ORIGINAL ARTICLE

Comparison of the Gut Microbiome between Single-Vessel Disease and Multivessel Disease in Chronic Coronary Syndrome Patients

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Background: Chronic coronary syndrome (CCS) has a high mortality rate worldwide, especially in developing countries. The degree of vessel stenosis and the number of stenotic vessels impact the mortality rate in coronary artery disease (CAD) patients. Additionally, multivessel disease (MVD) significantly influences clinical outcomes more than single-vessel disease (SVD).

Objective: To examine if gut microbial communities could be used as biomarkers to discriminate patients with varying numbers of coronary stenotic vessels.

Materials and Methods: Patients between 35 and 70 years old hospitalized at Chulabhorn Hospital were recruited between February 25 and July 15, 2023. Participants were divided into three groups, CCS patients with SVD, CCS patients with MVD, and healthy participants. On the day of the assessment, blood was drawn from each patient. The day before an appointment, the feces of every patient were collected. The present study was cross-sectional.

Results: Forty-nine patients were included, and were divided into 11 SVD patients, 19 MVD patients, and 19 healthy participants. The patients were 38.78% female, with a mean age of 59.38±7.66 years, and 48.98% had hypertension. Based on sequencing of the V3-V4 regions of the 16S rRNA gene, it was revealed that the relative abundance of the *Prevotella* and *Veillonella* genera was significantly higher in MVD than in SVD patients. In the present study, MVD patients had differences in the diversity and composition of the gut microbiome compared to SVD patients and healthy participants.

Conclusion: The development of SVD and MVD is correlated with changes in the gut microbiome, which may create a diagnostic marker of CAD to distinguish MVD patients from SVD patients and may be useful for further therapy and prevention.

Keywords: Cardiovascular disease; Gut microbiome; Single-vessel disease; Multivessel disease; Chronic coronary syndrome; 16S rRNA sequencing

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Coronary artery disease (CAD) is one of the leading causes of death worldwide, with 17.8 million deaths annually. Between 1990 and 2020, it is predicted that CAD mortality rates will double in developing countries due to exposure of people to more CAD risk factors, including dyslipidemia, diabetes mellitus, hypertension, and smoking[1]. Acute coronary syndrome (ACS) and chronic coronary syndrome (CCS) are two subtypes of CAD that differ in the severity of clinical symptoms[2]. The likelihood of mortality rate in CAD patients is influenced by the vessel's degree of stenosis and the number of stenotic vessels[3]. Moreover, multivessel disease (MVD) has an impact on clinical outcomes, for instance, a higher incidence of re-myocardial infarction, urgent revascularization, and decreased left ventricular remodeling than single-vessel disease (SVD)[4].

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CAD is associated with atherosclerosis, in which lipid deposition forms a plaque in the blood vessel walls[5]. Koren et al. found that *Veillonella* and *Streptococcus* were found in atherosclerotic plaque samples. Bacteria from the oral cavity and the gastrointestinal tract may be related to atherosclerosis disease markers[6].

There are trillions of microorganisms in the gut microbiota, a complex ecology. Gut dysbiosis is linked to several diseases, including CAD[7]. Patients with CAD and altered gut microbiota are related not only by correlation but also by causal relationships. The relative abundance of Enterobacteriaceae and *Streptococcus* spp. was higher in CAD patients[8]. Moreover, *Escherichia-Shigella* and *Enterococcus* had a significant increase in the microbial community, whereas *Faecalibacterium*, *Subdoligranulum*, *Roseburia*, and *Eubacterium rectale* were significantly decreased in the CAD patients' group[9]. According to the study by Liu et al., *Escherichia*, *Shigella*, *Veillonella*, *Klebsiella*, and *Haemophilus* increased with the severity of CAD[10].

Despite studies of coronary heart disease featuring gut microbiota modification, the data on the interrelation between the number of coronary stenotic vessels and gut microbiota changes in CCS patients is limited. To address this subject, the authors analyzed the characteristics differences in gut microbial community diversity and composition based on 16S rRNA V3-V4 region sequencing of 49 patients, including 11 CCS patients with SVD, 19 CCS patients with MVD, and 19 healthy participants. Moreover, the authors examined if gut microbial communities could be used as biomarkers to discriminate patients with varying numbers of coronary stenotic vessels.

Materials and Methods

Subject enrollment

Patients aged between 35 and 70 years old hospitalized at Chulabhorn Hospital were recruited between February 25 and July 15, 2023. The present study was cross-sectional.

Coronary angiography was used to confirm the diagnosis of CAD in those with 70% or greater stenosis in coronary vessels larger than 2.5 mm in one view, 50% or greater stenosis in coronary vessels in two views, and 50% or greater stenosis in the left main coronary artery[11]; and they signed the consent documents.

The SVD group included CCS patients who had just one coronary artery stenosis by coronary angiography. The MVD group included CCS patients who had two or more stenotic coronary arteries. Additionally, the control group consisted of healthy participants who had no cardiovascular risk factors, were asymptomatic, and had no previous history of CAD.

Patients with the following criteria were excluded, 1) use antibiotics, probiotics, and laxatives within four weeks, 2) liver disease such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) more than five times normal, or jaundice, chronic kidney disease, cancer, immunodeficiency, intestinal diseases, 3) history of infections within four weeks, 4) pregnant or lactating, 5) alcoholism, and 6) smoking.

Using statistical matching techniques with age and gender, participants were separated into three groups, SVD group, MVD group, and healthy participants. The present study was conducted following the Declaration of Helsinki and approved by Chulabhorn Hospital and Srinakharinwirot University Research Ethics Committees (IEC No. 174/2564 and SWUEC/E/M-100/2565E, respectively), and the study was conducted in accession with the Good Practices for Clinical Research in Thailand. The study was approved by the Thai Clinical Trails Registry (TCTR) (TCTR20230428002). All study participants provided their written, informed consent.

According to "Hypothesis testing and power calculations for taxonomic-based human microbiome data"[12] and the Rarefaction analysis method, the sample size was established as at least ten cases per group from the incidence rate of CAD, which is 160.28 instances per 100,000 people (0.0016)[13].

Sampling and sequencing

Blood samples from each patient were taken to assess for fasting blood sugar (FBS), hemoglobin A1C (HbA1C), total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), AST, ALT, creatinine, and high-sensitivity C-reactive protein (hs-CRP) levels. One day before the appointment, fresh stool was collected in DNA/RNA shield fecal collection tubes (Zymo Research, CA, USA) and promptly frozen at –20°C for 48 hours before further analysis.

DNA was extracted from feces samples using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, CA, USA). DNA quantity and quality were assessed using nanodrop and electrophoresis. The 16S rRNA gene's V4 hypervariable region was amplified by polymerase chain reaction (PCR) using

the 515 F and 806 R primers and 2X KAPA hot-start ready mix. The PCR reaction was performed using an initial denaturation step at 94°C for 3 minutes, followed by 25 cycles of 98°C for 20 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The indexing PCR reaction was subsequently carried out using a Nextera XT index kit with eight cycles of the previous PCR condition. The 16S amplicons generated from each step were purified using AMPure XP beads. Finally, the PCR products were pooled in preparation for cluster generation and Illumina® MiSeq™ 250-bp paired-end read sequencing.

Sequencing data analysis

Microbiome bioinformatics was performed using QIIME 2 2019.10[14]. The q2-demux plugin was used to demultiplex the raw sequence data, and DADA2's denoising software (via q2-dada2) rejected reads with expected errors (maxEE) greater than 3.0. Short sequences were inserted into the sepp-refs-gg-13-8. qza reference phylogenetic tree using the SEPP q2-plugin to create a phylogeny. After samples were rarefied (subsampled without replacement) to a minimum read, the alpha-diversity metric, beta-diversity metric, and Principle Coordinate Analysis (PCoA) were computed using q2-diversity. Utilizing the Greengenes 13_8 99% operational taxonomic units (OTUs) reference sequences and the classify-sklearn naive Bayes taxonomy classifier, taxonomic classification for amplicon sequence variants (ASVs) was carried out.

Additionally, determination of differentially abundant microbiota was carried out using linear discriminant analysis effect size (LEfSe). The Random Forests Classification model was trained at the genus level to find a group of the most crucial predictive microbiota able to differentiate between SVD and MVD patient groups.

Statistical analysis and visualization

Stata/SE 16.1 (StataCorp, College Station, Texas 77845 USA) was used to analyze the statistical data. Statistics were considered significant when p-value was less than 0.05. All study variables were subjected to descriptive statistics analysis, provided as frequency (%) for categorical data. Quantitative data displayed mean ± standard deviation for normal distribution or median (interquartile range) for non-normal distribution. One-way analysis of variance (ANOVA) statistic and post hoc analysis using the Scheffe test with p-value less than 0.050 were utilized if the distribution of the quantitative data, such as age and laboratory results, were normal. The Kruskal-Wallis and post hoc Mann-Whitney U tests with p-value less than 0.017 were used if the data were not normally distributed. Using Kruskal-Wallis and permutational multivariate analysis of variance (PERMANOVA) (number of permutations 999), statistical tests of alpha and beta diversity were conducted. To choose attributes that were differently distributed among classes ($p<0.05$), non-parametric factorial Kruskal-Wallissum-rank tests were first used. Their effect sizes were calculated using the linear discriminant analysis (LDA) model and validated by 30-fold bootstrapping (cutoff=logarithmic LDA score of ≥2.0). Additionally, using the Benjamini and Hochberg false discovery rate correction, significant p-values associated with the microbial significantly differential features by LEfSe were adjusted for multiple hypothesis testing. All study figures were processed using MicrobiomeAnalyst website tools[15].

Results

Clinical characteristics

Forty-nine patients were included and divided into three groups as CCS patients with SVD, CCS patients with MVD, and healthy participants, with 11, 19, and 19 patients in each group, respectively. To be mentioned, 53 patients were recruited, but the microbiota data of one SVD patient and two MVD patients were missing because stool samples could not be collected. Therefore, the authors examined 49 samples of the gut microbiome. The patients were 38.78% female, with a mean age of 59.38±7.66 years, and 48.98% had hypertension. There was no statistically significant difference in age or gender among the groups. Baseline characteristics are shown in Table 1.

The average age in the MVD group was slightly higher than the SVD group at 60.89±6.43 and 58.64±10.34, respectively. Compared to other groups, the MVD group had the highest proportion of obesity, metabolic syndrome, diabetes mellitus, and dyslipidemia at 52.63%, 36.84%, 36.84%, and 57.89%, respectively. The use of statins in the MVD group and SVD group was at 100% and 90.91%, respectively. Additionally, the MVD group had the highest level of hs-CRP at 3.4 mg/dL and the lowest LDL-C at 69 mg/dL.

Diversity of the gut microbiota

The Shannon and observed diversity indexes were analyzed for alpha diversity (Figure 1a, b).

Table 1. Clinical characteristics of the patients (n=49)

Parameters	Total (n=49)	SVD (n=11)	MVD (n=19)	Healthy (n=19)	p-value
Age (years); mean±SD	59.38±7.66	58.64±10.34	60.89±6.43	58.37±7.14	0.577
Male; n (%)	30 (61.22)	7 (63.64)	13 (68.42)	10 (52.63)	0.597
BMI (kg/m^2); mean±SD	23.64±3.20	23.74±2.29	25.14±3.96	22.08±1.93	0.010
Waist circumference (cm); mean±SD	83.42±9.63	87.00±6.87	88.89±10.03	75.87±4.56	<0.001
History of CAD; n (%)	17 (34.69)	5 (45.45)	12 (36.16)	0 (0.00)	<0.001
Medication; n (%)					
Antiplatelets	30 (61.22)	11 (100)	19 (100)	0 (0.00)	<0.001
Antihypertensive drugs	24 (48.98)	9 (81.82)	15 (78.95)	0 (0.00)	<0.001
Oral antidiabetic drugs	7 (14.29)	1 (9.09)	6 (31.58)	0 (0.00)	0.012
Statins; n (%)	29 (59.18)	10 (90.91)	19 (100)	0 (0.00)	<0.001
Statin intensity					0.532
• Low intensity	0 (0.00)	0 (0.00)	0 (0.00)	-	
• Moderate intensity	2 (6.90)	0 (0.00)	2 (10.53)	-	
• High intensity	27 (93.10)	10 (100)	17 (89.47)	-	
Duration ≥ 3 months	29 (59.18)	10 (90.91)	19 (100)	0 (0.00)	<0.001
Obesity+; n (%)	13 (26.53)	3 (27.27)	10 (52.63)	0 (0.00)	0.001
Abdominal obesity*; n (%)	13 (26.53)	3 (27.27)	10 (52.63)	0 (0.00)	<0.001
Hypertriglyceridemia**; n (%)	8 (16.33)	1 (9.09)	7 (36.84)	0 (0.00)	0.006
Low HDL-C#; n (%)	13 (26.53)	3 (27.27)	10 (52.63)	0 (0.00)	0.001
Impaired fasting glucose@; n (%)	22 (44.90)	8 (72.73)	13 (68.42)	1 (5.26)	<0.001
Metabolic syndrome; n (%)	10 (20.41)	3 (27.27)	7 (36.84)	0 (0.00)	0.006
Hypertension; n (%)	24 (48.98)	9 (81.82)	15 (78.95)	0 (0.00)	<0.001
Diabetes mellitus; n (%)	9 (18.37)	2 (18.18)	7 (36.84)	0 (0.00)	0.009
Dyslipidemia; n (%)	17 (34.69)	6 (54.55)	11 (57.89)	0 (0.00)	<0.001
Heart failure; n (%)	1 (2.04)	1 (9.09)	0 (0.00)	0 (0.00)	0.224
Stroke; n (%)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1.000
PAD; n (%)	1 (2.04)	1 (9.09)	0 (0.00)	0 (0.00)	0.224
SBP (mmHg); median (IQR)	120 (114 to 128)	122 (114 to 138)	123 (113 to 132)	120 (115 to 123)	0.408
DBP (mmHg); mean±SD	73.22±8.58	74.45±6.89	71.00±8.12	74.74±9.77	0.359
Laboratory data					
FBS (mg/dL); median (IQR)	98 (90.5 to 113)	103 (99 to 140)	103 (98 to 128)	90 (86 to 93)	<0.001
HbA1C (mg/dL); median (IQR)	5.5 (5.1 to 6.0)	5.8 (5.5 to 6.1)	5.9 (5.4 to 6.6)	5.2 (5.0 to 5.5)	0.006
Total Cholesterol (mg/dL) ; median (IQR)	165 (132 to 185)	134 (109 to 154)	143 (120 to 170)	184 (172 to 189)	<0.001
Triglyceride (mg/dL); median (IQR)	86 (69 to 126)	81 (69 to 126)	119 (80 to 189)	74 (63 to 92)	0.006
LDL-C (mg/dL); median (IQR)	94 (66 to 115.6)	74.2 (49 to 105)	69 (50.3 to 99)	115.6 (99 to 128.1)	<0.001
HDL-C (mg/dL); median (IQR)	50 (40 to 64)	42 (38 to 45)	40 (34 to 52.9)	64 (58 to 81)	<0.001
Serum creatinine (mg/dL); mean±SD	0.91±0.21	0.99±0.27	0.98±0.14	0.79±0.18	0.007
AST (IU/L); median (IQR)	20 (17 to 23)	19 (17 to 23)	22 (19 to 27)	16 (14 to 22)	0.005
ALT (IU/L); median (IQR)	17 (13 to 28)	21 (14 to 30)	28 (15 to 35)	14 (10 to 16)	0.002
hs-CRP (mg/dL); median (IQR)	1.36 (0.73 to 3.40)	1.18 (0.62 to 2.13)	3.4 (1.9 to 11.34)	0.76 (0.45 to 1.58)	<0.001
PCI; n (%)	17 (56.67)	6 (54.55)	11 (57.89)	-	1.000

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; CAD=coronary artery disease; DBP=diastolic blood pressure; FBS=fasting blood sugar; HbA1C=hemoglobin A1C; Healthy=healthy participants; hs-CRP=high-sensitivity C-reactive protein; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; MVD=multivessel disease patients; PAD=peripheral artery disease; PCI=percutaneous coronary intervention; SBP=systolic blood pressure; SVD=single-vessel disease patients; IQR=interquartile range; SD=standard deviation

+ Obesity: BMI ≥25 kg/m^2; * Abdominal obesity, waist circumference >90 cm for male, waist circumference >80 cm for female; ** Hypertriglyceridemia: triglyceride ≥150 mg/dL; # Low HDL-C: HDL-C <40 mg/dL for male, HDL-C <50 mg/dL for female; @ Impaired fasting glucose: FBS ≥100 mg/dL

According to the Shannon index, MVD patients had a lower diversity than SVD patients and healthy participants, however, SVD patients had slightly higher than healthy participants in the observed index.

Regarding beta diversity, the Bray-Curtis

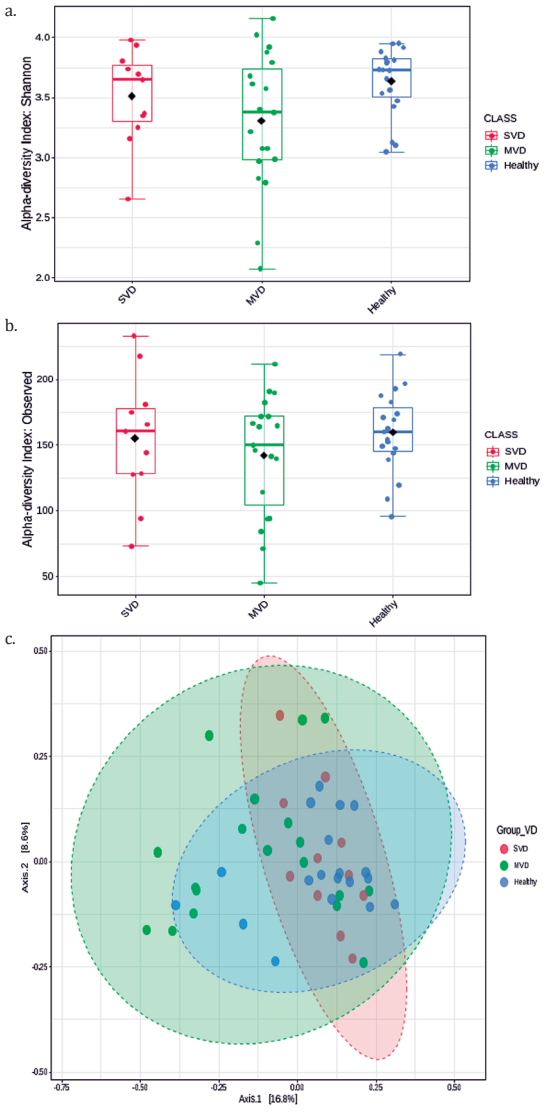

Figure 1. Analysis of alpha- and beta-diversity of microbial composition in the three patient groups. Diversity within bacterial communities was measured by the Shannon diversity index (a), and the observed index (b). The principal coordinate analysis (PCoA) of beta diversity is based on the Bray-Curtis index (c). Kruskal-Wallis H test was used in the statistical test of the alpha diversity. PERMANOVA (permutational multivariate analysis of variance) test was used in the statistical test of the beta diversity.

SVD=single-vessel disease patients; MVD=multivessel disease patients; Healthy=healthy participants

index was used to determine the similarities and differences in the composition structure of microbial communities. Comparing SVD and MVD patients, MVD patients and healthy participants were statistically significant different, however, SVD patients and healthy participants had no statistical significance (Figure 1c).

Assessment of bacterial taxonomic composition of SVD, MVD patients, and healthy participants

At the phylum level, the most common bacterial phyla were Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, respectively. The relative abundance of Proteobacteria was increased in CCS patients with MVD. The percentage of Proteobacteria in MVD patients, SVD patients, and healthy participants: 8.35, 7.76, and 4.28, respectively. On the other hand, in CCS patients with MVD, Actinobacteria and Verrucomicrobia had the lowest relative abundance proportion. The Firmicutes and Bacteroidetes ratio was higher in SVD patients than in the others (Figure 2a).

At the family level, Lachnospiraceae and Bacteroidaceae were shown to be the two most common families. Prevotellaceae, Enterobacteriaceae, and Streptococcaceae families were proportionally more common in CCS patients with MVD than in other groups (Figure 2b).

At the genus level, *Prevotella* and *Streptococcus* genera had higher relative abundance in CCS patients with MVD than in other groups with statistical significance (Figure 2c). Moreover, the relative abundance of *Roseburia* (the percentage of *Roseburia* in MVD patients, SVD patients, and healthy participants were 2.60, 5.29, and 4.78, respectively), *Ruminococcus* (the percentage of *Ruminococcus* in MVD patients, SVD patients and healthy participants: 2.51, 2.63, and 3.40, respectively), and *Faecalibacterium* genera (the percentage of *Faecalibacterium* in MVD patients, SVD patients and healthy participants: 6.54, 7.70, and 8.41, respectively) were the lowest in CCS patients with MVD than in the others. Moreover, the relative abundance of *Subdoligranulum* and *Collinsella* was significantly increased in healthy participants compared with SVD and MVD patients.

The bacterial microbiome characteristic of MVD patients

In CCS patients with MVD, the relative abundance of Prevotellaceae, Enterobacteriaceae, Streptococcaceae, and f_S24_7 in phylum Proteobacteria, had significantly risen at the family level compared with healthy participants, according to the LEfSe. At the genus level, the LEfSe revealed that *Prevotella*, *Veillonella*, and *Enterococcus* genera had significantly higher relative abundances in CCS patients with MVD compared to healthy participants (Figure 3b).

In this analysis of SVD and MVD patients at

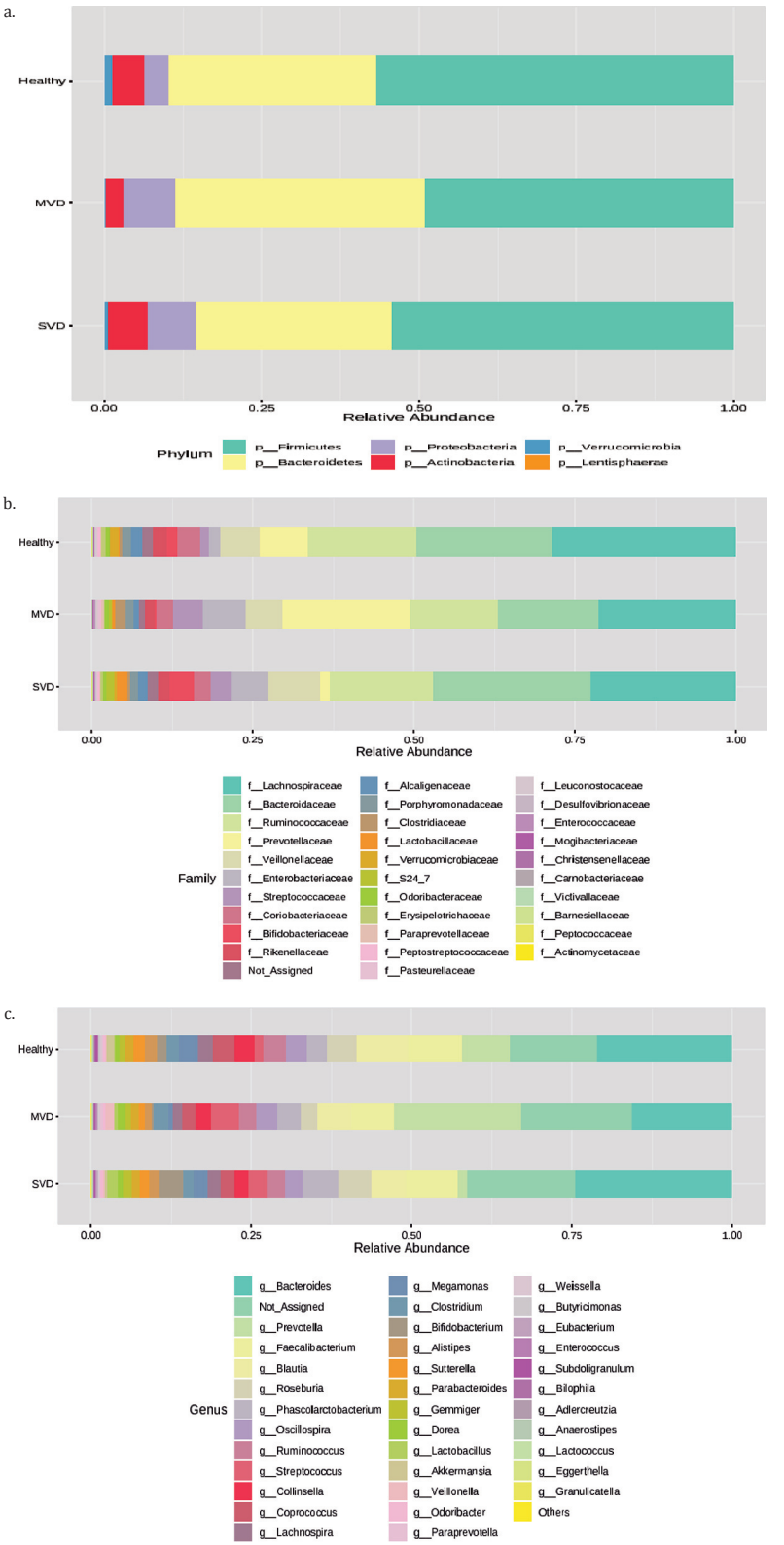

Figure 2. The relative abundance of bacterial taxonomic profile in the feces at the phylum (a), family (b), and genus (c) level.

Healthy=healthy participants; MVD=multivessel disease patients; SVD=single-vessel disease patients

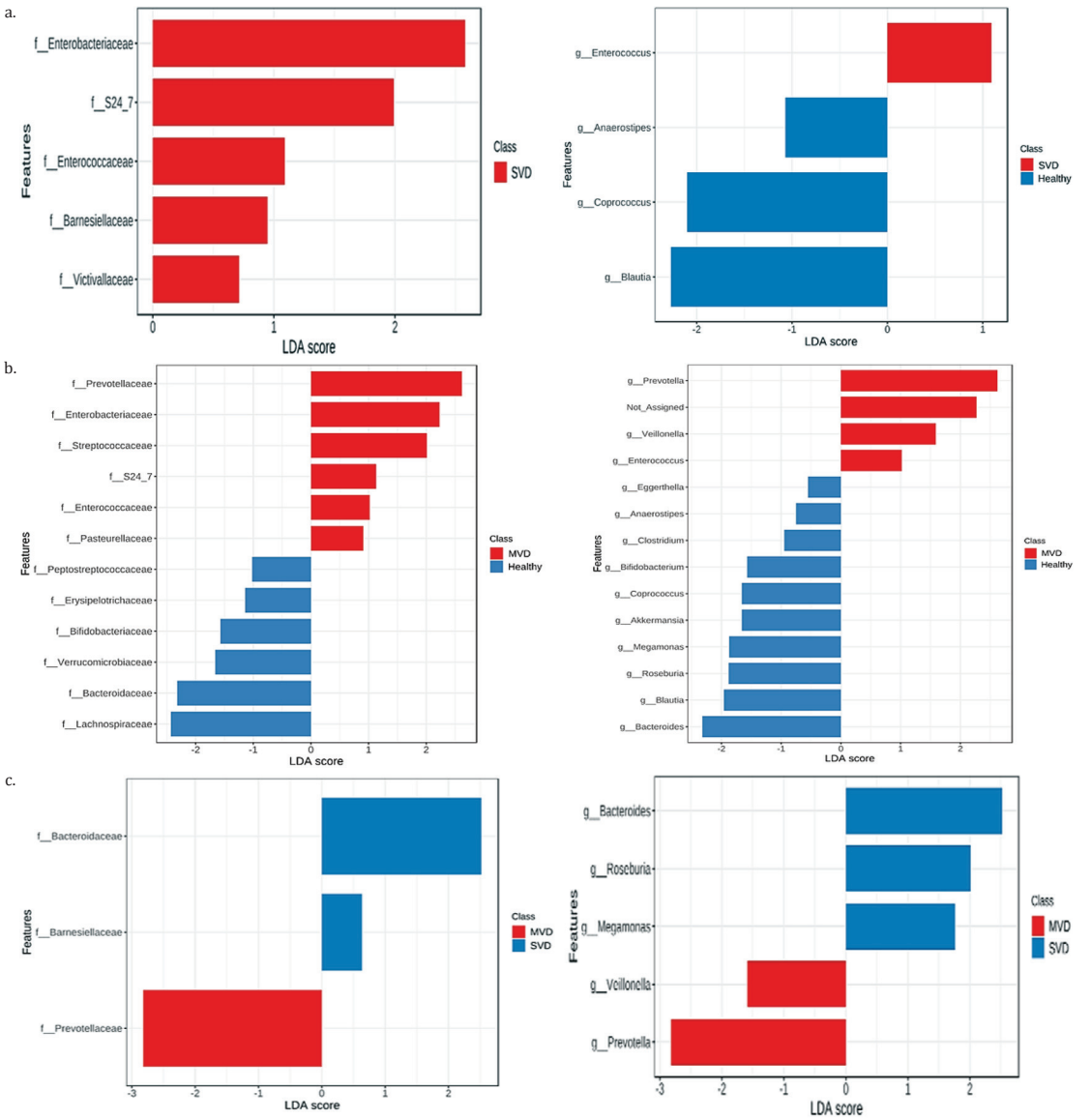

Figure 3. The linear discriminant analysis effect size (LEfSe) in comparison of bacterial microbiota between SVD patients and healthy participants (a), MVD patients and healthy participants (b), and SVD patients and MVD patients (c).

Healthy=healthy participants; MVD=multivessel disease patients; SVD=single-vessel disease patients

the family level, Prevotellaceae was more relatively abundant in MVD patients than SVD patients (Figure 3c). At the genus level, *Prevotella* and *Veillonella* were more enriched in MVD patients compared to SVD patients (Figure 3c).

Prediction of gut microbiota biomarkers to discriminate between SVD and MVD patients

To identify which bacteria are the crucial candidates in accounting for differences due to SVD and MVD in CCS patients, the random forest analysis was performed and demonstrated that *Prevotella* had the highest accuracy for discriminating MVD patients from SVD patients, followed by *Veillonella*, *Enterococcus*, *Oscillospira*, *Catenibacterium*, *Lachnobacterium*, *Holdemania*, and *Streptococcus*, respectively. On the other hand, *Roseburia*, *Faecalibacterium*, *Bacteroides*, *Acidaminococcus*, *Bifidobacterium*, *Parabacteroides*, and *Megamonas*, in that order, provided the best value for separating SVD patients from MVD patients (Figure 4).

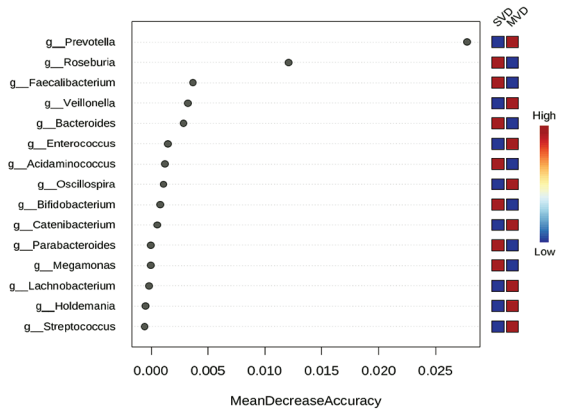

Figure 4. The Random Forest classification model was used on the 16S rRNA abundance data to identify the top 15 most important microbial biomarkers to discriminate between SVD and MVD patients. Predictive attributions are ranked by their involvement in classification accuracy which is Mean Decrease Accuracy. MVD, multivessel disease patients; SVD, single-vessel disease patients.

Discussion

The present study is the first research in Southeast Asia that examined patients with different numbers of coronary artery stenosis in CCS patients undergoing coronary angiography. In the present study, CCS patients with MVD had differences in the diversity and composition of the gut microbiome compared to CCS patients with SVD and healthy participants. The reduction in diversity in MVD patients compared to the other two groups in more complex diseases is consistent with other studies. For example, there was a trend to less diversity in CAD with non-alcoholic fatty liver disease (NAFLD) patients comparing CAD patients[16].

The present study indicated that CCS patients with MVD increased in Proteobacteria, *Prevotella*, and *Streptococcus*. Proteobacteria is linked to gut dysbiosis and a high concentration of bacteria-producing trimethylamine-N-oxide (TMAO), a gut metabolite linked to a high risk of developing CAD[17]. The direct effects of these microorganisms on CCS patients with MVD have not been studied. CAD and atherosclerosis were closely connected with the *Prevotella* and *Streptococcus* genera[18].

On the other hand, beneficial microorganisms, such as *Roseburia*, *Ruminococcus*, and *Faecalibacterium* genera had the lowest relative abundance in CCS patients with MVD. *Roseburia* has been connected to mice's enhanced glucose tolerance and weight reduction, as well as atherosclerosis patients. *Ruminococcus* is reduced with the progression of CAD[10]. In addition, *Faecalibacterium* has an anti-inflammatory property[19].

The development of CAD is significantly affected by the gut microbiome. In Yu et al.'s study, the abundance of *Escherichia-Shigella* was significantly increased in the MVD and SVD groups and positively correlated with LDL-C, while the abundance of *Subdoligranulum* and *Collinsella* was significantly decreased compared with the control group[20]. These findings correlated with the present study. The Proteobacteria phylum and the Enterobacteriaceae family were more common in CCS patients with MVD than in others. Most bacteria generating TMAO precursors are Proteobacteria, particularly those in the Enterobacteriaceae family and some Firmicutes[21].

Prevotella's influence on human health is controversial. There was no study about *Prevotella* in varying numbers of coronary stenotic vessels in CCS patients. *Prevotella* has been associated with diets high in complex carbohydrates from plants, fruits, and vegetables and can be found in healthy humans[22]. *Bacteroides* and *Prevotella* were decreased in CAD patients[23]. The genetic variety of *Prevotella* strains may explain the variances in how it responds to dietary and health conditions in different persons[24]. On the other hand, *Prevotella* abundance is increased in metabolic syndrome, insulin resistance, and low-grade systemic inflammation due to the stimulation of epithelial cells to produce interleukin-1 (IL-1), interleukin-8 (IL-8), interleukin-6 (IL-6), and interleukin-23 (IL-23), and the stimulation of mucosal helper T-cell (Th17) immune responses[25]. For instance, *P. copri* may be a significant risk factor for CAD patients due to a positive connection between this bacterium and LDL-C and it may have pro-inflammatory properties[26]. The latter finding supports our investigation that *Prevotella* was higher in CCS patients with MVD than in the other groups.

The present research has limitations, including the fact that it did not experimentally investigate the specific functions and metabolites of the gut microbiota. Additionally, the study's sample size was limited because participation in coronary angiogram has a risk, and the procedure was voluntary. However, the methods used to collect and analyze data were robust, a statistical power analysis was used to determine the minimum sample size needed for the present study, and the authors were unable to control other potential confounding factors such as age and gender of patients, which may have affected the results of the present study. Although the present

research provides information on links between gut microbiota modification and the extent of coronary stenosis, larger and underlying mechanism studies are warranted to strengthen and extend these findings before translational applications.

Conclusion

In the present study, MVD patients had differences in the diversity and composition of the gut microbiome compared to SVD patients and healthy participants. Gut microbiotas were associated with the atherosclerosis process and CAD. *Prevotella* and *Veillonella* were more enriched in MVD patients compared to SVD patients. As a result, the development of SVD and MVD is correlated with changes in the gut microbiome, which may create a diagnostic marker of CAD to distinguish MVD patients from SVD patients and may be useful for further therapy and prevention.

What is already known on this topic?

The degree of vessel stenosis and the number of stenotic vessels impact the mortality rate in CAD patients. Additionally, MVD significantly influences clinical outcomes more than SVD.

What does this study add?

The development of SVD and MVD is correlated with changes in the gut microbiome, which may create a diagnostic marker of CAD to distinguish MVD patients from SVD patients.

Authors' contributions

Conceptualization, WL, PP, and MT; data curation, WL, PP, and MT; format analysis, WL and MT; funding acquisition, WL and MT; investigation, WL and MT; methodology, WL and MT; project administration, WL, NS, PS, KS, CW, PJ, KS, and TT; resources, WL, PP, and MT; software, WL, PP, and MT; supervision, PP, MT, and EE; validation, WL, PP, and MT; visualization, WL, PP, MT, and VM; writing-original draft preparation, WL; writing-review and editing, WL, PP, MT, and AC; All authors have read and agreed to the published version of the manuscript.

Data availability

The raw sequence data are available under BioProject PRJNA1000984 from the following link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1000984?reviewer=kpesdmrsqch7ijls8j4i6ai1bo, and Biosample SAMN36786011.

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Conflicts of interest

The authors declare no conflict of interests.

References

1. Okrainec K, Banerjee DK, Eisenberg MJ. Coronary artery disease in the developing world. Am Heart J 2004;148:7-15.
2. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2020;41:407-77.
3. Min JK, Shaw LJ, Devereux RB, Okin PM, Weinsaft JW, Russo DJ, et al. Prognostic value of multidetector coronary computed tomographic angiography for prediction of all-cause mortality. J Am Coll Cardiol 2007;50:1161-70.
4. Tarantini G, Napodano M, Gasparetto N, Favaretto E, Marra MP, Cacciavillani L, et al. Impact of multivessel coronary artery disease on early ischemic injury, late clinical outcome, and remodeling in patients with acute myocardial infarction treated by primary coronary angioplasty. Coron Artery Dis 2010;21:78-86.
5. Momiyama Y, Adachi H, Fairweather D, Ishizaka N, Saita E. Inflammation, atherosclerosis and coronary artery disease. Clin Med Insights Cardiol 2014;8 Suppl 3:67-70.
6. Koren O, Spor A, Felin J, Fåk F, Stombaugh J, Tremaroli V, et al. Human oral, gut, and plaque microbiota in patients with atherosclerosis. Proc Natl Acad Sci USA 2011;108 Suppl 1:4592-8.
7. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. Nature 2016;535:85-93.
8. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun 2017;8:845.
9. Zhu Q, Gao R, Zhang Y, Pan D, Zhu Y, Zhang X, et al. Dysbiosis signatures of gut microbiota in coronary artery disease. Physiol Genomics 2018;50:893-903.
10. Liu H, Chen X, Hu X, Niu H, Tian R, Wang H, et al. Alterations in the gut microbiome and metabolism with coronary artery disease severity. Microbiome 2019;7:68.
11. Jiangping S, Zhe Z, Wei W, Yunhu S, Jie H, Hongyue W, et al. Assessment of coronary artery stenosis by coronary angiography: a head-to-head comparison with pathological coronary artery anatomy. Circ Cardiovasc Interv 2013;6:262-8.

12. La Rosa PS, Brooks JP, Deych E, Boone EL, Edwards DJ, Wang Q, et al. Hypothesis testing and power calculations for taxonomic-based human microbiome data. PLoS One 2012;7:e52078.
13. Hata J, Kiyohara Y. Epidemiology of stroke and coronary artery disease in Asia. Circ J 2013;77:1923-32.
14. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852-7.
15. Chong J, Liu P, Zhou G, Xia J. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat Protoc 2020;15:799-821.
16. Zhang Y, Xu J, Wang X, Ren X, Liu Y. Changes of intestinal bacterial microbiota in coronary heart disease complicated with nonalcoholic fatty liver disease. BMC Genomics 2019;20:862.
17. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. mBio 2015;6:e02481.
18. Madhogaria B, Bhowmik P, Kundu A. Correlation between human gut microbiome and diseases. Infect Med (Beijing) 2022;1:180-91.
19. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut 2014;63:1275-83.
20. Yu H, Li L, Deng Y, Zhang G, Jiang M, Huang H, et al. The relationship between the number of stenotic coronary arteries and the gut microbiome in coronary heart disease patients. Front Cell Infect Microbiol 2022;12:903828.
21. Dalla Via A, Gargari G, Taverniti V, Rondini G, Velardi I, Gambaro V, et al. Urinary TMAO levels are associated with the taxonomic composition of the gut microbiota and with the choline TMA-Lyase gene (cutC) harbored by enterobacteriaceae. Nutrients 2019;12.
22. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011;334:105-8.
23. Emoto T, Yamashita T, Sasaki N, Hirota Y, Hayashi T, So A, et al. Analysis of gut microbiota in coronary artery disease patients: A possible link between gut microbiota and coronary artery disease. J Atheroscler Thromb 2016;23:908-21.
24. Ley RE. Gut microbiota in 2015: Prevotella in the gut: choose carefully. Nat Rev Gastroenterol Hepatol 2016;13:69-70.
25. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology 2017;151:363-74.
26. Liu Z, Li J, Liu H, Tang Y, Zhan Q, Lai W, et al. The intestinal microbiota associated with cardiac valve calcification differs from that of coronary artery disease. Atherosclerosis 2019;284:121-8.