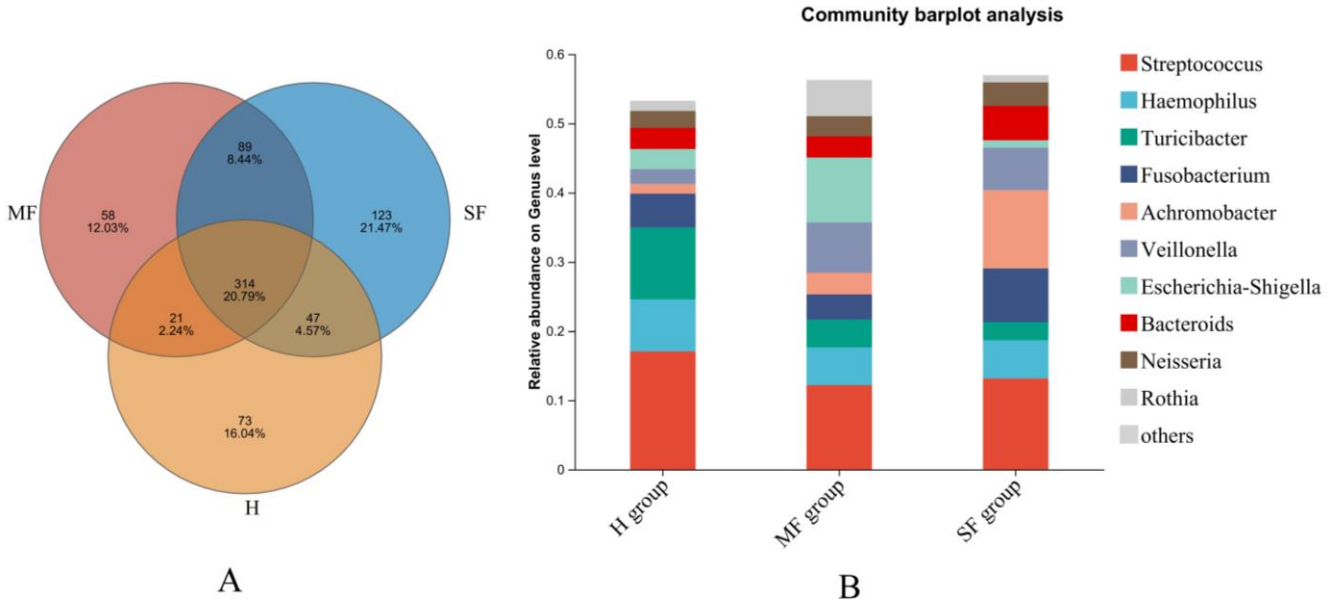

Figure 3. (A) Venn diagram of composition at the genus level; **(B)** bar chart of compositional structure at the genus level.

3.4. Analysis of species differences

The Kruskal-Wallis test was conducted for comparison among multiple groups on the genus level (**Figure 4**). The results showed that compared with the healthy group, the abundances of *Prevotella*, *Leptotrichia*, and *Porphyromonas* increased significantly ($P < 0.001$). *Leptotrichia*, *Prevotella*, and *Porphyromonas* are all common oral bacteria, which are related to periodontitis and pulp infection. The abundances of *Fusobacterium*, *Lactobacillus*, and *Veillonella* in the MAFLD group were relatively reduced.

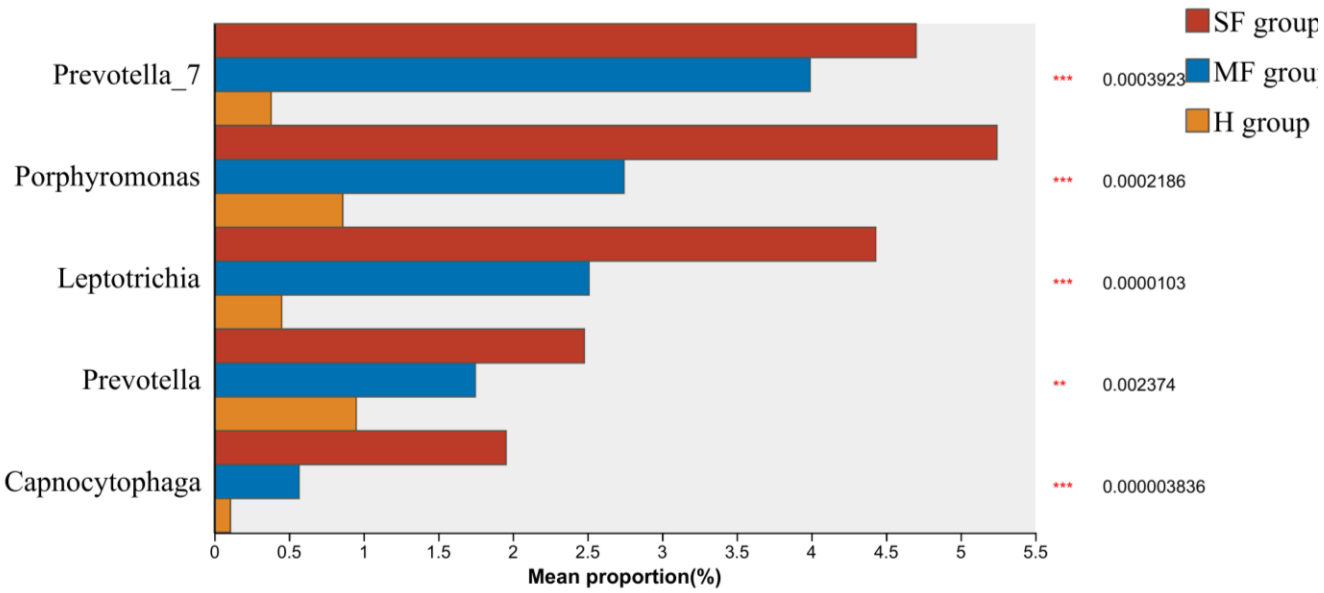

Figure 4. Multiple groups kruskal-wallis *H* test bar plot on genus level.

4. Discussion

In view of the fact that metabolic dysfunction-associated fatty liver disease (MAFLD) exhibits a high global incidence rate and imposes a substantial disease burden, it becomes imperative to conduct investigations into its pathogenesis and to engage in the development of novel pharmaceutical agents. This urgency is compounded by the growing prevalence of obesity and related metabolic disorders, which significantly contribute to the incidence of MAFLD. Understanding the complex interactions between metabolic pathways and liver health is essential for identifying effective treatment strategies. The US FDA has approved the first MAFLD drug, Resmetirom, for the treatment of adult MASH (non-alcoholic steatohepatitis) patients with moderate to severe liver fibrosis (stages F2–F3) [9]. This approval represents a pivotal advancement in the medical management of MAFLD, offering a specific therapeutic option for patients who previously had limited choices. The introduction of Resmetirom not only highlights the potential for targeted therapies but also encourages further research into other pharmacological interventions. New drugs for weight loss and improvement of metabolic disorders also show certain therapeutic promise. Tirzepatide, a dual GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1) receptor agonist, is in the phase II clinical trial stage [10]. The dual mechanism of Tirzepatide may provide a multifaceted approach to treating MAFLD, addressing both weight management and metabolic dysregulation simultaneously. As clinical trials progress, the outcomes will be critical in determining its role in the comprehensive treatment landscape for MAFLD. However, the safety and efficacy of these drugs still need further evaluation. Drugs targeting CCR2/5, ASK-1, FGF-21, etc., such as Cenicriviroc, Selonsertib, and Pegbelfermin, have all halted their development due to failure to meet the primary endpoints. This situation underscores the inherent challenges in drug development, where promising candidates may not always yield the expected results. It emphasizes the necessity for rigorous clinical testing and adaptive trial designs to enhance the likelihood of success for

future therapeutic agents. With the emerging development of gut microbiota research, some scholars have found that the gut microbiota can affect MAFLD by regulating the bile acid farnesoid X receptor (FXR) and related signaling pathways [11]. This emerging understanding of the gut-liver axis opens exciting possibilities for novel therapeutic approaches. By targeting gut microbiota, researchers may discover new ways to modulate liver function and improve metabolic health. The interplay between gut bacteria and bile acids is particularly intriguing, as it suggests that enhancing or modifying the microbiome could lead to significant improvements in MAFLD outcomes. More research on this target can provide a basis and ideas for the development of new drugs. Investigating specific microbial species and their metabolic products may reveal biomarkers for disease progression and treatment response, ultimately paving the way for personalized medicine approaches in managing MAFLD.

The gut microbiota is closely related to factors such as age, diet, daily routine, and the intestinal environment of the population. These factors collectively shape the composition and functionality of the gut microbiome, which in turn influences the host's metabolic processes. An individual's dietary habits, for instance, can lead to significant shifts in microbial diversity, affecting overall health and disease susceptibility. It not only affects the host's metabolism but also has a close connection with bile acids, important endogenous signaling molecules, and regulates multiple signaling pathways closely related to MAFLD. Bile acids play a crucial role in digestion and metabolism, and their interaction with gut microbiota can modulate various metabolic pathways, further linking the gut and liver health. This relationship underscores the importance of maintaining a balanced microbiome to support metabolic function and prevent liver diseases. Previous studies have found that in MAFLD and related diseases, the structural composition of the gut microbiota changes significantly. The abundances of *Firmicutes* and *Proteobacteria* increase, accompanied by a decrease in the abundances of *Coprococcus* and *Eubacterium*. These shifts in microbial populations suggest a dysbiotic state that may contribute to the pathogenesis of MAFLD. The increase in potentially harmful bacteria like *Firmicutes* and *Proteobacteria* could lead to increased inflammation and metabolic disturbances, which are hallmarks of MAFLD. In this study, we also found that the abundances of *Fusobacterium* and *Veillonella* in the fatty liver group of MAFLD patients were relatively reduced. This reduction may indicate a loss of beneficial microbial species that play a protective role in maintaining gut integrity and metabolic health. The overall balance of microbial communities is essential for preventing disease progression. Wei et al. [12] found that *Enterococcus* had an increased abundance in children with obesity and non-alcoholic fatty liver disease, and the transplantation of Enterococcus B6 (Enterococcus faecium B6) from patients could significantly induce NAFLD (non-alcoholic fatty liver disease) symptoms in mice. This highlights the potential pathogenic role of specific bacterial strains in the development of fatty liver disease. The ability of Enterococcus B6 to induce NAFLD symptoms in animal models suggests that similar mechanisms may occur in humans, warranting further investigation into its impact on liver health. *Enterococcus* B6 can produce tyramine, an important metabolite that may activate PPAR-γ, leading to liver lipid accumulation, inflammation, and fibrosis. This metabolic pathway illustrates

how gut-derived metabolites can influence liver pathology, emphasizing the need for a deeper understanding of the gut-liver axis. In this study, we found that bacteria enriched in the fatty liver group included *Prevotella*, *Leptotrichia*, and *Porphyromonas*, which are mainly colonized in the oral cavity. The presence of these oral bacteria in the gut microbiota of MAFLD patients suggests possible translocation, where bacteria migrate from the oral cavity to the gut, further contributing to dysbiosis and disease progression. These findings suggest that bacterial translocation and dysbiosis play a certain role in the development of MAFLD. Understanding these mechanisms could lead to novel therapeutic strategies aimed at restoring microbial balance and improving liver health.

The study found that *Turicibacter* had a high abundance in the healthy control group, and its abundance was inversely proportional to the severity of fatty liver, suggesting that *Turicibacter* gradually decreased in the intestines of patients. This observation indicates that *Turicibacter* may play a protective role in maintaining liver health, and its depletion could be a contributing factor to the progression of fatty liver disease. The relationship between *Turicibacter* levels and liver severity highlights the potential of this bacterium as a biomarker for early detection and monitoring of MAFLD. *Turicibacter* participates in bile acid metabolism by directly hydrolyzing TCA (taurocholic acid) and glycodeoxycholic acid and converting CA (cholic acid) and CDCA (chenodeoxycholic acid) into secondary bile acids through 7α-dehydroxylase [13]. This metabolic function is crucial as bile acids are not only important for digestion but also serve as signaling molecules that influence various metabolic pathways. The ability of *Turicibacter* to modify bile acids suggests that it could play a significant role in regulating lipid metabolism and inflammation in the liver. Previous studies have shown that in high-fat-induced animal models, *Turicibacter* was gradually depleted, and its abundance was positively correlated with the metabolite butyrate, which plays an important role in various chronic inflammatory diseases [14,15]. The correlation between *Turicibacter* and butyrate emphasizes the importance of microbial metabolites in mediating host health. Butyrate is known for its anti-inflammatory properties and its ability to strengthen the gut barrier, which may help mitigate the effects of a high-fat diet on liver health. The colonization of a single *Turicibacter* strain can lead to changes in the host's bile acid profile, which is consistent with the bile acid profile produced in in vitro experiments. This finding suggests that *Turicibacter* has the potential to be utilized in therapeutic interventions aimed at restoring healthy bile acid profiles in patients with MAFLD. The establishment of bacteria with bile-modifying genes from the *Turicibacter* strain in mice can reduce serum cholesterol, triglycerides, and adipose tissue mass [16]. This reduction in lipid levels indicates that *Turicibacter* may have a role in combating metabolic syndrome, further supporting its significance in maintaining metabolic health. The genes in the *Turicibacter* strain can change the host's bile acid and lipid metabolism and position *Turicibacter* bacteria as regulators of host fat biology. *Lactobacillus*, as the most common probiotic, plays an important protective role in the homeostasis of the gut microbiota [17]. Its presence in the gut can help prevent dysbiosis, which is often associated with various metabolic disorders, including MAFLD. Metabolomic analysis found that valeric acid was the most enriched metabolite of *Lactobacillus*, and its upregulation was confirmed in the liver and portal

vein of mice. Valeric acid significantly inhibited the formation of NAFLD to HCC (hepatocellular carcinoma) induced by a high-fat diet in mice [17], and could improve the integrity of the intestinal barrier. This protective effect of valeric acid underscores the potential of *Lactobacillus* and its metabolites in the prevention and management of liver diseases, highlighting the importance of maintaining a healthy gut microbiome for overall liver health.

Previous endoscopic sampling studies used direct biopsy forceps for sampling. This method has the risk of sample contamination by digestive tract bacteria, resulting in a certain degree of bias in the results. Contamination can lead to misleading conclusions about the microbial composition and diversity in the sampled tissues, which is particularly problematic in studies aiming to understand the gut microbiome's role in health and disease. Such biases can obscure the true relationship between microbial communities and various gastrointestinal conditions. Shanahan [18] designed the BABD biopsy forceps, which can perform targeted biopsies under aseptic conditions. However, it has disadvantages such as a small amount of sample tissue and interference of host DNA in metagenomic sequencing. The limited sample size can hinder comprehensive analyses, making it challenging to draw definitive conclusions regarding the microbiota's contributions to specific diseases. Additionally, the presence of host DNA can complicate the interpretation of sequencing results, as it may mask the microbial signals that are crucial for understanding gut health. Mottawea [19] rinsed the forceps channel with sterile water before sampling. Although the operation method is simple, a serious drawback of this method is the dilution of bacteria in the lumen after rinsing with sterile water, resulting in a decrease in the concentration of PCR products for extracting microbial DNA. This dilution effect can significantly reduce the sensitivity of microbial detection, potentially leading to an underestimation of the microbial diversity present in the samples.

In this study, we designed a new endoscopic working channel plug accessory to keep the channel sterile during operation, avoiding contamination by miscellaneous bacteria or intestinal contents. This innovative accessory is designed to maintain a sterile environment, thus enhancing the reliability of microbial sampling. By preventing contamination, the accessory ensures that the microbial profiles obtained are more representative of the actual microbial communities present in the targeted tissue. Compared with 3D-printed capsules or BABD biopsy forceps [20,21], this method has significant advantages in terms of cost and ease of operation, making it an ideal choice for clinical research applications. The affordability of this method allows for wider adoption in clinical settings, which is crucial for advancing research on gut microbiota. Its simplicity also means that more healthcare professionals can utilize it effectively, potentially increasing the volume of high-quality data collected in microbiome studies. Moreover, this method can also be applied to other parts of the digestive tract, such as the duodenum and colon, to obtain the microbiota characteristics of specific sites. By enabling targeted sampling across various gastrointestinal regions, researchers can gain deeper insights into how microbial communities differ throughout the digestive tract and their implications for localized diseases. Taking advantage of the sterile state in the forceps channel, researchers can perform more operations or surgeries by using devices such as suction tubes, biopsy forceps, and puncture needles. This versatility allows for a broader range of

interventions during endoscopic procedures, facilitating comprehensive assessments of gut health. Of course, this method also has certain limitations. Endoscopic operation is essentially invasive and requires skilled operators. The need for skilled personnel underscores the importance of training and experience in minimizing the risks associated with such procedures. At the same time, further research is needed to evaluate the impact of intestinal cleansing preparation, operation time, and patient tolerance. Understanding these factors will be essential for optimizing the procedure and ensuring patient safety while maximizing the quality of the microbiota samples obtained. Subsequent studies need to expand the sample size and incorporate patients of different ages, genders, regions and living habits, thus enhancing the universality and reliability of the research findings. Also, diet, exercise, drugs and other factors will be further considered and long-term dynamic follow-up studies will be conducted to provide strong support for the early prevention and treatment of MAFLD.

5. Conclusion

This study aimed to investigate the characteristics of ileal microbiota in patients with metabolic associated fatty liver disease (MAFLD). A total of 72 patients were recruited, categorized into mild fatty liver (MF group, n = 36) and moderate to severe fatty liver (SF group, n = 36) based on Controlled Attenuation Parameter (CAP) values, alongside a healthy control group (H, n = 36). The ileal microbiota was sampled using innovative endoscopic techniques, followed by 16S rDNA sequencing for comprehensive analysis. The findings revealed significant alterations in the ileal microbiota among MAFLD patients compared to the control group. Notably, there was an increase in alpha diversity of the ileal flora, indicating a more varied microbial community. However, a gradual decrease in the percentage of *Turicibacter* was observed, alongside reduced abundances of *Lactobacillus* and *Veillonella*. In contrast, there was a marked increase in the abundance of *Prevotella*, *Leptotrichia*, and *Porphyromonas*. These results suggest that the diversity and composition of ileal microbiota are closely linked to the severity of MAFLD. The reduction of *Turicibacter* and the influx of oral colonizing bacteria emerge as distinctive features of the ileal microbiota in MAFLD patients. This study revealed the changes in the biomechanical characteristics of the ileal microbiota in MAFLD patients, providing a new perspective for in-depth understanding of the pathogenesis of MAFLD. These findings complement the results of previous studies on the overall gut microbiota and jointly promote the research progress on the relationship between MAFLD and the gut microbiota.

Author contributions: Conceptualization, TW and TL; methodology, TL; validation, TW, GD and YT; formal analysis, YT; investigation, TW; resources, TW; data curation, TW; writing—original draft preparation, TW; writing—review and editing, YT; visualization, TW; supervision, YT; project administration, GD; funding acquisition, GD. All authors have read and agreed to the published version of the manuscript.

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Ethical approval: The study was conducted in accordance with the Declaration of Helsinki. The studies involving humans were approved by Ethics Committee of Wuxi Hospital of Traditional Chinese Medicine [SWJW2022062701], The China Clinical Trial Registry [ChiCTR2400089346]. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Conflict of interest: The authors declare no conflict of interest.

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