Postprint: Genetically Predicted Causal Effects of Gut Microbiota on Metabolic Syndrome

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Abstract

Background Many observational studies have identified associations between gut microbiota and metabolic syndrome (MetS) and its components. However, the causal relationship between them remains unclear.

Objective This study aimed to investigate the bidirectional causal relationship between gut microbiota and MetS and its components.

Methods Single nucleotide polymorphisms (SNPs) associated with gut microbiota were obtained from MiBioGen, and summary statistics for MetS and its components were derived from the UK Biobank, Complex Trait Genetics (CTG), and other consortium studies. Bidirectional two-sample Mendelian randomization analysis was conducted to assess the causal relationships. Additionally, a series of sensitivity analyses were performed to validate the robustness of the Mendelian randomization results. To obtain a more stringent causal interpretation, Bonferroni correction was applied to test the strength of the causal relationships between gut microbiota and MetS.

Results Inverse variance weighted (IVW) estimation revealed a significant negative causal effect of Bifidobacteriaceae on MetS (OR=0.96, 95%CI=0.93-0.98, P=1.49E-03). Certain gut microbiota taxa showed significant positive causal effects on waist circumference, including class.Melainabacteria (OR=1.02, 95%CI=1.01-1.03, P=1.90E-03), order.Gastranaerophilales (OR=1.02, 95%CI=1.01-1.03, P=1.61E-03), order.NB1n (OR=1.02, 95%CI=1.01-1.03, P=2.00E-03), and genus.Eubacteriumhalliigroup (OR=1.03, 95%CI=1.01-1.04, P=6.97E-04), among others. However, reverse Mendelian randomization analysis did not support a causal relationship in the opposite direction. Sensitivity analyses indicated no heterogeneity or horizontal pleiotropy.

Conclusion This bidirectional Mendelian randomization study provides evidence for a significant causal effect of gut microbiota on MetS and its components, but does not support reverse causality. These findings offer new insights into

the prevention and treatment of MetS. Further randomized controlled trials are warranted to elucidate the effects of microbial agents such as probiotics on MetS.

Full Text

A Study of the Causal Effect of Gut Microbiota on Genetic Prediction of Metabolic Syndrome

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Abstract

Background: Many observational studies have identified associations between the gut microbiome and metabolic syndrome (MetS) and its components, but the causal relationship remains unclear. **Objective:** This study aimed to investigate the bidirectional causal relationship between gut microbiota and MetS and its components. **Methods:** We obtained gut microbiota-associated single nucleotide polymorphisms from MiBioGen and summary statistics for MetS and its components from the UK Biobank, Complex Trait Genetics (CTG), and other consortium studies. Bidirectional two-sample Mendelian randomization (MR) analyses were performed to assess causal relationships, supplemented by a series of sensitivity analyses to verify the robustness of results. To obtain more rigorous causal interpretations, Bonferroni correction was applied to test the strength of causal relationships between gut microbiota and MetS. **Results:** Inverse variance weighted (IVW) estimation revealed a significant negative causal relationship between Bifidobacteriaceae and MetS (OR=0.96, 95%CI=0.93-0.98, P=1.49E-03). Several gut microbiota taxa showed significant positive causal relationships with waist circumference, including class.Melainabacteria (OR=1.02, 95%CI=1.01-1.03, P=1.90E-03), order.Gastranaerophilales (OR=1.02, 95%CI=1.01-1.03, P=1.61E-03), order.NB1n (OR=1.02, 95%CI=1.01-1.03, P=2.00E-03), and genus.Eubacteriumhalliigroup (OR=1.03, 95%CI=1.01-1.04, P=6.97E-04). However, reverse MR analysis did not support causal relationships in the opposite direction. Sensitivity analyses indicated no heterogeneity or horizontal pleiotropy. **Conclusion:** This bidirectional MR study provides evidence for a clear causal effect of gut microbiota on MetS and its components but does not support reverse causality. These findings offer new insights for MetS prevention and management. Further randomized controlled trials are needed to elucidate the effects of microbial agents such as probiotics on MetS.

Keywords: Gut microbiota; Metabolic syndrome; Mendelian randomization; Causal relationship

Introduction

Metabolic syndrome (MetS) is defined by the WHO as the presence of at least three of five conditions: elevated fasting glucose (FG), reduced high-density lipoprotein cholesterol (HDL-C), hypertension (HBP), elevated triglycerides (TG), and increased waist circumference (WC) [1]. Globally, approximately one-quarter of the population meets MetS diagnostic criteria, affecting over one billion people [2-3]. MetS represents a highly prevalent worldwide disease and a serious cardiometabolic risk factor that promotes cardiovascular disease, type 2 diabetes, stroke, and even cancer [4]. Therefore, identifying risk factors for MetS and implementing preventive measures are crucial for cardiovascular disease management.

The gut microbiota has received increasing attention in recent years, with demonstrated involvement in numerous diseases through various axes including the brain-gut, lung-gut, gut-liver, brain-kidney-gut, and gut-liver-kidney pathways [5-6]. Research has established that gut microbiota participates in the pathogenesis of metabolic diseases such as type 2 diabetes, obesity, cardiometabolic disorders, non-alcoholic liver disease, and malnutrition [7]. These effects are mediated by various bioactive metabolites produced by microbial metabolism that reach the peripheral circulation via the portal vein, acting as substrates or signaling molecules [1]. Disease-associated metabolites can now be detected through mass spectrometry and multi-omics technologies, enabling integrated analysis of microbes, metabolites, and host phenotypes to identify potential pathogenic mechanisms [8]. Observational studies have found that many MetS risk factors may cause MetS through alterations in gut microbial composition, suggesting potential therapeutic targets [9]. For example, circadian rhythm-disrupting lifestyles disturb host metabolism, energy balance, and oxidative stress pathways, altering gut microbiota composition and consequently affecting MetS [10]. Studies have shown that fecal transplantation from volunteers treated with antibiotics into germ-free mice improved glucose metabolism in high-fat diet-fed, obesity- and diabetes-prone C57BL/6J mice [11]. Wang et al. [12] conducted systematic analysis of host genome, gut microbiome (16S rRNA), BMI, and blood lipids in 893 participants from the LifeLines-DEEP cohort (a Dutch prospective population cohort), estimating that gut microbiota explained 4.5% of BMI variation, 6.0% of TG variation, and 4% of HDL-C variation. These findings indicate that gut microbiota is an important regulator of human metabolic function, though establishing causal relationships and specific mechanisms remains challenging.

Previous research on gut microbiota and MetS has primarily relied on observational studies for etiological inference, which are susceptible to confounding

by age, diet, lifestyle, and other limitations including subjective interference, large sample requirements, and long study periods. These factors constrain exploration of causal relationships between gut microbiota and MetS. Mendelian randomization (MR) is a novel epidemiological approach for revealing causality that has developed rapidly in recent years [13]. Unlike cohort studies and other observational designs, exposure is determined before birth, making MR less susceptible to confounding and reverse causation, thereby effectively reducing bias [13-14]. In essence, MR utilizes the random allocation of genotypes in nature to infer the effect of phenotypes on disease outcomes. Rapid advances in genomic technology and the emergence of large-scale, multi-center databases have provided convenient conditions for widespread MR application. This bidirectional MR study aimed to clarify causal relationships between gut microbiota and MetS and its components, providing insights for developing effective prevention strategies and therapeutic interventions.

Methods

1.1 Study Design This study employed a two-sample bidirectional MR design, with the workflow illustrated in Figure 1 [Figure 1: see original paper]. MR analysis requires three core assumptions [15]: (1) **Relevance assumption**: instrumental variables (IVs) must be strongly associated with the exposure; (2) **Independence assumption**: IVs must be independent of confounders affecting the exposure-outcome relationship; and (3) **Exclusion restriction assumption**: genetic variants affect the outcome only through the exposure, not through other pathways.

1.2 Data Sources We selected genome-wide association study (GWAS) data on human gut microbiota from the international MiBioGen consortium [16]. This large-scale, multi-ethnic GWAS included 18,340 participants from 24 cohorts across the United States, Canada, Israel, South Korea, Germany, Denmark, Netherlands, Belgium, Sweden, Finland, and other countries, with 72.3% being of European ancestry. The database integrated 16S rRNA gene sequencing profiles and genotyping data to reveal associations between human genetic variation and gut microbiome composition. At $P<10^{-5}$, 211 taxonomic units were obtained (131 genera, 35 families, 20 orders, 16 classes, 9 phyla) with a sample size of 14,587. Fifteen bacterial traits lacking specific species names (3 families and 12 genera) were excluded, leaving 196 bacterial traits for analysis. Based on WHO definitions for MetS, we included GWAS summary statistics for MetS, WC, FG, HDL-C, HBP, and TG. MetS data were obtained from CTG (291,107 participants: 59,677 cases and 231,430 controls). Detailed data for MetS components came from the UK Biobank (UKB): WC (n=462,166), FG (n=58,074), HDL-C (n=403,943), HBP (n=463,010), and TG (n=441,016). These cohorts consisted primarily of individuals of European ancestry. To minimize racial mismatch, our analysis focused predominantly on participants of European descent.

1.3 Instrumental Variable Selection According to the three core MR assumptions, and considering the limited number of genetic instruments for gut microbiota, we selected single nucleotide polymorphisms (SNPs) at P<1$×10^{-5}$ as IVs. Second, SNPs had to be independent of each other, requiring removal of linkage disequilibrium (LD). LD was set to exclude SNPs within a 1,000 kb window with R^2>0.1, retaining only the SNP with the lowest P-value. Additionally, we identified potential confounders between gut microbiota and MetS through literature review, including age, sex, education level, smoking status, alcohol consumption, physical activity, depression, and low mood. To ensure IV independence from confounders affecting the exposure-outcome relationship, we queried PhenoScanner V2 to identify and exclude SNPs associated with other potential confounding traits at the genome-wide level. Finally, we calculated the F-statistic for each IV to assess strength. An F-statistic >10 indicates a valid genetic instrument [17-19]; weak instruments introduce bias into analyses. The F-statistic was calculated as:

F=R2/1-R2×N-K-1/K

R2=2×$ $2×EAF×(1−EAF)/2×$$ $2×EAF×(1−EAF)+2×SE2×N×EAF×$(1-EAF)

where N represents sample size, β is the SNP effect size on exposure, SE is the standard error of β, and EAF is the effect allele frequency.

1.4 MR Analysis We primarily used the inverse-variance weighted (IVW) method to assess causal relationships between genetically predicted gut microbiota and MetS and its components, supplemented by additional methods including MR Egger regression, weighted median (WME), simple mode (SM), and weighted mode (WM). These statistical methods assume all IVs included in MR analysis are valid, affecting the outcome only through exposure. When IVW results showed P<0.008 with directionally consistent results from MR Egger, SM, WME, and WM, we considered this a relatively stable causal association. Associations with 0.008<P<0.05 were considered potential relationships between gut microbiota and MetS or its components.

1.5 Sensitivity Analysis Cochrane' s Q test assessed heterogeneity among SNPs associated with each bacterial genus [20]. In the presence of heterogeneity (P<0.05), random-effects IVW provided more conservative but robust estimates. We examined horizontal pleiotropy bias by analyzing the MR Egger intercept term (Egger-intercept) and its P-value; an intercept not deviating from 0 suggests absence of horizontal pleiotropy. We also applied MR-PRESSO outlier testing to evaluate the impact of removing individual SNPs sequentially from MR analysis, calculating the P-value for pleiotropy significance. If the global test P-value>0.05, no effect was indicated. Conversely, if global test P-value<0.05, deviant SNPs existed among the included IVs, requiring removal of outlier genetic instruments and re-analysis of remaining SNPs. This process was repeated until MR-PRESSO test P-values became non-significant (P>0.05) [22].

1.6 Statistical Analysis Given the large number of tests performed, statistical significance for primary results was set at P<0.008 after Bonferroni correction (0.05/6). We also conducted reverse MR analysis. For reverse MR, many SNPs were identified at P<1$\times10^{\{-5\}, so the IV selection threshold was set at } P < 5\times10^{\{-8\}}$. LD was set to exclude SNPs within a 10,000 kb window with $R^{\wedge}\{2\}$>0.001. These threshold modifications for IV selection in reverse MR have been widely applied [23-25]. The detailed MR workflow is shown in Figure 1b. Bacterial taxa showing significant associations in forward MR were selected for reverse MR analysis. All MR analyses were performed using the TwoSampleMR package in R version 4.3.1 (http://www.r-project.org).

Results

Using bidirectional MR analysis, we explored causal relationships between gut microbiota and MetS and its components (WC, HBP, TG, HDL-C, FG). All significant results are presented in Table 1 and Table 2 .

2.1.1 Causal Effect of Gut Microbiota on MetS Genetically predicted gut microbiota showed significant associations with MetS and its components. MR-Egger regression intercepts did not deviate from zero, indicating no potential horizontal pleiotropy (all p-Egger_{intercept}>0.05). Specifically, after Bonferroni correction, Bifidobacteriaceae showed a significant negative causal relationship with MetS (OR=0.96, 95%CI=0.93-0.98, P=1.49E-03). Several bacterial taxa showed positive causal relationships with MetS. Wald ratio statistics indicated class.Gammaproteobacteria (OR=1.09, 95%CI=1.01-1.18, P=0.04) had a potential positive association with MetS. Additionally, genus.Enterorhabdus (OR=1.03, 95%CI=1.01-1.06, P=0.03) and genus.Allisonella (OR=1.03, 95%CI=1.01-1.05, P=0.04) showed potential associations with MetS.

2.1.2 Causal Effect of Gut Microbiota on WC When exploring genetic susceptibility between gut microbiota and WC, we identified several taxa with significant positive causal relationships: class.Melainabacteria (OR=1.02, 95%CI=1.01-1.03, P=1.90E-03), order.Gastranaerophilales (OR=1.02, 95%CI=1.01-1.03, P=1.61E-03), order.NB1n (OR=1.02, 95%CI=1.01-1.03, P=2.00E-03), and genus.Eubacteriumhalliigroup (OR=1.03, 95%CI=1.01-1.04, P=6.97E-04). Additionally, genus.Barnesiella (OR=1.02, 95%CI=1.00-1.04, P=0.02) showed a potential positive association with WC, while genus.Lactobacillus (OR=0.99, 95%CI=0.97-1.00, P=0.04) showed a potential negative association. Scatter plots and leave-one-out plots in Figures 2 [Figure 2: see original paper] and 3 [Figure 3: see original paper] demonstrate robust causal relationships between these taxa and MetS and WC.

2.1.3 Causal Effect of Gut Microbiota on HBP IVW estimation indicated genus.Olsenella (OR=1.01, 95%CI=1.00-1.01, P=2.40E-03) had a

detrimental effect on HBP. However, MR-Egger and other statistical methods showed opposite directions, so this result was excluded. IVW estimates for other taxa suggested potential associations with HBP, including family.Alcaligenaceae (OR=1.01, 95%CI=1.00-1.01, P=0.04), family.Veillonellaceae (OR=1.01, 95%CI=1.00-1.01, P=0.04), and genus.Eubacteriumfissicatenagroup (OR=1.01, 95%CI=1.00-1.01, P=0.03).

2.1.4 Causal Effect of Gut Microbiota on TG MR analysis did not support a causal relationship between gut microbiota and TG after Bonferroni correction (P<0.008). However, IVW estimates showed potential protective relationships with genus.Ruminococcustorquesgroup (OR=0.98, 95%CI=0.96-1.00, P=0.05), genus.Dorea (OR=0.98, 95%CI=0.96-1.00, P=0.03), and genus.RuminococcaceaeUCG010 (OR=0.95, 95%CI=0.92-0.99, P=0.01).

2.1.5 Causal Effect of Gut Microbiota on HDL-C IVW estimation indicated genus.Ruminiclostridium9 (OR=1.05, 95%CI=1.02-1.07, P=2.60E-04) had a significant detrimental effect on HDL-C. However, MR-Egger showed opposite direction, making the causal relationship non-robust, so this result was excluded. Additionally, genus.RuminococcaceaeUCG009 showed MR-PRESSO Global test P<0.001; after removing outliers and re-analyzing, the new IVW result was non-significant (P=0.276), so this genus was also excluded. Several taxa showed potential positive associations with HDL-C, including class.Erysipelotrichia (OR=1.026, 95%CI=1.003-1.049, P=0.028), family.Bifidobacteriaceae (OR=1.022, 95%CI=1.000-1.045, P=0.046), genus.Bifidobacterium (OR=1.025, 95%CI=1.001-1.050, P=0.039), genus.LachnospiraceaeNK4A136group (OR=1.023, 95%CI=1.006-1.040, P=0.008), genus.Parabacteroides (OR=1.030, 95%CI=1.006-1.054, P=0.013), and genus.RuminococcaceaeUCG010 (OR=1.043, 95%CI=1.008-1.079, P=0.015). Other taxa showed potential negative associations, including genus.Olsenella (OR=0.985, 95%CI=0.974-0.997, P=0.016) and genus.Peptococcus (OR=0.990, 95%CI=0.979-1.000, P=0.047).

2.1.6 Causal Effect of Gut Microbiota on FG After Bonferroni correction, no significant causal relationship was identified. However, potential protective relationships were found with family.Lactobacillaceae (OR=0.96, 95%CI=0.93-1.00, P=0.03), genus.Bifidobacterium (OR=0.96, 95%CI=0.93-1.00, P=0.02), and genus.Ruminococcusgauvreauiigroup (OR=0.96, 95%CI=0.93-1.00, P=0.05). Potential detrimental relationships were observed with genus.Bilophila (OR=1.05, 95%CI=1.00-1.09, P=0.05), genus.Eisenbergiella (OR=1.02, 95%CI=1.00-1.05, P=0.03), and genus.Parasutterella (OR=1.04, 95%CI=1.01-1.03, P=0.02).

2.2 Reverse MR Analysis Reverse MR results did not support causal relationships for taxa showing significant associations in forward MR analysis (Table 2).

Discussion

Using GWAS summary statistics from MiBioGen, CTG, and UKB, we performed bidirectional MR analysis of gut microbiota with MetS, WC, FG, HDL-C, HBP, and TG. IVW results identified several gut microbial taxa with causal relationships to MetS and its components. We found that the core human genus Bifidobacterium had a significant negative causal relationship with MetS. Class.Melainabacteria, order.Gastranaerophilales, order.NB1n, and genus.Eubacteriumhalliigroup showed significant positive causal relationships with WC. Additionally, several taxa showed potential associations with outcomes.

The influence of gut microbiota on MetS essentially reflects dysbiosis—suppression of beneficial bacteria and proliferation of harmful bacteria. Bifidobacterium, a member of Actinobacteria widely used as a probiotic component, demonstrates protective effects in metabolic diseases such as diabetes [26-27]. Our findings align with these observations, showing that Actinobacteria and its component Bifidobacteriaceae are protective factors negatively associated with MetS. Bifidobacterium provides physiological functions including biological barrier maintenance, nutritional effects, anti-tumor activity, immune enhancement, gastrointestinal function improvement, and anti-aging [16]. Mechanistically, Bifidobacteriaceae participates in short-chain fatty acid (SCFA) metabolism. SCFAs, primarily acetate, propionate, and butyrate, are major end products of colonic bacterial metabolism with broad physiological effects on the host [28].

Class.Melainabacteria showed a positive causal relationship with WC, with increased abundance associated with WC growth. Literature indicates that class.Melainabacteria abundance increases in patients with neurodegenerative diseases and gastrointestinal, hepatic, metabolic, and respiratory diseases [29]. Eubacterium is a Gram-positive bacterium belonging to the family Eubacteriaceae and phylum Firmicutes. Our study also found a positive causal relationship between order.Gastranaerophilales and WC. Research reports that Gastranaerophilales is an important indole-producing bacterium; indole promotes synthesis of indolepropionic acid, which has anti-inflammatory and anti-cancer effects in the gastrointestinal tract [30]. Order.NB1n belongs to Mollicutes, previously detected in patients with chronic gastritis and relatively low in healthy individuals [31]. NB1n may also be positively associated with gastroduodenal ulcers [32], though its specific association with WC remains understudied. Eubacterium is a core gut genus widely colonizing the intestines and oral cavity of most individuals, playing important roles in nutritional metabolism and intestinal homeostasis [33-34]. Many Eubacterium species produce SCFAs, particularly butyrate, which serves as a specific nutrient and energy source for intestinal epithelium, protects intestinal barrier function, reduces inflammation, and enhances gastrointestinal motility [35]. The relationship between Eubacterium and obesity remains controversial [36-38]. Some studies report positive associations between Eubacterium and obesity [39-40], consistent with our finding that increased genus.Eubacteriumhalliigroup abundance has a positive causal rela-

tionship with WC. However, other studies report reduced Eubacterium in obese individuals, limiting microbial utilization of complex carbohydrates [41-43].

Multiple mouse experiments have revealed metabolic effects of gut microbiota. Fei et al. [44] isolated an endotoxin-producing Enterobacter cloacae from morbidly obese volunteers and colonized germ-free C57BL/6J mice, inducing obesity and insulin resistance phenotypes under high-fat diet, while normal-diet transplanted mice did not develop these phenotypes. Notably, germ-free control mice on high-fat diet did not show the same disease phenotypes. The mechanism may involve endotoxin-mediated inflammatory responses from Enterobacter under high-fat diet promoting obesity. Morbidly obese volunteers following high-fiber diets rich in whole grains, traditional Chinese herbs, and prebiotics showed significant weight loss and improved blood glucose and blood pressure, demonstrating that high-fat diet significantly impacts gut microbiota and that dietary patterns mediate obesity development through gut microbes. Additionally, Zhou et al. [45] used 16S rRNA gene sequencing to analyze fecal samples from four male Zucker diabetic fatty (ZDF) rats, showing that fecal microbiome changes were associated with age and disease progression. During weeks 8-15, fecal microbiota were dominated by Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria; while Lactobacillus and Turicibacter were dominant in 8-10-week-old rats, Bifidobacterium, Lactobacillus, Ruminococcus, and Allobaculum were more abundant in 15-week-old rats. Random blood glucose was negatively correlated with Lactobacillus and Turicibacter but positively correlated with Ruminococcus and Allobaculum.

Trimethylamine N-oxide (TMAO) is a plasma metabolite derived from nutrient precursors (choline, phosphatidylcholine, and L-carnitine) produced by gut microbiota through metabolism of choline, betaine, or other trimethylamine-containing nutrients, generating trimethylamine that is transported via portal circulation to the liver for rapid conversion to TMAO by host hepatic flavin monooxygenase 3 [28, 46]. Multiple studies demonstrate that TMAO levels are significantly higher in diabetic subjects than non-diabetic controls, and TMAO increases mortality risk in type 2 diabetes and MetS, primarily through higher adverse cardiac events independent of glycemic control [47-48].

Many factors influence MetS, with gut microbiota representing one component. Other confirmed causal factors for MetS and its components include sleep duration, depression, exercise, diet, and smoking/alcohol consumption lifestyles [49-51]. These factors may influence MetS through gut microbiota and metabolites. As the most diverse microbial community, gut microbiota forms a symbiotic relationship with the host and deeply participates in regulating host gene expression, intestinal barrier function, nutrition, metabolism, and immunity [52]. Serving as a "second genome," gut microbiota plays a crucial role in maintaining human health homeostasis [7]. Gut microbiota-targeted therapies including probiotics, prebiotics, synbiotics, fecal microbiota transplantation (FMT), and antibiotics may be effective interventions for MetS [53-54]. Probiotics, defined as live microorganisms beneficial to human health, primarily improve gas-

trointestinal health, stimulate the immune system, inhibit pathogenic microbial growth, improve lactose intolerance, and reduce various disease risks. Several studies indicate probiotics can serve as important dietary supplements to reduce diabetes-related glucose metabolism factors, showing beneficial effects on glycemic control [54-55]. Randomized controlled trials have also demonstrated that probiotic supplementation significantly affects weight loss and WC through gut microbiota [56-57].

FMT, previously known as donor fecal transplantation or fecal bacterial therapy, has been shown to effectively improve insulin resistance and glucose metabolism while increasing gut microbial diversity, including notable increases in butyrate-producing strains, when administered to male subjects with MetS [53]. Butyrate, a four-carbon SCFA produced by anaerobic fermentation of indigestible carbohydrates by gut microbiota [58], enhances mitochondrial activity through gene expression and hormonal regulation, thereby preventing metabolic endotoxemia [59]. It also activates intestinal gluconeogenesis, triggering beneficial metabolic effects. However, some in vitro and in vivo studies suggest excessive SCFA production may increase energy accumulation and potentially produce adverse effects promoting obesity [60-61].

Lifestyle management is also an effective approach to reverse or slow MetS progression. Reducing sedentary time, regular walking or exercise, increasing low-fat high-fiber food intake, and eliminating smoking and alcohol consumption are effective methods to control MetS incidence. Research shows gut microbiota digestion of dietary fiber produces short-chain unsaturated fatty acids, and low fiber intake is associated with T2DM development [28, 62]. Exercise-induced changes in gut microbiota are closely associated with improved glucose metabolism and insulin sensitivity, manifested by increased SCFA synthesis and enhanced catabolism of branched-chain amino acids [51].

This study provides evidence for causal relationships between gut microbiota and MetS and its components, offering insights for MetS improvement through microbiota modulation. However, several limitations exist [63-64]. First, most GWAS summary statistics used participants of European ancestry, while some gut microbiota data were obtained from datasets including other ethnicities, potentially introducing bias and limiting generalizability to other populations. Second, population stratification effects cannot be ignored as they reduce MR statistical power. Critically, MR analysis of complex MetS pathogenesis mechanisms remains immature. Notably, the three MR assumptions are difficult to fully satisfy in practice; issues including horizontal pleiotropy, LD, genetic heterogeneity, and weak instrument bias affect MR statistical power. While some methods address these limitations, they only partially resolve these problems.

Although bidirectional two-sample MR effectively inferred causal relationships between gut microbiota and MetS, and previous observational studies in experimental animals established relevant associations, the specific mechanisms and pathways remain uncertain and controversial. Future clinical studies are needed to explore the effects of gut microbiota on MetS and its role in prevention and

treatment.

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