

Relationship between Human Gut Microbiota and Nutrition Intake in Hypertensive Discordant Monozygotic Twins

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Abstract

Background and Objectives: Intestinal bacteria digest substances, produce metabolites, and influence the host. Blood pressure increases with age, even in healthy people, and hypertension is said to affect one in four people worldwide. It is a major risk factor for stroke and stroke-related death. Although it is known that blood pressure is correlated with the ratio of Firmicutes and Bacteroides in gut microbiota and that the intake of peptides in fermented dairy products lowers blood pressure in humans, the relationship with the gut microbiota at the genus level is still inconsistent. Here, we aimed to examine the association between high blood pressure, gut microbiota, and nutrient intake by removing gender, age, and genetic effects.

Materials and Methods: We selected 25 hypertensive discordant Japanese monozygotic twins and confirmed their zygosity by matching 15 short tandem repeat loci. Their fecal samples were subjected to 16S rRNA sequencing and bioinformatics analyses to identify and compare fluctuations in intestinal bacteria.

Results: Four genera were extracted by comparing age- and gender-unified hypertensive and non-hypertensive groups, and 15 genera: *Actinomyces*, *Butyricoccus*, *Coprobacter*, *Coprococcus 1*, *Eubacterium fissicatena* group, *Eubacterium rectale* group, *Eubacterium ruminantium* group, *Eubacterium eligens* group, *Lachnospira*, *Prevotellaceae UCG 001*, *Ruminiclostridium 9*, *Ruminococcaceae NK 4A 214* group, *Ruminococcaceae UCG 004*, *Ruminococcaceae UCG 005* and *Ruminococcaceae UCG 014* were extracted by focusing on differences between pairs to account for genetic effects. The correlation between the 15 hypertension-associated bacteria extracted and nutrient intake showed that most minerals, vitamins, and lipids, including plant fatty acids, had a negative correlation with *Actinomyces*, while some had a positive correlation with the *Eubacterium rectale* group.

Conclusions: When comparing monozygotic twins with hypertension discordance, age, sex, and genetic factors were excluded, and 15 hypertension-related genera were extracted, including *Actinomyces* and *Eubacterium rectale* group, which were associated with several nutrients.

Keywords: Gut microbiome • Hypertension • High blood pressure • Monozygotic twins • Nutrients

Introduction

The human intestinal tract is composed of over 100 trillion microorganisms with more than 1,000 species [1], which form the gut microbiota. The gut microbiota has been shown to be involved in the pathogenesis of metabolic diseases, immune diseases, and malignant tumors. An association with cardiovascular diseases and intestinal bacteria has also been reported. The Firmicutes: Bacteroidetes (F/B) ratio also increased in a hyper-tensive rat model [2]. This result suggests that activation of the renin-angiotensin system (RAS), which is a cause of hypertension, may lead to dysbiosis.

In humans, a decrease in intestinal microbiota diversity has been confirmed in hypertensive patients [3]. Additionally, it has been reported that the

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intestinal microflora of rats in which hypertension was induced by a high-fat diet became Firmicutes-dominant, and short-chain fatty acid (SCFA) butyrate decreased [4]. Olfr78, an olfactory receptor (OR), is found in the renal arteries and paraglomerular cells of the kidney, and its binding to SCFAs such as acetic acid and propionic acid causes an increase in blood pressure [5], whereas Gpr41, which is expressed in blood vessels, has a hypotensive effect when bound to SCFAs [6]. Thus, Olfr78 and Gpr41 share a common ligand, propionic acid, and have antagonistic effects on blood pressure regulation.

The prevalence of hypertension (blood pressure of 140/90 mmHg or higher or on antihypertensive medication) accounts for 60% of men and 40% of women in the range of 40–70 years, and the proportion of hypertensive patients increases with age. Seventy four percent of men and 70% of women aged 75 years and older fall into this category, with no differences noted between men and women [7].

Hypertension is a multifactorial genetic disease, and the average systolic blood pressure level of the population has been decreasing owing to the progress and spread of hypertension treatment, lower salt intake, and changes in lifestyle and living environment.

However, persistent high blood pressure causes arteriosclerosis, which is a cause of various complications which include life-threatening stroke,

myocardial infarction, and kidney disease. Given that hypertension is a disease with few subjective symptoms and a large number of patients, it has a substantial impact on health and welfare and the medical economy.

In addition to susceptibility to diseases other than single genetic disorders, all human phenotypes are affected by genetic and environmental factors. In addition to hypertension, the composition of the gut microbiota is known to be affected by genetic and environmental factors [8]. Therefore, the only human study that can assess the environmental effects on gut microbiota whilst controlling genetic effects is a twin study. Monozygotic twins share 100% of the intrauterine environment and growth environment in addition to their genetic background. Thus, differences due to environmental factors can be evaluated by comparison between twins.

Japan has been one of the longest-lived countries in the world for many years, and it is known that the composition of the Japanese intestinal flora is unique [9]. As both hypertension and gut microbiota are strongly influenced by diet, this study is the first to focus on the relationship between hypertension and gut microbiota, as well as nutrient intake in a pair of Japanese identical twins, one of whom had hypertension and the other did not.

The aim of this study was to focus on the differences between monozygotic twin pairs and their gut microbiota by eliminating the influence of genetic factors, identify bacteria associated with hypertension, and examine which nutrients these bacteria were associated with.

Materials and Methods

Subjects

Japanese monozygotic twins were recruited from a registry established by the Center for Twin Research, Osaka University Graduate School of Medicine, and written informed consent was obtained from all 52 discordant individuals (MZ 26 pairs) in whom one of the twins was hypertensive and the other was not, for analysis in this study.

Hypertension was determined based on either confirmation of self-reported current history by a health care professional or the mean of three blood pressure readings taken in the sitting position during the face-to-face survey, which was greater than or equal to degree I hypertension (140 mmHg \leq systolic blood pressure and/or 90 mmHg \leq diastolic blood pressure) of the Hypertension Treatment Guidelines 2019 [10].

The zygosity of the subjects was confirmed by matching 15 short tandem repeat loci using the PowerPlex®16 system (Promega, Madison, Wisconsin, USA).

This study was approved by the Ethics Committee of Osaka University and the Ethics Committee of the National Institute of Biomedical Innovation, and was conducted in accordance with their guidelines.

Nutrient Intake Data

The brief-type self-administered diet history questionnaire has been developed for nutritional epidemiology studies and has been shown to be accurate and valid [11]. In this study, the value per 1000 kcal of energy was calculated and used for analysis, considering the effect of energy intake. Responses were obtained simultaneously with fecal sample collection.

Fecal Sample Collection

Fecal samples were collected from two twin pairs simultaneously. The samples were placed in 15 mL vials containing 3 mL of guanidine thiocyanate solution (TechnoSuruga Laboratory Co., Ltd, Shizuoka, Japan), mixed well, and stored at 4°C until DNA extraction.

DNA Extraction and 16S rRNA Gene Amplicon Sequencing

The fecal sample mixture was mechanically disrupted using the bead beating method. DNA was extracted using a Gene Prep Star PI-80X device (Kurashiki Boseki Co., Ltd.). Following DNA extraction, the V 3-V 4 region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq

(Illumina, San Diego, CA, USA). The complete flow from fecal sampling to 16SrRNA sequencing was performed according to the protocol described by Hosomi et al [12].

Bioinformatic Analysis

The resulting paired-end FASTQ data were trimmed and merged before selecting operational taxonomic units (OTUs). OTU classification and diversity analyses were performed using the QIIME pipeline (v. 1.9.1) [13]. All steps from FASTQ file trimming to gut microbiota diversity analysis were performed automatically according to a previously described method [14]. OTUs were clustered against the SILVA 128 reference database [15] with 97% similarity using the USEARCH algorithm [16]. Taxonomic classification was performed using the SILVA 128 reference database down to the genus level.

Statistical Analyses

The output of the QIIME pipeline in the Biom table format was imported and analyzed using R (version 3.5.1). Alpha-diversity indices were calculated using the estimate_richness function in the “phyloseq” R-package. Hierarchical clustering analysis was performed using the R package “vegan”, “stats” based on the Bray-Curtis distance matrix at the genus level and by using the ward.D 2 method. The beta-diversity index, calculated by the Bray-Curtis distance matrix using genus-level data, was generated using the vegdist function in the “vegan” R-package. Principal coordinates analysis (PCoA) was performed using the dudi.pco function in the “ade4” R-package. PCoA figures were created using the R package “ggplot2”. Covariates of gut microbiome β -diversity were identified by calculating the association between continuous or categorical phenotypes and genus-level community coordinates with the envfit function in the “vegan” R-package. This function performs the MANOVA and linear correlations for categorical and continuous variables, respectively. We used the Wilcoxon rank sum test (wilcox.test function in “stats” R-package) for comparison analysis. To completely rule out genetic effects, we focused on the differences between each twin pair. For bacterial relative abundance, the control was subtracted from Hypertension (HT) to create an integrated table. In addition, to confirm the relationship between hypertension and gut microbiota more robustly, the calculated values were converted into increasing, unchanged, and decreasing categorical variables, and comparative analysis was performed using Welch's two-sample t-test. Furthermore, for the confirmed hypertension-related bacteria, the nutrient intake was determined using a nutrient intake table that was also integrated by subtracting the control from HT (Spearman correlation analysis, cor function in “stats” R-package).

Heatmaps were created using the “corrplot” and “superheat” R-package, and PCoA figures and Boxplots were created using the R package “ggplot2”. All statistical tests were two-sided, with a significance level of $p < 0.05$.

Results

Characteristics of Participants

We analyzed 26 monozygotic twin pairs aged 65.4 ± 13.1 (31–85) years, of which 12 were male and 14 females. The subjects did not heavily consume alcohol and had not consumed any antibiotic medication for two weeks prior to the investigation.

There was no significant difference in nutrient intake between hypertensive and control twins except for iron (Table 1).

Examination of the Effects of Hypertension Medication

We performed a comparative analysis using the hypertension group only to determine whether hypertension medication affects the gut microbial community. Hypertension medication was not significantly correlated with the PCoA ordination of the microbial community structure ($p = 0.608$) (Figure 1).

Table 1. Participant characteristics and mean scores of nutrient intakes.

Variables			all(n = 52)		Hypertensive (n = 26)	Control (n = 26)
			Mean ± SD	(Min - Max)	Mean ± SD	(Min - Max)
male/ female	pair	14 / 12				
Age		65.4 ± 13.1	31 - 85			
Body mass index	kg/m ²	23.4 ± 3.3	17.4 - 33.2	24 ± 3.2	22.8 ± 3.3	
Nutrients						
EN	energy	kcal	1870.5 ± 610.7	826.8-3256	1782.5 ± 531.5	1958.5±669.3
PRT	protein	g/1000 kcal	40.8 ± 8	20.8 - 57.4	40.2 ± 8.6	41.5 ± 7.3
APRT	animal protein	g/1000 kcal	24.3 ± 8.1	7.3 - 41	24.3 ± 8.5	24.3 ± 7.8
VPRT	vegetable protein	g/1000 kcal	16.5 ± 2.9	10.3 - 23.3	15.9 ± 2.5	17.2 ± 3.2
FAT	fatty acid	g/1000 kcal	33.1 ± 7.6	12 - 49.3	32.5 ± 7.7	33.6 ± 7.6
AFAT	animal fatty acid	g/1000 kcal	15.4 ± 5	4.3 - 28.3	15.3 ± 5.1	15.6 ± 4.8
VFAT	vegetable fatty acid	g/1000 kcal	17.6 ± 5.4	6 - 32.5	17.3 ± 5.6	18 ± 5.1
CHO	carbohydrate	g/1000 kcal	124.9 ± 20.8	89 - 187.7	124.7 ± 19	125.1 ± 22.5
NA	sodium	mg/1000 kcal	2453.4 ± 562.1	1181.9 - 3749	2500.3 ± 573.2	2406.6 ± 546.7
K	potassium	mg/1000 kcal	1605.2 ± 406.7	566.1 - 2497	1541 ± 450.6	1669.5 ± 345.7
CA	calcium	mg/1000 kcal	363.7 ± 113.9	93.6 - 710.3	358 ± 130	369.3 ± 94.7
MG	magnesium	mg/1000 kcal	152.3 ± 32.7	71.1 - 211.3	147.7 ± 35.9	156.8 ± 28.5
PP	phosphorus	mg/1000 kcal	633.5 ± 134.2	293.9 - 908.7	624.3 ± 150.6	642.7 ± 114.9
FE	iron	mg/1000 kcal	4.8 ± 1.2	2 - 10	4.7 ± 1.4	5 ± 1*
ZN	zinc	mg/1000 kcal	4.6 ± 0.7	3.3 - 6.7	4.6 ± 0.8	4.7 ± 0.6
CU	copper	mg/1000 kcal	0.6 ± 0.1	0.3 - 0.9	0.6 ± 0.1	0.6 ± 0.1
MN	manganese	mg/1000 kcal	1.8 ± 0.5	1 - 3.5	1.7 ± 0.4	1.8 ± 0.6
RTE	retinol equivalent	µg /1000 kcal	488.5 ± 241.8	88.3 - 1649	472.3 ± 275.8	504.7 ± 200.7
VD	vitamin D	µg/1000 kcal	8.7 ± 5	1.1 - 27.4	8.6 ± 5.8	8.8 ± 4.1
ATC	alfa tocopherol	µg/1000 kcal	4.8 ± 1.2	1.4 - 8.4	4.6 ± 1.3	4.9 ± 1.1
VK	vitamin K	µg/1000 kcal	219.9 ± 91.3	41 - 591.6	207.2 ± 103.5	232.5 ± 75.2
VB1	vitamin B1	mg/1000 kcal	0.5 ± 0.1	0.2 - 0.7	0.5 ± 0.1	0.5 ± 0.1
VB2	vitamin B2	mg/1000 kcal	0.8 ± 0.2	0.3 - 1.5	0.8 ± 0.2	0.8 ± 0.2
VB6	vitamin B6	mg/1000 kcal	0.7 ± 0.2	0.3 - 1.2	0.7 ± 0.2	0.8 ± 0.2
VB12	vitamin B12	µg/1000 kcal	5.3 ± 2.4	1 - 12.4	5.2 ± 2.7	5.3 ± 2.1
VC	vitamin C	mg/1000 kcal	74.7 ± 28.9	24.5 - 148.5	71.4 ± 32.3	77.9 ± 24.7
SFA	saturated fatty acid	g/1000 kcal	8.9 ± 2.4	3 - 14	8.8 ± 2.4	9.1 ± 2.4
MFA	monounsaturated fatty acid	g/1000 kcal	11.8 ± 3	4.2 - 18.5	11.7 ± 2.9	11.9 ± 3.2
PFA	polyunsaturated fatty acid	g/1000 kcal	7.9 ± 1.8	3.1 - 12.2	7.7 ± 1.9	8 ± 1.6
CHL	cholesterol	mg/1000 kcal	254.5 ± 92.4	43.3 - 663.6	266.6 ± 110.2	242.5 ± 68.1
WDF	provide soluble fiber	g/1000 kcal	1.9 ± 0.6	0.4 - 3.2	1.8 ± 0.5	2.1 ± 0.6
NDF	insoluble dietary fiber	g/1000 kcal	5.4 ± 1.4	2.1 - 9.6	5.2 ± 1.5	5.6 ± 1.3
TDF	total dietary fiber	g/1000 kcal	7.6 ± 2	2.6 - 12.8	7.2 ± 2	7.9 ± 1.9
N3	n-3 fatty acid	g/1000 kcal	1.6 ± 0.5	0.6 - 3.2	1.6 ± 0.5	1.7 ± 0.5
N6	n-6 fatty acid	g/1000 kcal	6.2 ± 1.4	2.5 - 10.1	6.1 ± 1.6	6.4 ± 1.2

SD: standard deviation. Wilcoxon rank sum test between two groups *p <0.05.

The nutrient intake scores were calculated from the results of brief-type self-administered diet history questionnaire (BDHQ).

Gut Microbiota Diversity in Hypertension group and Control Group

We calculated Chao 1, Shannon diversity index, and Simpson diversity index and found no significant difference between the two groups (p = 0.405, 0.146, 0.151) (Table 2).

As a result of comparative analysis between the HT group and the control group using PCoA ordination of the microbial community structure, no difference was observed. After controlling for genetic changes, it was confirmed that hypertension had no effect on the microbial community of the gut (p = 0.608) (Figure 2).

Characteristics of Bacteria in Hypertension group and Control Group

The characteristic bacteria found in the case and control groups were the *Eubacterium ruminantium* group, *Lachnospira*, *Ruminococcaceae* UCG 004 and four other genera, all of which had higher occupancy rates in the control group (Table 3).

Characteristics of Bacteria in Co-Twins

We focused on the differences between each twin pair to completely rule out genetic effects, and 15 genera were extracted: *Actinomyces*, *Butyrivibrio*, *Coprobacter*, *Coprococcus* 1, *Eubacterium fissicatena* group, *Eubacterium rectale* group, *Eubacterium ruminantium* group, *Eubacterium eligens* group, *Lachnospira*, *Prevotellaceae* UCG 001, *Ruminiclostridium* 9, *Ruminococcaceae* NK 4A 214 group, *Ruminococcaceae* UCG 004, *Ruminococcaceae* UCG 005, and *Ruminococcaceae* UCG 014. All four genera mentioned above were also included (Table 4).

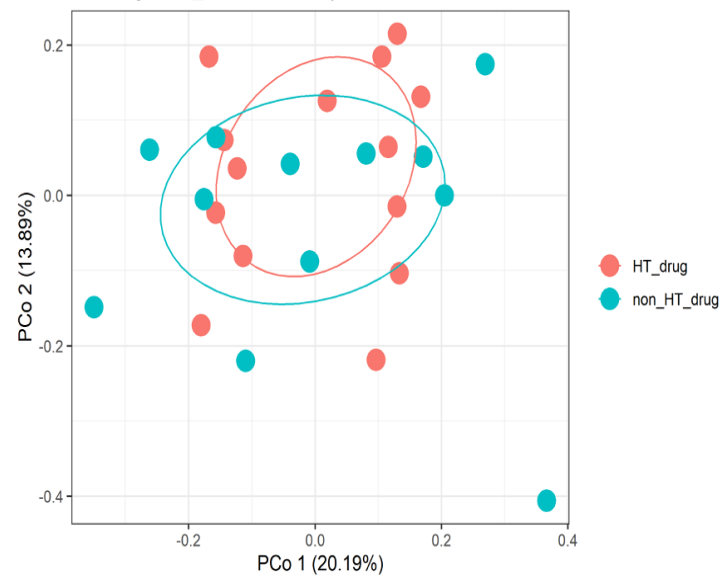


Figure 1. Principal coordinates analysis (PCoA) ordination of microbial community structure in the presence and absence of therapeutic drugs for hypertension.

Table 2. Alpha diversity at genera level between Hypertension group and Control group.

Estimators	HT_mean	Control_mean	FDR	p-value
Chao1	1447.583	1550.957	0.405	0.405012
Shannon	3.949	4.115	0.377	0.145685
Simpson	0.932	0.941	0.377	0.150819

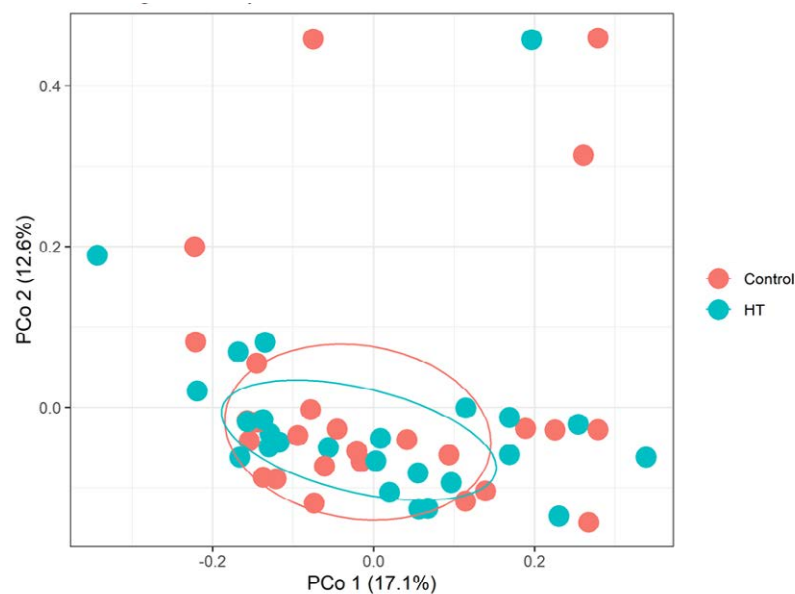


Figure 2. Principal coordinates analysis (PCoA) ordination of microbial community structure between hypertension and control groups.

Nutrient Meta-Analysis with the 15 extracted hypertension-associated bacteria

The correlation between the 15 extracted hypertension-associated bacteria and nutritional intake was calculated (Figure 3). As indicated by the dendrogram, nutrients were divided into three major groups. Most minerals, vitamins, and some lipids and proteins were largely classified. Dietary fiber was grouped with vegetable protein, vegetable fat, manganese, and vitamin C. Energy and carbohydrates were classified in the last group.

Several minerals, vitamins, and fats, including vegetable fatty acids, were negatively correlated with Actinomycetes and some positively correlated with the Eubacterium rectale group.

In addition, minerals, vitamins, and fats are inversely correlated to carbohydrates (Figure 3).

Discussion

In the present study, we investigated the human gut microbiota and its nutrient intake in relation to hypertension by removing genetic effects using identical Japanese twins discordant for hypertension.

Additionally, in the Healthy Life in an Urban Setting study, gut microbiota accounted for 4.4% of the total unadjusted systolic blood pressure variance; however, the proportion of variance varied widely by ethnicity (4.8% in the

Table 3. Four genera extracted from two group comparisons of high blood pressure group and healthy group.

Phylum	Genus	HT_mean	Control_mean	fdr	p-value
Firmicutes	<i>Eubacterium ruminantium</i> group	0.769	55.769	0.886	0.037329
Firmicutes	<i>Lachnospira</i>	71.038	128.808	0.886	0.038243
Firmicutes	<i>Ruminococcaceae.UCG.004</i>	3.462	7.500	0.886	0.031139
Firmicutes	<i>Ruminococcaceae.UCG.014</i>	0.000	10.346	0.719	0.005245

Wilcoxon rank sum test

Table 4. Fifteen genera extracted by twin-pair difference.

Phylum	Genus	HT_plus (%)	Control_plus (%)	p-val
Actinobacteria	<i>Actinomyces</i>	38.462	15.385	0.031881
Firmicutes	<i>Butyricoccus</i>	69.231	30.769	0.01242
Bacteroidetes	<i>Coprobacter</i>	34.615	11.538	0.022199
Firmicutes	<i>Coprococcus 1</i>	23.077	57.692	0.048661
Firmicutes	<i>Eubacterium fissicatena</i> group	30.769	11.538	0.031326
Firmicutes	<i>Eubacterium rectale</i> group	57.692	30.769	0.043127
Firmicutes	<i>Eubacterium ruminantium</i> group	0.000	23.077	0.026433
Firmicutes	<i>Eubacterium eligens</i> group	34.615	11.538	0.019722
Firmicutes	<i>Lachnospira</i>	26.923	69.231	0.025172
Bacteroidetes	<i>Prevotellaceae UCG 001</i>	3.846	0.000	0.025191
Firmicutes	<i>Ruminiclostridium 9</i>	19.231	65.385	0.009561
Firmicutes	<i>Ruminococcaceae NK4A214</i> group	15.385	50.000	0.026421
Firmicutes	<i>Ruminococcaceae UCG 004</i>	19.231	53.846	0.033175
Firmicutes	<i>Ruminococcaceae UCG 005</i>	19.231	57.692	0.018991
Firmicutes	<i>Ruminococcaceae UCG 014</i>	0.000	26.923	0.006366

Welch's t-test

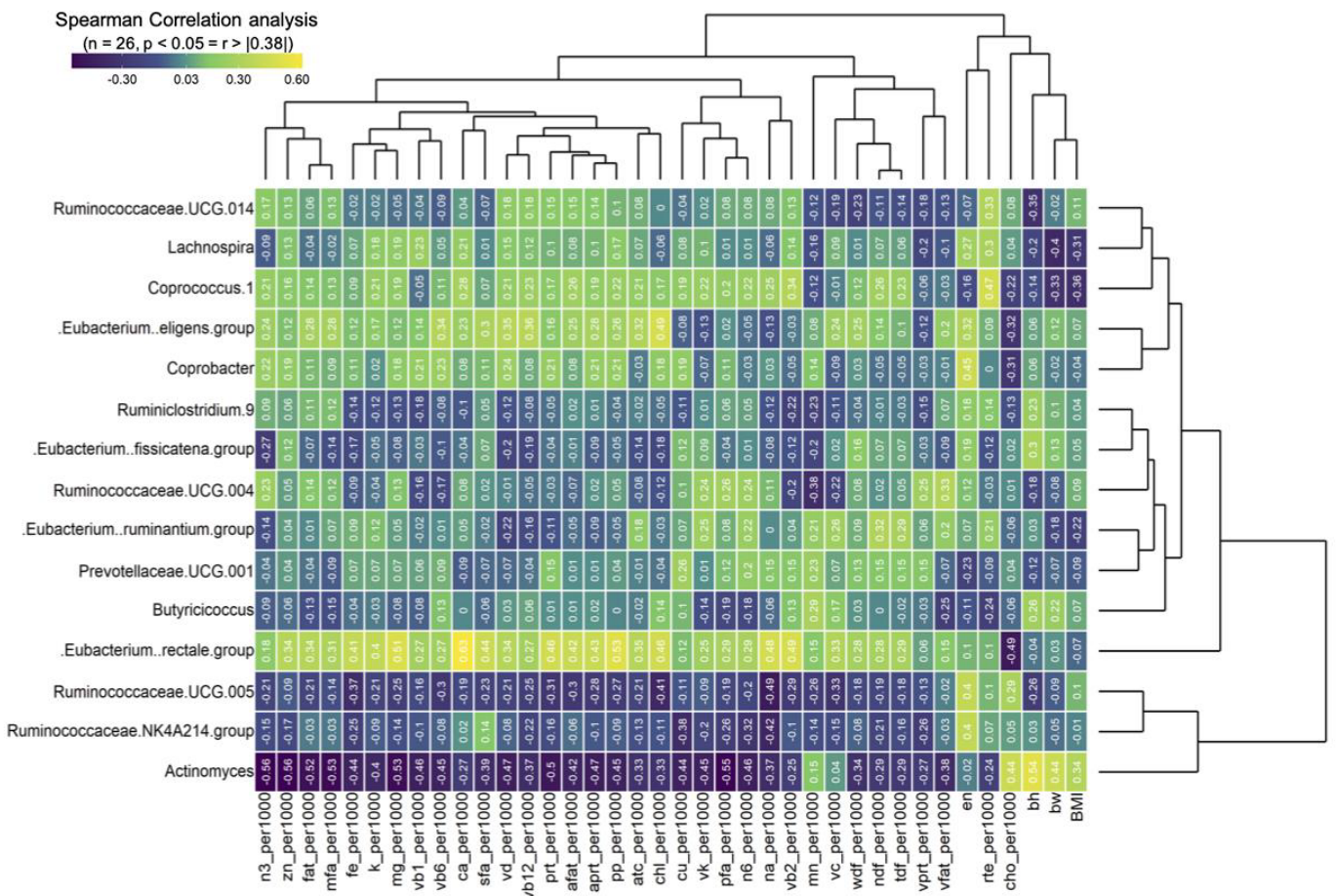


Figure 3. Association between hypertension-related bacteria and nutrient intake.

Dutch and less than 0.8% in others) [17], suggesting that a study of Japanese monozygotic twins would have important consequences.

In previous studies, hypertension decreased richness and diversity [2,3,18]; however, no difference in diversity was observed in this study. Although

diversity was observed in previous studies of patients with mild hypertension [3], the present study did not focus on the severity of hypertension because the hypertension group was identified using self-reported questionnaires, the number of years of medical history was not considered, and the criteria for judging the hypertension group from the measured values was the grade I hypertension group. Including and comparing more severe hypertensive cases should be considered in the future study.

By equally grouping one of the pairs into the hypertensive group and the other into the control group, four genera were extracted for comparison after adjusting for age and sex between the two groups. Age [19] and sex [20] contribute to microbiota variation; however, there was no need to adjust for age or gender because each pair of monozygotic twins was divided into two groups.

In addition, when the effects of age, sex, and genetic background were removed by focusing on the differences between pairs, 15 genera were extracted, including the four genera mentioned above, all of which showed higher occupancy in the control group. Twelve genera belonged to the phylum Firmicutes, two to the phylum Bacteroidetes, and the remaining one to the phylum Actinobacteria. At the phylum level, Firmicutes has been reported to be associated with coronary artery disease and Actinobacteria with cerebral infarction, suggesting that Bacteroidetes may be involved in the prevention of coronary artery disease and cerebral infarction. In addition, the F/B ratio has been found to be higher in studies of hypertension [2,21], and several genera extracted in this study were also found in the Firmicutes and Bacteroidetes phyla, supporting the previous findings.

Of the 15 genera selected for this study, six had already been reported to be associated with blood pressure.

Four genera showed the same trend as in a previous study [5,22-24].

Actinomyces was observed in a significant amount in the hypertensive group, showing the same trend as in a previous study [22], having a positive correlation with blood pressure indices.

The *Eubacterium rectale* group was observed in a significant (0.043) amount in the hypertensive group in this study, showing the same trend as in a previous study [23].

The *Eubacterium eligens* group had high occupancy in the hypertension group, similar to a study examining the differences in gut bacteria by diet when saturated fatty acid was replaced with walnuts and vegetable oil in people that were high risk for cardiovascular disease. An increase in the *Eubacterium eligens* group showed the same trend as reported [24], which correlated with a decrease in brachial mean arterial pressure, central mean arterial pressure, and central diastolic blood pressure (BP).

The *Ruminococcaceae* NK 4A 214 group was observed in a significant (0.026) amount in the control group, with the same trend in a previous study [5].

Two genera showed a different trend from the previous study.

Butyricoccus was observed in a significant (0.012) amount in the hypertension group, and showed an opposite trend to previous studies, which reported an association with enrichment in normotension or negatively associated with BP indices [3].

Coprobacter was found in a significant (0.022) amount in the hypertensive group, whereas it was high in the control group in a previous study [25], thus showing the opposite trend. The difference was that the previous study did not exclude patients who had received antihypertensive treatment.

Given that present study included hypertensive discordant monozygotic twins, indicates the possibility of age, sex, and genetic effects in these two genera.

Persistent high blood pressure causes arteriosclerosis and lead to cardiovascular diseases including cardiac disease. *Ruminococcaceae* UCG 014, which had a higher occupancy in the control group in this study, was not reported to be related to hypertension in previous studies, but it has been reported to be cardiac disease [26]. An increased presence of

Ruminococcaceae UCG 005, *Ruminococcaceae* UCG 014 and *Eubacterium fissicatena* group was noted in the acute coronary syndrome (ACS) group compared to the healthy control group, as well as increased serum trimethylamine N-oxide (TMAO) concentration [26]. Cardiovascular events, such ACS, are caused by the disruption of atherosclerotic plaques and thrombus occlusion. Atherosclerosis is a chronic inflammatory disease of the blood vessels that occurs under the influence of lifestyle-related diseases, including hypertension and smoking. Its mechanism has been clarified since the report that TMAO, a metabolite derived from intestinal bacteria, is associated with the onset of neovascular events [27]. Choline and L-carnitine contained in eggs, shrimp, and meat are metabolized to TMA by intestinal bacteria, absorbed from the intestinal tract, and converted to TMAO via enzymes in the liver [28]. TMAO increases cholesterol accumulation in macrophages and decreases cholesterol withdrawal from plaques. TMAO also increases platelet aggregation capacity, promotes thrombus formation, and increases cardiovascular events [27]. A previous study confirmed that serum TMAO correlated with ACS, suggesting that these bacteria may be predictive biomarkers for ACS development [26]; however, the results of this study showed that *Ruminococcaceae* UCG 005, *Ruminococcaceae* UCG 014 and *Eubacterium eligens* group may also be affected by age, gender, and genetics, and therefore further research concerning these factors needs to be considered.

In a previous study, *Ruminococcaceae* UCG 014 showed that the intestinal flora may mediate the effect of periodontitis on diabetes [26]. In the gut microbiota, some butyric acid-producing bacteria, such as *Eubacterium fissicatena* group and *Ruminococcaceae*_UCG-014 were reduced, and a negative correlation with serum HbA 1c was confirmed. These results suggest that *Ruminococcaceae* UCG 014 and *Eubacterium fissicatena* group may have similar functions; however the *Eubacterium fissicatena* group was observed in a significant amount in the HT group. And *Ruminococcaceae* UCG 005 was observed in a significant (0.033) amount in the control group. This was also observed in the results for intestinal diseases in the non-disease group [33].

Further studies, including those on its mechanism, are required.

For the other five bacteria extracted in this study, there were no reports showing an association with cardiovascular disease.

Lachnospira was observed in a significant (0.025) amount in the control group. Previous studies in humans also reported a decrease in patients with gallstones compared to the normal group [29] and significantly more in the normal group than in patients with idiopathic nephrotic syndrome with membranous nephropathy [30]. This was consistent with the present study in that it was more common in the normal group, despite differences in disease.

A study focusing on differences in gut microbiota between normal-weight and overweight children living in urban areas in the Philippines reported that *Lachnospira* was more common in the overweight group [31]; however, it is difficult to compare the two groups because the median body mass index in this study was 22.8 and there were differences in age.

In addition, *Lachnospira* is associated with SNP rs59846192 (DMRTB1), and it has been reported that populations with the major allele (GG) are rich in *Lachnospira* [32]. However, this study classified monozygotic twin pairs with identical genetic backgrounds into two groups; therefore, this effect was not considered.

Prevotellaceae UCG 001 was observed in a significant (0.025) amount in the hypertensive group, whereas a decrease in this amount was observed in chronic kidney disease patients [34]. A significant decrease was also observed in the high fat diet group in a mouse experiment [35]; however, it increased when combined with alcoholic beverages. This also caused an increase in the value and activity of antioxidant enzymes and a decrease in the content of lipid peroxidation products [36], suggesting that it may be influenced by diet.

Coprococcus 1 was observed in a significant (0.049) amount in the control group. There are a few human studies on *Coprococcus* 1, and it has been

reported to be associated with weight loss [37]; however, there is still much to be learned.

Eubacterium ruminantium group, an opportunistic bacterium, was not found in the hypertensive group. Few studies have been conducted on humans, and it was reported that it was more common in patients that had miscarriages [38].

Ruminiclostridium 9 was observed in a significant (0.010) amount in the control group. To date, no study has reported its relationship with the disease.

Ruminococcaceae UCG 004 was observed in a significant amount in the control group. There was a report associated with inattentive symptoms in a study of people with attention deficit/hyperactivity disorder [39].

In a cohort study, no association was found between 68 different microbiota markers and self-reported hypertension after correction for multiple testing [40]; however, we suggest that 15 bacteria are hypertension-associated bacteria by studying hypertensive discordant monozygotic twins. Even though subtypes of hypertension, isolated systolic hypertension, and diastolic hypertension share different gut microbiota profiles [25], for rigor, actual measurements and severity of hypertension should be considered. In a previous study, the association between gut microbiota and hypertension was not significant in the British, Africans, Ghanaians, South Asians, and Turks, and significant associations were observed in Americans, Finns, Moroccans, and the Dutch. Only the gut microbiota of the Dutch cohort showed a high level of explained variance [40], suggesting that the Japanese may be the ethnic group that shows an association between gut bacteria and hypertension.

The gut microbiome is affected by diet, and the relationship between diet and nutrients has already been reported in the 15 genera identified in this study.

For example, *Actinomyces* and *Lachnospira* have been found to be affected by dietary fiber; for *Actinomyces*, it has been reported that the higher the fiber intake, the lower the percentage of *Actinomyces* [23], and the present results are not statistically significant [24]. However, as in previous studies, the higher the fiber intake, the lower the percentage of *Actinomyces* and *Actinomyces* was lower in the control group. Alternatively, for *Lachnospira*, L-Theanine increased the percentage of *Lachnospira* and an increase in total SCFA (acetate, propionate, butyrate) was reported [41]. The present results also showed a positive correlation between dietary fiber intake and *Lachnospira*, although this was not statistically significant ($r = -0.1$).

A diet high in dietary fiber has an antihypertensive effect in hypertensive patients [42], and the results are consistent with the association between these two bacteria and dietary fiber. One of the mechanisms is the response of the intestinal bacterial tumor metabolite SCFA to its receptors Olfr78 [5] and Gpr41 [6]. In addition, dietary fiber is effective in lowering blood pressure by adsorbing sodium and removing it from the body; however, when we focused on sodium, which has a negative impact on BP and cardio-vascular health, we found a significant positive correlation with *Eubacterium rectale* group ($r = 0.48$), and *Eubacterium rectale* group was more common in the hypertensive group. Therefore, *Eubacterium rectale* group may play some role in this study. In addition, there was a negative correlation between *Ruminococcaceae NK 4A 214* group and *Ruminococcaceae UCG 005*.

In addition, fruits that increase SCFA are rich in potassium. Potassium intake was negatively correlated with *Actinomyces* ($r = -0.4$) and positively correlated with the *Eubacterium rectale* group ($r = 0.4$). In a previous study, *Lachnospira* was also positively correlated with fruit [43]; however, this result was not statistically significant ($r = 0.18$). Here, a positive correlation with potassium was observed, although this was not statistically significant.

Conclusions

This study had several limitations. The target sample size was insufficient for strong statistical power (26 pairs), which may have led to bias in the data. Owing to these limitations, we were unable to detect several genera associated with Blood Pressure, as identified in previous studies. Further

studies with a larger number of subjects are needed to confirm these relationships in the future.

Our results extracted 15 genera of gut microbiota associated with hypertension, taking into account age, gender, and genetic factors that affect gut microbiota, by targeting hypertensive discordant Japanese monozygotic twins. Furthermore, by investigating the relationship between the extracted hypertension-related bacteria and nutrients that strongly affect the intestinal bacteria, *Actinomyces* and *Eubacterium rectale* groups were associated with some nutrients, but other bacteria were not associated with nutrients. The cross-sectional of this study inherently limits our ability to delineate fully the observed associations among hypertension and gut bacteria and nutrition. Further exploration of specific associations presented here will require comparison with cohort studies or increasing the number of subjects.

Author Contribution

Rie Tomizawa and Jonguk Park contributed equally to this work.

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Conflicts of Interest

The authors declare no conflict of interest.

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