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Retrospective Study

Associations of gut microbiota with dyslipidemia based on gender differences in subjects from Northwestern China

Associations of GM with dyslipidemia

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Abstract

BACKGROUND

Gut microbiota (GM) has been proved to play a role in the regulation of host lipid metabolism, which provides a new theory about the pathogenesis of dyslipidemia. However, the associations of GM with dyslipidemia based on gender differences remain unclear, and warrant elucidated.

AIM

To investigate the associations of GM features with serum lipid profiles based on gender differences in a Chinese population.

METHODS

This study finally recruited 142 participants (73 females and 69 males) in Honghui Hospital, Xi'an Jiaotong University. The anthropometric and blood metabolic parameters of all participants were measured and detected. According to their serum lipid concentrations, participants were classified into a high triglyceride (H_TG) group, a high total cholesterol (H_CHO) group, a low high-density lipoprotein cholesterol (L_HDL-C) group, and a control (CON) group with normal serum lipid concentrations in females and males, respectively. Fresh fecal samples were collected for 16S rRNA gene sequencing analysis. UPARSE software, QIIME software, RDP classifier and FAPROTAX database were used for sequencing analysis.

RESULTS

The GM composition at the phylum level included Firmicutes and Bacteroidetes as the core GM. Different GM features were identified between females and males, and the associations between GM and serum lipid profiles were different in females and males. The GM features in different dyslipidemia subgroups changed both in female patients and male patients. Proteobacteria, Lactobacillaceae, *Lactobacillus* and *Lactobacillus_salivarius* were enriched in H_CHO females compared with CON females,

while Coriobacteriia were enriched in L_HDL-C females. In the comparison of the three dyslipidemia subgroups in females, *Lactobacillus_salivarius* were the enriched GM taxon in H_CHO females, and Prevotellaceae were the enriched GM taxon in L_HDL-C females. Compared with CON or H_TG males, Prevotellaceae, *unidentified_Ruminococcaceae*, *Roseburia* and *Roseburia_inulinivorans* decreased in L_HDL-C males (P value < 0.05), while LEfSe analysis indicated an enrichment of these GM taxa in H_TG males in the comparison to other male subgroups. Additionally, *Roseburia_inulinivorans* abundance was positively correlated with serum TG and TC concentrations, and *Roseburia* were positively correlated with serum TG. Furthermore, Proteobacteria (0.724, 95%CI: 0.567~0.849), Lactobacillaceae (0.703, 95%CI: 0.544~0.832), *Lactobacillus* (0.705, 95%CI: 0.547~0.834) and *Lactobacillus_salivarius* (0.706, 95%CI: 0.548~0.835) could distinguish H_CHO females from CON, while Coriobacteriia (0.710, 95%CI: 0.547~0.841), Coriobacteriales (0.710, 95%CI: 0.547~0.841), Prevotellaceae (0.697, 95%CI: 0.534 to 0.830), *Roseburia* (0.697, 95%CI: 0.534~0.830) and *Roseburia_inulinivorans* (0.684, 95%CI: 0.520~0.820) could discriminate H_TG males from CON. Based on the predictions of GM metabolic capabilities with the FAPROTAX database, a total of 51 functional assignments were obtained in females, while 38 were acquired in males. This functional prediction suggested that cellulolysis increased in L_HDL-C females compared with CON females, but decreased in L_HDL-C males compared with CON males.

CONCLUSION

This study indicates associations of GM with serum lipid profiles, supporting the notion that GM dysbiosis may participate in the pathogenesis of dyslipidemia, and gender differences should be considered.

Key Words: Dyslipidemia; Gut microbiota; 16S rRNA; Sequencing analysis; Gender differences; Northwestern China

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Core Tip: Dyslipidemia is the circulating lipid expression of metabolic syndrome, and alterations of gut microbiota (GM) are indicated to participate in the pathogenesis of dyslipidemia; however, little evidence was found in literatures on these associations based on gender differences. Our results demonstrated the GM features in different dyslipidemia subgroups in females and males, suggesting a complex interactivity between GM and lipid metabolism. Our observations may provide new evidence that different GM taxa may be associated with distinct lipids, and GM may affect specific aspects of lipid metabolism. More studies are required to propose specific taxa that have the potential to ameliorate dyslipidemia.

INTRODUCTION

Obesity has become a worldwide public health challenge, with its prevalence nearly tripled since 1975[1]. Obesity is defined as a chronic accumulation of excessive lipids in tissues[2], which is related to the disruption of lipid metabolism[3]. As a defect in lipid metabolism, dyslipidemia is defined as any abnormality in blood lipid levels, and is characterized by an elevation of circulating triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), or a decrease of low high-density lipoprotein cholesterol (HDL-C). Since observational studies have shown that 60%~70% of adults have lipid levels outside the recommended range[4, 5], it is essential to reveal the underlying mechanism of dyslipidemia. Circulating lipid levels are known to have an important genetic contribution from over 500 single-nucleotide polymorphisms (SNPs) in more than 150 Loci, explaining ~40% of the total individual variation[6]. However, the unexplained 60% variation has been attributed to undiscovered rare elements and unquantified environmental factors, such as dietary intake and physical activity[7, 8]. In recent years, considerable progress has been made in elucidating the mechanism

responsible for dyslipidemia, and accumulated evidence has shown that gut microbiota (GM) may play a potential role in obesity and related metabolic diseases, such as dyslipidemia[9-12].

The human gastrointestinal tract harbors over 100 trillion microorganisms[13], and the gut bacteria having effects on human health are the most prevalent and well-studied. In humans, the GM profiles vary between different ethnicities (host genetics) and genders[14, 15], and is mainly shaped by early life events and stabilizes in adolescence[16]. However, its composition and activity can be dynamic, and may be altered dramatically by multiple factors, such as medications, chronic dietary patterns and other environmental exposures[15, 17, 18]. This "microbial organ" has been recognized to perform various physiological functions[19-21], and is often called "a new virtual metabolic organ"[22, 23]. The first indication of associations between GM and disorder statuses were for inflammation[24], and altered GM community (dysbiosis) has now been established in the development of cardiometabolic phenotypes[25]. These lines of evidence have raised an interest in GM as an important candidate in accounting for the unexplained variation in serum lipid levels in humans, and a target for the therapeutic benefit of dyslipidemia[26]. Recent studies have convincingly linked GM to dyslipidemia, and GM was reported to explain substantial variation in TG and HDL-C independent of genetic factors in a Dutch study[26]. In addition, accumulating data from animal studies demonstrate that GM can affect host lipid metabolism through multiple direct and indirect biological mechanisms[27, 28]. Nevertheless, proof of associations between GM and host lipid metabolism remains a challenge in humans. Furthermore, gender is an important factor that may influence the GM profiles, and gender differences could also be observed in serum lipid profiles between females and males[29-31]. However, it remains inconclusive about the relationship between GM and dyslipidemia based on gender differences.

Collectively, ethnicity, geography and gender are potent factors that could influence the GM community[14, 15, 32]. Thus, this study focused on the associations of GM features with dyslipidemia based on gender differences in a northwestern Chinese population.

We will first reveal gender differences regarding the GM features, then introduce dyslipidemia, highlighting its intricate relationships with GM, and discuss possible altered GM functions.

MATERIALS AND METHODS

Study design

From July 2018 to January 2020, this study recruited 206 adult individuals (107 females and 99 males) from the outpatient clinics at Honghui Hospital, Xi'an Jiaotong University, China. Individuals were excluded if one of the following conditions existed: (1) with any gastrointestinal diseases, infectious or chronic diseases, serious systematic dysfunctions, or surgery histories of the gastrointestinal tract; (2) taking any medications that could disrupt the original GM community, such as probiotics or prebiotics, antimicrobial therapies, anti-inflammatory drugs, acid-suppressing drugs, immunosuppressants, or anti-dyslipidemia/anti-dysglycemia/anti-hypertension drugs within the past month prior to sampling[33]; and (3) females who were pregnant or lactating. The individuals who met the above conditions were included as participants in this study, and informed consent was obtained. Finally, 142 participants (73 females and 69 males) were recruited, including 81 dyslipidemia patients and 61 controls with normal serum lipid concentrations. Data privacy was ensured by using anonymized identifiers, and the study flow is shown in Figure 1. This study was approved by the Ethical Committee of Honghui Hospital, Xi'an Jiaotong University (Protocol Number: 201801022, approved January 8th, 2018).

Measurement of anthropometric parameters

On their first visit, related medical information was documented for all participants. Body weight (W) and height (H) were measured without shoes and heavy clothing to the nearest 0.1 cm and 0.1 kg, respectively. Waist circumference (WC) was measured in the middle of the lower rib margin and the iliac crest with a nonexpandable tape to the nearest 0.1 cm in the standing position. Body mass index (BMI) and waist

circumference/height ratio (WHR) were calculated accordingly. Blood pressure (BP) was assessed using a medical electronic sphygmomanometer (HEM-7130 professional, OMRON, Dalian, China) on the left arm positioned at the heart level with palm face up in a sitting position. The participants were required to rest in a seated position for at least 5 min before BP assessment, with triplicate measurements at 1-min intervals. All equipment was calibrated at the beginning of the study.

Detection and computation of metabolic indicators

Venous blood samples were drawn from an antecubital vein in the morning following an overnight (at least 8 h) fast. Concentrations of fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were detected by an automatic biochemical analyzer (Cobas c701, Roche, Mannheim, Germany). This instrument system was calibrated regularly. Moreover, the Chinese visceral adiposity index (CVAI) was calculated using the formula[34]: CVAI for females = −187.32 + 1.71×age + 4.23×BMI + 1.12×WC (cm) + 39.76×Log_{10}TG (mmol/L) − 11.66×HDL-C (mmol/L), and CVAI for males = −267.93 + 0.68×age + 0.03×BMI + 4.00×WC + 22.00×Log_{10}TG − 16.32×HDL-C.

Diagnostic criteria and grouping

In accordance with the "Guidelines for prevention and treatment of dyslipidemia in Chinese adults" (revised in 2016)[35], and the stratification standard of dyslipidemia in the primary prevention population of arteriosclerotic cardiovascular disease (ASCVD) in China[36], dyslipidemia was defined as the presence of one or more abnormal serum lipid concentrations without any lipid-lowering medication: TG ≥ 1.7 mmol/L, TC ≥ 5.2 mmol/L, LDL-C ≥ 3.4 mmol/L, and/or HDL-C < 1.0 mmol/L. Subsequently, participants were divided into subgroups according to their serum lipid profiles. Specifically, patients were classified into the high TG (H_TG) group if only high TG concentrations existed. Patients with increased serum TC and/or LDL-C concentrations and without HDL-C or TG abnormalities were classified into the high cholesterol

(H_CHO) group. Patients were classified into the low HDL-C (L_HDL-C) group if only low serum HDL-C concentrations existed. Participants with normal serum lipid profiles served as controls (CON).

Fecal sample collection, DNA extraction, and 16S rRNA gene sequencing

Fresh fecal samples were collected from each participant at home, and stored in foam boxes with frozen cold packs[37]. Within 6 h after defecation, all fecal samples were transported to the Clinical Laboratory of Honghui Hospital, Xi'an Jiaotong University, and immediately stored at -80 °C until further processing. Genomic DNA was extracted from all samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. After quality evaluation and concentration determination, DNA samples, greater than 1 μg and with an OD value between 1.8~2.0, were considered to be qualified for subsequent sequencing. Then, the V3~V4 regions of the 16S rRNA gene were amplified by universal primers (338F: 5'-ACT CCT ACG GGA GGC AGC AG-3'; 806R: 5'-GGA CTA CHV GGG TWT CTA AT-3') with barcodes, and all PCRs were performed using Phusion® High Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). Next, the PCR products were mixed and purified using a GeneJET™ Gel Extraction Kit (Thermo Scientific, Waltham, MA, USA). The sequencing library for each sample was constructed with the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA), and library quality was assessed on a Qubit® 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA). Finally, the generated libraries were pair-end (2×250 bp) sequenced on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

16S rRNA gene sequencing analysis

UPARSE (v7.0.1001) and QIIME software (v1.7.0) were introduced for sequencing analysis. According to a similarity threshold of 97%, acquired clean reads with high quality were de novo clustered into the same operational taxonomic unit (OTU), and the representative sequence of each OTU was screened and used to annotate taxonomic

information based on the RDP classifier (v2.2). GM diversity and composition were assessed based on the annotated OTUs. The alpha diversity (α-diversity) of GM was estimated by four indices, including Chao1, abundance coverage-based estimator (Ace), Shannon and Simpson. The comparisons of these indices between groups were conducted by the Wilcoxon rank-sum test, using function *wilcox.test* from the R package stats. To investigate the significance of differences in the GM community, beta diversity (β-diversity) was estimated using the unweighted UniFrac method to calculate the distances between samples, and visualized by the principal coordinates analysis (PCoA) model. The "*WGCNA*", "*stats*" and "*ggplot2*" packages in R were utilized herein. The top ten GM taxa sorted by higher relative abundances at the six taxonomic levels, including phylum, class, order, family, genus and species, were identified and visualized in each group. The linear discriminant analysis (LDA) effect size (LEfSe) algorithm was applied to identify the enriched significant taxa in each group. The LDA score threshold was set to 2 on a log10 scale. The FAPROTAX (v1.2.2) database, containing 90 different types of metabolic assignments, was introduced to obtain the functional information of the GM community[38]. The differences between subgroups above were assessed by the Wilcoxon rank-sum test, with a P value < 0.05 considered significant.

Statistical analysis

SPSS (v23.0.0.0, IBM SPSS Inc., Chicago, IL), R platform (v4.0.2, R Foundation, Vienna, Austria), GraphPad Prism (v8.4.3, GraphPad Software Inc., San Diego, CA, USA) and MedCalc (v19.0.4, MedCalc Software Bvba, Ostend, Belgium) were employed for statistical analysis and figure construction. The normal distribution of quantitative variables was assessed by the Shapiro-Wilk test. Clinical parameters are presented as the mean ± standard deviation (SD), and were analyzed among groups by the variance analysis (ANOVA) or Welch's ANOVA depending on the homogeneity of variance. In addition, the Games-Howell test was used for multiple comparisons if a P value < 0.05 existed. The "*ggcorrplot*" package in R was utilized for Spearman correlation analysis

between clinical parameters. Moreover, Spearman correlation analysis was also applied to evaluate the potential associations between GM features and clinical parameters, using the function *cor.test (method=spearman)* in R. Receiver operating characteristic (ROC) curve analysis, with areas under the curve (AUCs), was applied to evaluate the diagnostic performance of specific GM taxa. All statistical tests with a P value < 0.05 were considered significant.

The bioinformatics analysis and statistical methods/techniques mentioned in this study were conducted, verified and reviewed by our expert Ji-Han Wang, PhD, from Institute of Medical Research, Northwestern Polytechnical University, and Guo-Dong Wang, Master Degree, from Department of Quality Control, Xi'an Mental Health Center.

RESULTS

Basic information of the study population

The present study finally included 142 participants (73 females and 69 males), as shown in Figure 1. Table 1 shows the clinical characteristics of all the participants grouped by gender and serum lipid profiles. Serum lipid concentrations and CVAI showed differences among female subgroups, as well as height, WC and WHR (P value < 0.05). Meanwhile, serum lipid concentrations and CVAI showed differences among male subgroups as well (P value < 0.05). Please refer to Supplementary Table 1 for the detailed information and differences between females and males. The correlation analysis between the clinical characteristics of females and males is shown in Figure 2A and B, respectively, indicating positive correlations between these serum lipid indicators.

Diversity analysis of gut microbiota in the study population

To identify the associations of GM features with serum lipid profiles, we performed 16S rRNA gene sequencing analysis of GM from fecal samples. After quality control, sequencing reads from 142 fecal samples were processed to determine the OTUs. Our

data indicated that females had more unique OTUs, and the number of common OTUs shared by female subgroups was larger than that shared by male subgroups (data not shown).

We evaluated the diversity of GM community to assess the richness and evenness for females and males, and Shannon and Simpson indices suggested a higher α-diversity in females (P value < 0.05, Figure 3A). In addition, a dissimilarity between females and males could be observed according to the visualized PCoA model for β-diversity analysis (Figure 3B). Since gender is an important determinant in GM and serum lipid-related analysis[30, 32, 39], the study population was divided into female group and male group for the subsequent analysis.

To reveal the associations of GM diversity with serum lipid profiles based on gender differences, we conducted analogous analyses in the enrolled females and males, respectively. Our results showed that the α-diversity of GM changed in different dyslipidemia subgroups in females and males, respectively (Figure 3C and D). Of note, the α-diversity in L_HDL-C females was higher than that in CON females (P value < 0.05), while it was lower in L_HDL-C males than that in CON males (P value < 0.05). Additionally, the α-diversity of GM in H_TG males was higher than that in L_HDL-C males (P value < 0.05). The PCoA results suggested that the GM community in different dyslipidemia subgroups varied from that in the CON group in females and males, respectively, but could not be separated accurately and clearly (data not shown). Furthermore, the α-diversity of GM was found to be correlated with the serum lipid profiles and CVAI in males (Figure 3E). Specifically, the Chao1, Ace and Shannon indices were positively correlated with serum HDL-C concentrations, while the Chao1 and Ace indices were negatively correlated with CVAI. However, similar results could not be observed in females. The bioinformatics analysis above revealed the associations between the general state of GM and the human serum lipid profiles.

Taxonomic composition of gut microbiota in the study population

After diversity analysis, we focused on the relative abundances of GM taxa at six different taxonomic levels in different groups, and the top ten taxa with higher relative abundances at each level are shown in Supplementary Figure 1 for females and males. Similarly, the top ten taxa at each level were identified in female subgroups (Figure 4). Consistent with previous data, the GM composition at the phylum level included Firmicutes and Bacteroidetes as the core GM, with lower relative abundances of Actinobacteria, Proteobacteria and others. The most abundant GM taxa were Clostridia, Bacteroidia, Clostridiales, Bacteroides, Ruminococcaceae, Lachnospiraceae, Bacteroidaceae, *Faecalibacterium* and *Bacteroides*. GM taxa with relative abundances of no less than 0.0001 at each level were included in the following analysis. The relative abundances of Proteobacteria, Lactobacillaceae and *Lactobacillus* were no less than 0.010 at respective levels in H_CHO females, and were greater than those in CON females (P value < 0.05); while Coriobacteriia abundance was higher in L_HDL-C females than that in CON females (P value < 0.05). In the comparison of H_CHO and L_HDL-C females, Prevotellaceae abundance was greater in L_HDL-C females (P value < 0.05), and *Lactobacillus_salivarius* abundance was greater in H_CHO females (P value < 0.05). In the comparison groups of H_CHO and H_TG females, *Agathobacter* abundance was higher in H_TG females (P value < 0.05), while the relative abundances of *Ruminococcus_bromii* and *Lactobacillus_salivarius* were higher in H_CHO females (P value < 0.05). Additionally, Prevotellaceae abundance was greater in L_HDL-C females than that in H_TG females (P value < 0.05). Subsequently, analogous comparison analysis was conducted in male subgroups (Figure 5). The most abundant taxa were Clostridia, Bacteroidia, Clostridiales, Bacteroidales, Ruminococcaceae, Lachnospiraceae, *Faecalibacterium*, *Bifidobacterium*, *Bifidobacterium_pseudocatenulatum* and *Clostridium_disporicum*. In the comparison groups of L_HDL-C and CON males, the relative abundances of Bacteroidetes, Bacteroidia, Bacteroidales, Prevotellaceae, *unidentified_Ruminococcaceae*, *Roseburia* and *Roseburia_inulinivorans* decreased in L_HDL-C males (P value < 0.05). Meanwhile, Coriobacteriia, Coriobacteriales, Prevotellaceae, *unidentified_Ruminococcaceae*, *Roseburia* and *Roseburia_inulinivorans* decreased in L_HDL-

C males compared with H_TG males (P value < 0.05). Interestingly, no differences were observed in the comparison between H_TG group and CON group (P value > 0.05) in females and males, respectively.

LEfSe analysis was conducted in different dyslipidemia subgroups and the CON group in females and males. Compared with CON females (Figure 6), LEfSe analysis revealed the enrichment of Proteobacteria, Lactobacillaceae, *Lactobacillus* and *Lactobacillus_salivarius* in H_CHO females, and the enrichment of Coriobacteriia in L_HDL-C females. In the comparison among the three dyslipidemia subgroups in females, *Lactobacillus_salivarius* were enriched in H_CHO females, and Prevotellaceae were enriched in L_HDL-C females. Simultaneously, LEfSe analysis revealed that Prevotellaceae, *unidentified_Ruminococcaceae*, *Roseburia*, *Roseburia_inulinivorans*, Coriobacteriia, Coriobacteriales and Verrucomicrobiae were the enriched taxa in H_TG males compared with other males (Figure 7).

Associations of gut microbiota taxa with serum lipid profiles in the study population

To further explore the clinical implications of GM with dyslipidemia, Spearman correlation analysis was introduced to assess the associations between differential GM taxa and serum lipid profiles/CVAI, and a number of reliable correlations were revealed. In females, a positive correlation of *Bacteroides_coprocola* with serum TG was observed, while negative correlations of Bacteroidetes, Bacteroidia and Bacteroidales with CVAI were noted (P value < 0.05, Figure 8A). Moreover, more correlations were identified in males (P value < 0.05, Figure 8B). Specifically, the relative abundances of Actinobacteria, unidentified_Actinobacteria, Bifidobacteriales, Bifidobacteriaceae and *Bifidobacterium* were negatively correlated with serum TG and TC concentrations, while *Roseburia_inulinivorans* abundance was positively correlated with serum TG and TC concentrations, as well as the positive correlation of *Roseburia* with serum TG. In addition, the relative abundances of Leuconostocaceae, *Weissella* and *Weissella_cibaria* were correlated with increased serum TC and LDL-C concentrations, and *Bacteroides_vulgatus* abundance was correlated with decreased serum LDL-C

concentrations. Furthermore, we found that the relative abundances of Bacteroidetes, Bacteroidia, Bacteroidales, Coriobacteriales, Prevotellaceae, *unidentified_Ruminococcaceae* and *Roseburia_inulinivorans* were positively correlated with serum HDL-C concentrations, and the abundances of Bacteroidetes, Bacteroidia, Bacteroidales and *Bacteroides_vulgatus* were negatively correlated with CVAI in males.

To search for the specific GM taxa that may facilitate the differentiation of dyslipidemia patients from controls, ROC curve analysis was conducted in the female and male subgroups (*P* value < 0.05, Figure 9). In females, the GM taxa, effectively distinguishing H_CHO from CON, were Proteobacteria (0.724, 95%CI: 0.567~0.849), Lactobacillaceae (0.703, 95%CI: 0.544~0.832), *Lactobacillus* (0.705, 95%CI: 0.547~0.834) and *Lactobacillus_salivarius* (0.706, 95%CI: 0.548~0.835). In addition, Coriobacteriia (0.697, 95%CI: 0.546~0.822) may provide a clue for the discrimination of L_HDL-C females from CON. Moreover, the GM taxa in favor of the differentiation of H_TG males from CON were Coriobacteriia (0.710, 95%CI: 0.547~0.841), Coriobacteriales (0.710, 95%CI: 0.547~0.841), Prevotellaceae (0.697, 95%CI: 0.534 to 0.830), *Roseburia* (0.697, 95%CI: 0.534~0.830) and *Roseburia_inulinivorans* (0.684, 95%CI: 0.520~0.820). Additionally, seven GM taxa may play a role in distinguishing L_HDL-C males from CON: Bacteroidetes (0.676, 95%CI: 0.539 to 0.794), Bacteroidia (0.676, 95%CI: 0.539 to 0.794), Bacteroidales (0.676, 95%CI: 0.538 to 0.793), Prevotellaceae (0.685, 95%CI: 0.548 to 0.802), *unidentified_Ruminococcaceae* (0.687, 95%CI: 0.551 to 0.804), *Roseburia* (0.662, 95%CI: 0.524 to 0.782) and *Roseburia_inulinivorans* (0.682, 95%CI: 0.545 to 0.799).

Functional analysis of gut microbiota in the study population

Finally, we evaluated the functions of GM community using the FAPROTAX database, and obtained the main metabolisms of microorganisms associated with different biogeochemical cycles. A total of 51 functional assignments, with relative abundances larger than 0.0001 of the average level, were obtained in females, and 38 assignments were obtained in males. Apparently, GM functions showed different patterns between females and males, further supporting our strategy of separation and independent

analysis in females and males. The top twenty annotated functions with higher relative abundances in females and males are shown in Figure 10. Furthermore, comparison analysis between the different subgroups was conducted. Compared with CON females, the relative abundance of aerobic chemoheterotrophy was increased in H_CHO females (P value < 0.05), while cellulolysis abundance was increased in L_HDL-C females (P value < 0.05). Additionally, cellulolysis abundance was increased in L_HDL-C females when compared with H_CHO females (P value < 0.05). Compared with CON males, the relative abundances of sulfate respiration, respiration of sulfur compounds and cellulolysis were decreased in L_HDL-C males (P value < 0.05), while nitrate respiration increased in L_HDL-C males (P value < 0.05).

DISCUSSION

Dyslipidemia is considered as a defect of lipid metabolism in circulation, which is characterized by increased/decreased concentrations of serum lipids. Notably, it appears that the alterations in GM community may participate in the pathogenesis of dyslipidemia, and GM composition could be influenced by gender, host genotype and geographic location. To our knowledge, little evidence was found in the literature on the correlations between GM and serum lipids based on gender differences[39], hence an initial objective of this study was to identify the associations of GM features with serum lipid profiles based on gender differences in a Chinese population.

Considered as a whole, there were significant differences in serum lipid profiles and GM features between females and males. Specifically, females have higher GM diversity, and their GM composition was quite different from males, which is consistent with previous data[40]. In addition, the functional analysis of GM also showed an obvious dissimilarity between females and males, further supporting our strategy of respective GM analysis in females and males[14]. Of note, the associations between GM and dyslipidemia in humans have been investigated and demonstrated by various studies, suggesting the alteration of GM in patients with impaired lipid metabolism[28, 41]. For instance, Cotillard *et al*[42] noted that reduced GM richness, commonly observed

in obese patients, was linked with increased serum TG and TC. After that, more studies demonstrated negative correlations of circulating TG and LDL-C levels with GM diversity, and a positive correlation of HDL-C with GM richness[9, 26, 43, 44]. Moreover, certain GM taxa were identified to be correlated with specific lipid profiles, suggesting that different GM taxa may affect distinct classes of lipids[26, 42, 43]. Although present data have not clearly defined the GM pattern of patients with dyslipidemia, these observations provide new avenues for validation and follow-up studies. Therefore, dyslipidemia female and male patients in this study were divided into the H_TG group, H_CHO group and L_HDL-C group, respectively, for a more targeted investigation and interpretation.

As an important indicator of GM community and a general measure of gut health, higher GM diversity has been proposed to be associated with healthy lipid levels, such as increased HDL-C and decreased TG[26]. Our results showed a positive correlation between GM diversity and serum HDL-C content in males, consistent with previous data[26]. Additionally, GM diversity was negatively correlated with CVAI in males. As a valuable indicator of "adipose distribution and function", CVAI has been suggested to be a reliable and applicable indicator for the evaluation of visceral fat dysfunction in Chinese individuals, which is based on simple and obtainable clinical parameters and lipid concentrations[34, 45]. The CVAI is essentially correlated with lipid metabolism, and its negative correlation with GM diversity could further support the reliability of our data.

Butyrate has been suggested to facilitate the prevention and treatment of diet-induced obesity by reducing fat accumulation and insulin resistance[46, 47], and the ability to produce butyrate is widely distributed among gram-positive anaerobic bacteria[48]. As members of the butyrate-producing bacteria[48], *Roseburia* and *Roseburia_inulinivorans* have been investigated in certain diseases. By analyzing the GM composition of patients with symptomatic atherosclerosis, Karlsson FH *et al*[49] noted an enrichment of *Roseburia* in CVD patients, while a lower abundance of *Roseburia* could be observed in patients with diabetes[44]. In this study, we found that *Roseburia_inulinivorans* were positively

correlated with serum TG, TC and HDL-C, and *Roseburia* were positively correlated with serum TG in males. In addition, decreased *Roseburia* and *Roseburia_inulinivorans* could distinguish L_HDL-C males, while increased *Roseburia* and *Roseburia_inulinivorans* were able to differentiate H_TG males from CON males. Furthermore, it is known that *Bifidobacterium* and *Lactobacillus* are strains with potential for therapeutic purposes[50]. An HM *et al*[51] once described a comparable, positive anti-obesity and lipid-lowering effect of *Bifidobacterium* spp. in obese rats fed a high-fat diet. Consistent with this evidence, our results demonstrated that the relative abundances of Bifidobacteriales, Bifidobacteriaceae and *Bifidobacterium* were negatively correlated with serum TG and TC concentrations in males. However, certain favorable GM taxa, such as Lactobacillaceae, *Lactobacillus* and *Lactobacillus_salivarius*, were enriched in H_CHO females. As a probiotic supplement, *Lactobacillus* were noted to have a negative correlation with the serum lipid profiles[52]. An animal study, focused on mice fed a high-fat high-cholesterol diet and supplemented with *Lactobacillus curvatus* and/or *Lactobacillus plantarum*, revealed that these probiotic bacteria play important roles in normalizing lipid metabolism, such as decreasing TC in plasma and liver, and reducing the accumulation of hepatic TG[53]. Obviously, certain controversial issues exist, which should be further investigated. Nevertheless, these observations may provide a hint that different GM taxa associate with certain lipids, and may affect specific aspects of lipid metabolism[26, 42, 43].

A recent study on rats[54] demonstrated that a high fat diet could decrease Bacteroidetes and its genera *Bacteroides* and *Prevotella*, and a study on swine indicated that decreased Bacteroidetes proportions were accompanied by decreases in circulating TG[55]; however, whether it can alter cholesterol or TG levels in humans remains disputed. In humans, decreased Bacteroidetes were observed in patients with coronary artery disease, including *Bacteroides_vulgatus* and *Bacteroides_dorei*[56, 57]. In addition, some species of *Bacteroides* were shown to be decreased in patients with CVDs[49], and *Bacteroides* may be used as biomarkers for evaluating the alleviation of obesity[54]. Our results showed that *Bacteroides_vulgatus* were negatively correlated with serum LDL-C

but positively correlated with serum TG in females, while Bacteroidetes, Bacteroidia and Bacteroidales were negatively correlated with CVAI in females. Moreover, Bacteroidetes, Bacteroidia and Bacteroidales were positively correlated with serum HDL-C concentrations, facilitated the differentiation of L_HDL-C males from CON, and negatively correlated with CVAI in males. These observed associations may further support their roles in the favorable regulation of lipid metabolism. Interestingly, Prevotellaceae was enriched in L_HDL-C group in the comparison among the three dyslipidemia subgroups in females. However, the relative abundance of Prevotellaceae decreased significantly in L_HDL-C males, and could distinguish L_HDL-C males from CON. Additionally, increased Prevotellaceae may also facilitate the discrimination of H_TG males from CON. Of note, Kelly TN *et al*[58] indicated that genera within the family Prevotellaceae had different effects; some were associated with an increased and others with a decreased CVD risk profile. Meanwhile, Coriobacteriia were indicated to be enriched in L_HDL-C females, and could differentiate L_HDL-C females from CON. Moreover, we identified that Coriobacteriales were correlated with increased serum HDL-C contents in males, and increased Coriobacteriia and Coriobacteriales could distinguish H_TG males from CON. However, these taxa have not been studied thoroughly, and it is difficult to assess their metabolic functions in human lipid metabolism.

Different GM taxa may have distinct activities and modes of action[50, 59], and certain taxa may exert synergistic and cooperative interactions[60], indicating the importance of balance in the GM community. Our results may suggest a complex interactivity between GM and distinct lipid metabolisms based on gender differences, and provide new evidence of GM analysis with dyslipidemia. Nevertheless, more studies are required to determine which specific taxa have the potential to ameliorate dyslipidemia.

There were several limitations in this study, the most marked of which was the small sample size in each subgroup. This was a single-center study, recruiting Chinese participants near Xi'an, a central city in northwestern China. In addition, certain confounders could influence the GM composition, such as dietary habit[61], and it's

necessary to take them into consideration. However, it remains valuable to elucidate certain associations of GM taxa and host lipid metabolism herein. We hope that our observations will facilitate prospective studies investigating diverse aspects of GM influences on human dyslipidemia based on gender differences. In future studies, it is recommended that the sample size be increased, more confounders be considered, and various research methods be integrated to ascertain the potential associations.

CONCLUSION

Based on the thoroughly analysis of GM features with dyslipidemia in females and males, potent associations of GM-host relations based on gender differences were revealed, and the potential of GM for dyslipidemia diagnosis was demonstrated. Although this study could not provide a conclusive association between GM and dyslipidemia, it may provide new insights into the pathogenesis of dyslipidemia.

ARTICLE HIGHLIGHTS

Research background

Dyslipidemia is a common chronic disorder, and defined as any abnormality in blood lipid levels. In recent years, considerable progress has been made in elucidating the mechanisms of dyslipidemia, and gut microbiota (GM) has been indicated to play a pivotal role in its pathophysiology. However, the associations between GM and dyslipidemia remain elucidated.

Research motivation

Although recent studies have convincingly linked GM to dyslipidemia, the proof of associations between GM and host lipid metabolism remains a challenge in humans. In addition, ethnicity, geography and gender are potent factors that could influence the GM community. Therefore, it has significance to clarify the relationship between GM and dyslipidemia, and explore the importance of gender differences herein. We will first reveal gender differences regarding the GM features, then introduce dyslipidemia

highlighting its intricate relationships with GM, and discuss possible altered GM functions.

Research objectives

This study focused on the associations of GM features with dyslipidemia based on gender differences in a northwestern Chinese population.

Research methods

This study finally recruited 142 participants (73 females and 69 males) in Honghui Hospital, Xi'an Jiaotong University, who fulfilled the criteria for the diagnosis of dyslipidemia, according to the "Guidelines for prevention and treatment of dyslipidemia in Chinese adults". The anthropometric and blood metabolic parameters of all participants were measured and detected. According to their detected serum lipid concentrations, participants were classified into a high triglyceride (H_TG) group, a high total cholesterol (H_CHO) group, a low high-density lipoprotein cholesterol (L_HDL-C) group, and a control (CON) group with normal serum lipid concentrations in females and males, respectively. Fresh fecal samples were collected for 16S rRNA gene sequencing, and UPARSE software, QIIME software, RDP classifier and FAPROTAX database were used for sequencing analysis.

Research results

Different GM features were identified between females and males, and the associations between GM and serum lipid profiles were different between females and males. In the comparison of the three dyslipidemia subgroups in females, *Lactobacillus_salivarius* were the enriched GM taxon in H_CHO females, and Prevotellaceae were the enriched GM taxon in L_HDL-C females. Compared with CON or H_TG males, Prevotellaceae, *unidentified_Ruminococcaceae*, *Roseburia* and *Roseburia_inulinivorans* decreased in L_HDL-C males, while LEfSe analysis indicated an enrichment of these GM taxa in H_TG males in the comparison to other male subgroups. Additionally, *Roseburia_inulinivorans*

abundance was positively correlated with serum TG and TC concentrations, and *Roseburia* were positively correlated with serum TG. Furthermore, Proteobacteria, Lactobacillaceae, *Lactobacillus* and *Lactobacillus_salivarius* could distinguish H_CHO females from CON, while Coriobacteriia, Coriobacteriales, Prevotellaceae, *Roseburia* and *Roseburia_inulinivorans* could discriminate H_TG males from CON. Based on the predictions of GM metabolic capabilities from the FAPROTAX database, cellulolysis increased in L_HDL-C females compared with CON females, but decreased in L_HDL-C males compared with CON males.

Research conclusions

This study provides evidence of the associations between GM and serum lipid profiles based on gender differences, suggesting a complex interactivity between GM and distinct lipid metabolisms, and providing new insights into the pathogenesis of dyslipidemia.

Research perspectives

Future studies are needed to determine which specific taxa have the potential to ameliorate dyslipidemia, to investigate the underlying biological functions of the key GM in dyslipidemia, and to explore the difference in diet and other factors between females and males as possible causes for the differences.

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