

Lack of *Faecalibacterium prausnitzii* and *Bifidobacterium* is associated with a higher risk of metabolic associated fatty liver disease in young-onset type 2 diabetes

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Abstract

Background The incidence of comorbidity between type 2 diabetes mellitus (T2DM) and metabolic-associated fatty liver disease (MAFLD) is high, and patients tend to be younger. When people develop metabolic diseases such as T2DM and MAFLD, the original homeostasis of the gut microbiota in the body is disrupted, and gut flora drift occurs. This study investigated the relationship between the number of gut flora and MAFLD in young-onset T2DM.

Methods This retrospective study analyzed 44 adolescent T2DM patients who were divided into a non-MAFLD group and a MAFLD group. Anthropometric measurements, clinical and biochemical markers, inflammatory markers, thyroid function assessments, and stool specimens were collected. Real-time PCR was performed to quantify several important gut flora constituents at the genus level. Student's t-test and the chi-square test were applied for group comparisons, and binary regression models were used to explore the relationship between gut flora and MAFLD in young-onset T2DM.

Results Among the 44 subjects, 26 (59.1%) were diagnosed with MAFLD, and 18 (40.9%) were not. Compared with the non-MAFLD group, body mass index (BMI), abdominal circumference, and levels of blood uric acid and thyroid stimulating hormone (TSH) in the MAFLD group were significantly increased, and age level and high-density lipoprotein cholesterol (HDL-C) were significantly decreased ($p < 0.05$). Compared with the non-MAFLD group, the abundance of *Faecalibacterium prausnitzii* and *Bifidobacterium* in the MAFLD group was significantly reduced, and the abundance of *Enterococcus* and *Lactobacillus* was significantly increased ($p < 0.05$). In the multivariate regression analysis, *Faecalibacterium prausnitzii* and *Bifidobacterium* were independent protective factors for MAFLD in young-onset T2DM, after excluding confounding factors.

Conclusion In young-onset T2DM, there was a difference in gut flora between patients with MAFLD and those without MAFLD. *Faecalibacterium prausnitzii* and *Bifidobacterium* were independent protective factors for MAFLD in young-onset T2DM.

Keywords Young-onset T2DM · Metabolic associated fatty liver disease · Gut flora · *Faecalibacterium prausnitzii* · *Bifidobacterium*

Introduction

Type 2 diabetes mellitus (T2DM) has emerged as one of the most severe health concerns worldwide and is traditionally considered to be a chronic disease of older individuals. However, due to changing lifestyles, including a sedentary

lifestyle, sleep structure alterations, physical inactivity, and overeating, the largest increase in T2DM in this century has occurred among adolescents and adults under the age of 40. [1] We classified patients as having young-onset diabetes if they were diagnosed before the age of 40 years and as having late-onset diabetes if they were diagnosed at 40 years or older [2]. Metabolic-associated fatty liver disease (MAFLD) is a chronic liver disease that involves genetic susceptibility and metabolic and environmental factors, and its incidence rate has been increasing annually, affecting approximately 25% of the global population. [3] The diagnostic criteria for MAFLD are based on evidence of hepatic steatosis in addition to one of the following three criteria, namely, overweight/obesity, presence of type 2 diabetes mellitus, or evidence of metabolic

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dysregulation [4]. Metabolic disorders such as impaired glycemic control, insulin resistance (IR), and visceral obesity are considered to be the major risk factors for MAFLD, as well as T2DM. Therefore, the prevalence of MAFLD is high in T2DM patients. According to a meta-analysis that included 80 studies from 20 different countries, 47.3–63.7% of patients with T2DM are perceived to have MAFLD. [5] Huang et al. revealed that patients with MAFLD diagnosed by diabetes alone are more susceptible to hepatic fibrosis, which may eventually lead to cirrhosis and cardiovascular disease. [6]

The gut flora are considered the second genome in the human body. It has been established that crosstalk occurs between gut microbiota and human health, as the gut flora have the ability to aid in absorption and metabolism, fight against harmful bacteria, improve immunity, and fight tumors. [7] There is growing evidence that the gut microbiota can act as an endocrine organ involved in the dynamic regulation of the body's energy homeostasis and immune response. [8] When genetics, environmental factors, diet, and behavioral patterns change, the previously stable gut flora in the body drift. Recent years have shown that patients with T2DM and NAFLD exhibit drifting intestinal flora. A study examined the gut flora of people with normal glucose tolerance (NGT), pre-diabetes (pre-DM), and newly diagnosed T2DM. The results indicated that the abundance of butyrate-producing bacteria was higher in the NGT group than in the pre-DM group and that the abundance of the genus *Bacteroides* in the T2DM group was only half that of the NGT and pre-DM groups. [9] Loomba et al. found that among 86 patients with biopsy-proven MAFLD, those with advanced liver fibrosis had a higher abundance of the phylum Proteobacteria, those with stage 0–2 liver fibrosis had a higher abundance of the phylum Firmicutes, and 37 (of 40) predictors of advanced liver fibrosis were associated with gut flora [10]. Impaired intestinal barrier function and increased mucosal permeability in T2DM patients lead to increased absorption of lipopolysaccharide, which is the initiator of the inflammatory cascade and leads to chronic inflammation and insulin resistance by activating specific Toll-like receptor signaling pathways on the surface of hepatocytes, thereby accelerating the formation of MAFLD [11]. However, there is a lack of animal experiments and clinical studies on the changes in gut flora with the coexistence of MAFLD and T2DM, and we found many young-onset T2DM patients with MAFLD in clinical practice. The characteristics of gut flora drift in this group of patients are still unknown.

In this retrospective study, we collected clinical data from adolescent T2DM patients and compared the differences in several major gut flora (e.g., *Faecalibacterium prausnitzii*, *Escherichia coli*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Bacteroides*, and the phylum Firmicutes) between MAFLD patients and non-MAFLD patients. This study investigated the relationship between the number of gut flora and MAFLD in young-onset T2DM.

Materials and methods

Study participants

A total of 44 patients with T2DM who visited our hospital and received liver ultrasound from October 2020 to July 2021 were recruited for the study. The recruitment criteria were as follows: 1) adolescents and adults less than 40 years old, 2) sex not limited, 3) complete clinical data, and 4) informed consent. The exclusion criteria were as follows: 1) pregnant or lactating women; 2) acute complications such as diabetic ketoacidosis and hyperosmolar coma; 3) patients with acute and chronic infections; 4) patients with acute cardiac, hepatic, gastrointestinal, renal and cerebrovascular lesions; 5) patients with serious trauma or surgery within 6 months; 6) patients with malignant tumors and clinical connective tissue diseases; or 7) other serious endocrine metabolic diseases (such as hyperthyroidism, hypothyroidism, Cushing's syndrome). This retrospective study was approved by the Ethics Committee of XXX Hospital (No. 2020018), and the participants provided written informed consent for personal information collection. Finally, the participants were divided into a non-MAFLD group ($n=18$) and a MAFLD group ($n=26$) according to MAFLD diagnostic criteria. [4]

Real-time qPCR and microbial quantification

Early morning fecal samples were collected from all patients, and the fresh fecal samples (10 ± 5 g) were snap frozen in a -80 °C refrigerator within 2 h. Total microbial DNA was extracted from all stool specimens using a QIAamp DNA Stool Mini Kit (QIAGEN, Frankfurt, Germany). Quantitative real-time PCRs (qPCRs) were performed with FTC-3000TM real-time quantitative thermal cycler (Funglyn, Shanghai, China). All qPCRs were run with 3 replicates per DNA. Standard curves were set up by serially diluting the pMD18-T vector plasmid with the appropriate insert from 10^7 to 10^2 target gene copies μl^{-1} for every primer set. The standard curve was obtained using linear regression of threshold cycle numbers (ct) versus log copy numbers of targets. Real-time qPCRs were performed in 25 μl reaction mixtures that were composed of 12.5 μl SRBR Premix Ex Taq (Takara, Osaka, Japan), 1 μl of each forward and reverse primer, 5 μl of template DNA, and sterilized deionized water. The primers are described in Table 1. Melting curve analyses were performed from 60 to 96 °C with increments of 0.1 °C per cycle. The amounts of gut flora were log transformed for analysis.

Measurements

Baseline characteristics such as age, sex duration of diabetes, history of smoking, history of drinking, and family history of diabetes were collected by reviewing medical records.

Table 1 16S rRNA gene group-specific primer for quantitative real-time PCR

Target	Forward primers (5'–3')	Reverse primers (5'–3')
Total bacteria (16S V4V5)	GTGCCAGCMGCCGCGGTAA	CCGTCAATTCMTTGTAGTTT
<i>Enterococcus</i>	ACTCGTTGTACTTCCCATTGT	CCTTATTGTTAGTTGCCATCATT
<i>Escherichia coli</i>	GTTAATACCTTTGCTCATTGA	ACCAGGGTATCTAATCCTGTT
<i>Firmicutes</i>	TGAAACTYAAAGGAATTGACG	ACCATGCACCACCTGTC
<i>Bacteroides fragilis</i>	ATAGCCTTTCGAAAGRAAGAT	CCAGTATCAACTGCAATTTTA
<i>Faecalibacterium prausnitzii</i>	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA
<i>Lactobacillus</i>	GGAAACAGRTGCTAATACCG	CACCGCTACACATGGAG
<i>Bifidobacterium</i>	CTCCTGGAAACGGGTGG	GGTGTCTCTCCCGATATCTACA

Anthropometric measurements (height, weight, abdominal circumference) were collected according to a standard protocol. Measurements of routine blood tests, fasting plasma glucose (FPG), 2-h postprandial blood glucose (2hPG), glycosylated hemoglobin (HbA1c), triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum uric acid, serum creatinine, thyroid stimulating hormone (TSH), free triiodothyronine (fT3), and free thyroxine (fT4) were performed using standard laboratory methods.

Statistical analysis

IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Variables were examined for normality (normal plots), and nonnormally distributed variables were log transformed. The independent-samples t-test (for normally distributed variables) or the Mann–Whitney test (if nonparametric tests were required) was used to evaluate the significant differences in continuous variables, while the chi-squared test was used to evaluate the significant differences in categorical variables. Correlations of gut flora with BMI, abdominal circumference, FBG, and other variables were examined using Pearson correlation coefficients. Binary logistic regression analyses were used to evaluate the association between the amounts of gut flora and risk of MAFLD in young-onset T2DM after correction for potential confounders. A p value < 0.05 was considered statistically significant.

Results

Participant characteristics

A total of 44 participants were divided into a non-MAFLD group ($n = 18$) and a MAFLD group ($n = 26$). There was no statistically significant difference between the two groups in sex, DM course, history of smoking or alcohol consumption, or family history of diabetes ($p > 0.05$). The MAFLD group was younger than the non-MAFLD group ($p < 0.05$).

The MAFLD group had a larger BMI (28.97 ± 5.12 kg/m² versus 23.75 ± 3.95 kg/m², $p < 0.05$) and abdominal circumference (98.79 ± 13.24 cm versus 86.33 ± 9.00 cm, $p < 0.05$) than the non-MAFLD group. Regarding biochemical features, HDL-C in the MAFLD group was lower than that in the non-MAFLD group (0.97 ± 0.21 mmol/l versus 1.14 ± 0.32 mmol/l, $p < 0.05$), while the blood uric acid level was higher than that in the non-MAFLD group (460.66 ± 190.90 μ mol/l versus 367.07 ± 91.89 μ mol/l, $p < 0.05$). Regarding thyroid function, TSH was higher in the MAFLD group than in the non-MAFLD group (1.90 ± 0.88 mIU/l versus 1.47 ± 0.37 mIU/l, $p < 0.05$). No significant difference was observed in FPG or 2hPG between the two groups, although the values were relatively abundant in the MAFLD group compared to the non-MAFLD group ($p = 0.123$ and $p = 0.418$, respectively). HbA1c, other lipid profiles (TC, TG, and LDL-C values), and inflammation indicators (NLR, PLR) did not differ between the two groups. The participant characteristics are reported in Table 2.

Quantitative PCR analysis of gut flora

The MAFLD group had lower amounts of *Faecalibacterium prausnitzii* (8.76 ± 0.69 versus 9.45 ± 0.74 , $p < 0.05$) and *Bifidobacterium* (6.56 ± 0.78 versus 7.35 ± 1.13 , $p < 0.05$) and larger amounts of *Enterococcus* (7.27 ± 1.01 versus 6.66 ± 0.81 , $p < 0.05$) and *Lactobacillus* (7.11 ± 0.70 versus 6.51 ± 1.03 , $p < 0.05$). We did not observe any significant differences in the amounts of other gut flora (*Bacteroides fragilis*, *Escherichia coli*, Firmicutes) between the MAFLD groups and the non-MAFLD group (Table 3).

Correlation between gut flora and clinical data

Pearson correlation analysis was performed between the significantly different gut flora and related clinical indicators between the two groups. We reported a negative correlation between abdominal circumference, blood uric acid level, NLR, and the abundance of *Faecalibacterium prausnitzii*.

Table 2 Characteristics of young-onset T2DM

Characteristics	Non-MAFLD group (n = 18)	MAFLD group (n = 26)	p value
Sex, males, n (%)	12 (66.7%)	20 (76.9%)	z = 0.564 p = 0.453
Age (years)	35.4 ± 7.3	29.2 ± 7.3	z = 2.807 p = 0.008
Newly diagnosed diabetes, n (%)	3 (16.7%)	8 (30.8%)	$\chi^2 = 1.128$ p = 0.288
Alcohol drinkers, n (%)	4 (22.2%)	2 (7.7%)	$\chi^2 = 1.907$ p = 0.167
HBsAg positive, n (%)	1 (5.6%)	2 (7.7%)	$\chi^2 = 0.076$ p = 0.782
Family history of diabetes, n (%)	13 (72.2%)	16 (61.5%)	$\chi^2 = 0.504$ p = 0.462
BMI (kg/m ²)	23.75 ± 3.95	28.97 ± 5.12	z = -3.631 p = 0.001
Abdominal circumference (cm)	86.33 ± 9.00	98.79 ± 13.24	z = -3.469 p = 0.001
FPG (mmol/l)	12.04 ± 6.42	14.68 ± 4.70	z = -1.573 p = 0.123
2hPG (mmol/l)	15.64 ± 6.55	17.04 ± 6.24	z = -0.711 p = 0.481
HbA1c (%)	11.46 ± 3.29	11.54 ± 1.86	z = -0.104 p = 0.925
TG (mmol/l)	3.40 ± 2.64	4.76 ± 4.17	z = -1.222 p = 0.229
TC (mmol/l)	5.63 ± 1.87	5.81 ± 1.71	z = -0.377 p = 0.738
HDL-C (mmol/l)	1.14 ± 0.32	0.97 ± 0.21	z = 2.229 p = 0.049
LDL-C (mmol/l)	3.57 ± 1.31	3.48 ± 0.97	z = 0.262 p = 0.795
SUA (umol/l)	367.07 ± 91.89	460.66 ± 190.90	z = -1.926 p = 0.037
NLR	2.03 ± 0.77	2.05 ± 0.93	z = -0.086 p = 0.749
PLR	98.91 ± 32.64	93.73 ± 29.94	z = 0.516 p = 0.606
TSH (mIU/l)	1.47 ± 0.37	1.90 ± 0.88	z = -1.874 p = 0.040
fT3 (pmol/l)	3.94 ± 0.89	4.25 ± 1.01	z = -1.017 p = 0.315
fT4 (pmol/l)	13.90 ± 1.75	14.24 ± 1.84	z = -0.576 p = 0.568

2hPG, 2-h postprandial plasma glucose; BMI, body mass index; FPG, fasting plasma glucose; fT3, free triiodothyronine; fT4, free thyroxine; HbA1c, glycosylated hemoglobin; HBsAg, hepatitis B surface antigen; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-lymphocyte ratio; SUA, serum uric acid; TG, triglyceride; TSH, thyroid stimulating hormone

We also found that the amount of *Enterococcus* was positively correlated with abdominal circumference; the amount of *Lactobacillus* was positively correlated with abdominal circumference, HbA1c, and LDL cholesterol; and the amounts of *Bifidobacterium* were negatively correlated with abdominal circumference and NLR (Table 4).

Association between the amounts of gut flora and risk of MAFLD in young-onset T2DM

Multivariate binary linear regression analysis was performed to assess the influence of gut flora on the risk of MAFLD (Table 5). The model was adjusted for age, sex, whether

Table 3 Comparison of the amounts of gut flora between the non-MAFLD and MAFLD group

Gut flora	log copies/gram stool		<i>p</i> value
	Non-MAFLD group	MAFLD group	
Total bacteria (16S V4V5)	11.16 ± 0.34	10.97 ± 0.69	<i>z</i> = 0.978 <i>p</i> = 0.293
<i>Enterococcus</i>	6.68 ± 0.85	7.27 ± 1.01	<i>z</i> = -2.007 <i>p</i> = 0.045
<i>Escherichia coli</i>	8.11 ± 0.88	7.85 ± 1.25	<i>z</i> = 0.755 <i>p</i> = 0.455
<i>Firmicutes</i>	10.23 ± 0.32	10.04 ± 0.55	<i>z</i> = 1.363 <i>p</i> = 0.180
<i>Bacteroides fragilis</i>	10.26 ± 0.47	9.87 ± 0.91	<i>z</i> = 1.662 <i>p</i> = 0.104
<i>Faecalibacterium prausnitzii</i>	9.45 ± 0.74	8.76 ± 0.69	<i>z</i> = 3.145 <i>p</i> = 0.003
<i>Lactobacillus</i>	6.51 ± 1.03	7.11 ± 0.70	<i>z</i> = -2.267 <i>p</i> = 0.043
<i>Bifidobacterium</i>	7.35 ± 1.13	6.51 ± 0.75	<i>z</i> = 2.960 <i>p</i> = 0.010

Table 4 Correlation of gut flora with metabolic indicators in young-onset T2DM

Variables	<i>Enterococcus</i>		<i>F. prausnitzii</i>		<i>Lactobacillus</i>		<i>Bifidobacterium</i>	
	<i>r</i>	<i>p</i> value	<i>R</i>	<i>p</i> value	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
BMI (kg/m ²)	0.269	0.078	-0.189	0.219	0.237	0.122	-0.307*	0.042
AC (cm)	0.317*	0.036	-0.301*	0.047	0.309*	0.042	-0.384*	0.010
FPG (mmol/l)	-0.068	0.663	-0.188	0.221	0.021	0.894	-0.184	0.232
2hPG (mmol/l)	-0.193	0.209	-0.250	0.102	0.053	0.732	-0.045	0.770
HbA1c (%)	-0.170	0.270	0.218	0.156	0.374*	0.012	0.141	0.361
HDL-C (mmol/l)	-0.090	0.560	0.108	0.485	-0.155	0.315	0.249	0.102
LDL-C (mmol/l)	-0.180	0.243	0.062	0.689	0.371*	0.013	0.243	0.113
SUA (umol/l)	0.038	0.806	-0.432**	0.003	0.228	0.136	-0.213	0.166
NLR	-0.175	0.286	-0.407*	0.01	-0.045	0.785	-0.365*	0.023
PLR	0.005	0.977	-0.118	0.476	0.001	0.997	0.038	0.819
TSH (mIU/l)	0.165	0.285	0.044	0.778	0.240	0.117	-0.109	0.482
FT3 (pmol/l)	0.079	0.613	0.034	0.827	-0.240	0.121	0.074	0.637
FT4 (pmol/l)	0.130	0.444	-0.045	0.793	-0.149	0.378	-0.290	0.081

r = Pearson's correlation coefficient; * *p* < 0.05; ** *p* < 0.001

diabetes was newly diagnosed, and BMI. The results suggested that after adjusting for confounders, *Faecalibacterium prausnitzii* (OR = 0.197, 95% CI = 0.047–0.820) and *Bifidobacterium* (OR = 0.340, 95% CI = 0.121–0.959) remained as independent protective factors for MAFLD in adolescent T2DM. In other words, as the amounts of *Faecalibacterium prausnitzii* and *Bifidobacterium* increased, the probability of MAFLD decreased in young-onset T2DM.

Discussion

The main risk of T2DM is the damage to target organs caused by its chronic hyperglycemic state, and MAFLD is the main form of liver damage in T2DM. [12] For the past

Table 5 Association between the amounts of gut flora and risk of MAFLD in young-onset T2DM

	Multivariate OR (95% CI), <i>p</i> value	
	Unadjusted model	Adjusted model
<i>Enterococcus</i>	2.071 (0.961–4.467), 0.063	-
<i>F. prausnitzii</i>	0.191 (0.056–0.650), 0.008	0.197 (0.047–0.820), 0.028
<i>Lactobacillus</i>	2.332 (1.055–5.156), 0.036	2.255 (0.895–5.683), 0.085
<i>Bifidobacterium</i>	0.358 (0.162–0.794), 0.011	0.340 (0.121–0.959), 0.041

Adjusted model: adjusted for age, sex, whether newly diagnosed diabetes and BMI

few years, it has been shown that gut flora can participate in the development of T2DM, MAFLD, and other metabolic diseases by regulating host energy metabolism and improving the inflammatory response. [13–15] In this study, we compared the clinical characteristics and differences in gut flora between MAFLD and non-MAFLD patients in young-onset T2DM and explored the relationship between the amounts of gut flora and MAFLD in young-onset T2DM. We found that *F. prausnitzii* and *Bifidobacterium* were protective factors for MAFLD in young-onset T2DM, independent of age, sex, DM course, and other confounders.

F. prausnitzii is considered one of the most important members of *Firmicutes* and is one of the most abundant flora in the intestines of healthy individuals, and its absence inevitably induces gut microbiota disturbance. [16] Recent studies have shown that *F. prausnitzii* is strongly associated with intestinal inflammatory diseases such as ulcerative colitis and Crohn's disease; a LACK of *F. prausnitzii* is associated with the diseases and that its presence improves health benefits. [17, 18] *F. prausnitzii* induces the secretion of IL-10, an anti-inflammatory cytokine, in peripheral blood mononuclear cells (PBMCs). Munukka et al. reported that *F. prausnitzii*-treated mice had lower liver fat content, aspartate aminotransferase, and alanine aminotransferase and increased fatty acid oxidation and adiponectin signaling in the liver compared to high-fat control mice. [19] Its exact mechanism is related to an increase in adiponectin expression in visceral adipose tissue, an increase in insulin sensitivity, and decrease in inflammation in subcutaneous and visceral adipose tissue. Our study found that the amounts of *F. prausnitzii* were lower in the MAFLD population and were negatively correlated with SUA and NLR. SUA causes oxidative stress in mitochondria, activates the NLRP3 inflammatory complex, and exacerbates the hepatic inflammatory response and insulin resistance (IR), while NLR is considered a biomarker that reflects the inflammatory state of the body, and when NLR is increased, MAFLD patients are more likely to further develop hepatitis and liver fibrosis. [20] Martin et al. isolated strains of *F. prausnitzii* from the feces of healthy volunteers. [21] By studying their products, antibiotic resistance, immunomodulatory functions, and metabolic characteristics, the researchers found that *F. prausnitzii* has good anti-inflammatory effects and is a good candidate for the next generation of probiotics.

Bifidobacterium is extensively used as a probiotic, and 72 subspecies have been identified and sequenced that play an essential role in maintaining intestinal function and human homeostasis. [22] A metagenomics analysis of the duodenal microbiota of obese patients with T2DM revealed that the abundance of *Bifidobacterium* was significantly lower in obese individuals and obese patients with T2DM than in non-obese individuals. [23] Using a multiomics approach, Turrone et al. analyzed the interactions between four strains

of human intestinal commensals (*Bifidobacterium bifidum* PRL2010, *Bifidobacterium adolescentis* 22L, *Bifidobacterium breve* 12L, and *Bifidobacterium longum* subsp. *infantis* ATCC15697) in the intestine of mice. [24] The results revealed that *Bifidobacterium* cooperated and established a symbiotic relationship with each other in a competitive environment through glycan harvesting, glycan breakdown, and crossfeeding behavior. The composition of the rat cecal microflora significantly changed with the introduction of *Bifidobacterium*, and the abundance of sugar-degrading flora such as *Lactobacillus* increased. Therefore, the intestinal degradation of plant carbohydrates and host polysaccharides was enhanced. Animal research demonstrated that probiotics from the combination of *Lactobacillus* and *Bifidobacterium* delayed the progression of high-fat-diet-induced NAFLD in rats, with significant reductions in body weight, serum free fatty acid, TG, ALT, IL-1 β , and IL-18 levels; significant reductions in Gpr109a expression in liver and adipose tissue; and significant increases in butyric acid levels. [25] The aforementioned study suggested that *Bifidobacterium* was negatively associated with obesity, low-grade inflammation, insulin resistance, and glucose metabolism disorders. Our study also found that the amounts of *Bifidobacterium* were lower in MAFLD patients and were negatively correlated with BMI, abdominal circumference, and NLR, but not significantly correlated with glucose metabolism indicators (FBG, 2hPG, HbA1c).

Although they were not an independent risk factor for MAFLD in young-onset T2DM, we observed that the amounts of *Enterococcus* and *Lactobacillus* were higher in patients with MAFLD. *Enterococcus* are considered the second most common cause of nosocomial infections (following *Escherichia coli*). Based on mouse models and human data, Llorente et al. found that proton pump inhibitors can lead to liver inflammation and hepatocyte death by increasing the amounts of *Enterococcus* in the intestine and promoting their translocation. [26] Interestingly, as a probiotic, several studies have observed a positive correlation between *Lactobacillus* and obesity. [27–29] It has also been found that *Lactobacillus rhamnosus* GG, the most common probiotic, prevents the development of fatty liver by competing with the host intestine for fatty acids and inhibiting fatty acid metabolism and absorption in the intestine to inhibit liver fat accumulation. [30] Since dysbiosis of *Lactobacillus* is common in patients with T2DM and MAFLD, the mechanisms by which it acts on these two metabolic diseases need to be further explored.

In addition, exploring the clinical characteristics of patients with combined MAFLD in T2DM, we found that even among adolescents and adults less than 40 years old, patients with MAFLD were younger (35.4 ± 7.3 years versus 29.2 ± 7.3 years, $p = 0.008$), which may be related to the multiple burdens of study, career, and family in younger T2DM patients and more unhealthy living, eating, and sleeping

habits, thus increasing the metabolic disorder risk. We also found that adolescent T2DM combined with MAFLD was characterized by high BMI, high abdominal circumference, low HDL-C, and high SUA ($p < 0.05$). It is worth mentioning that TSH levels were higher in the MAFLD group. A cross-sectional study showed that in a T2DM population with $HbA1c \geq 7\%$, elevated levels of TSH were associated not only with the prevalence of MAFLD but also with the degree of MAFLD steatosis. [31] Existing research has shown the following mechanism: TSH acts on hormone-sensitive triglyceride lipase (HSL) and adipose triglyceride lipase (ATGL) to promote adipocyte differentiation and thus inhibit adipose tissue breakdown. In addition, TSH increases triglyceride synthesis by regulating the expression activity of sterol regulatory element-binding protein-1c (SREBP-1c) in hepatocytes. [32]

This study entailed some limitations. First, the sample size was small, and the results were limited by geographic constraints. Second, a detailed medication history was not taken, and the effect of glucose-lowering drugs on gut flora could not be excluded. Third, due to the retrospective nature of this study, a causative relationship between gut flora and MAFLD in young-onset T2DM could not be established. Therefore, in further research, we should design multicenter prospective studies based on large sample sizes and collect medication histories that may affect the gut flora.

Conclusion

In conclusion, our study revealed that the gut flora of MAFLD patients and non-MAFLD patients in young-onset T2DM were different. The amounts of *F. prausnitzii* and *Bifidobacterium* were lower and the amounts of *Enterococcus* and *Lactobacillus* were higher in the MAFLD group. *F. prausnitzii* and *Bifidobacterium* were protective factors for MAFLD in young-onset T2DM, independent of age, sex, DM course, and other confounders. These results suggest that we might prevent or treat MAFLD in young onset with T2DM by regulating the corresponding gut flora.

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Data Availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Conflict of interest This retrospective study was approved by the Ethics Committee of XXX Hospital (No. 2020018), and the participants

provided written informed consent for personal information collection.

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