Original Article

Integrated metagenome and metabolome analyses of blood pressure studies in early postmenopausal Chinese women

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Objective: We carried out sensitivity analyses on gut microbiota metagenomic sequencing, untargeted metabolome, targeted metabolome for short-chain fatty acids (SCFAs) and human whole genome sequencing from 402 early postmenopausal Chinese women to search for early omics-biomarkers and gain novel insights into the potential mechanisms of BP regulation in postmenopausal women.

Methods: Clusters of co-abundant gut bacterial species and serum untargeted metabolites were identified by weighted gene co-expression network analysis (WGCNA). Partial least square analysis and joint analysis were performed to detect BP-associated omics-variables. Partial Pearson correlation was conducted to identify the interactions of microbe–host for host BP variation. Mendelian randomization analysis and causal inference test were used to examine causal relationships among gut microbiota, metabolites and BP variation.

Results: In the present study, 651 bacterial species and 296 metabolites were binned into 53 and 26 co-abundance clusters by WGCNA, respectively. Then, we totally identified four gut bacterial species, one host metabolites and two SCFAs that were significantly associated with both SBP and DBP. Moreover, we found that gut microbiota would play important roles in host metabolic activity. Finally, our results revealed that increased *Bacteroides fragilis* could elevate BP via decreased caproic acid, and phenylacetylglutamine mediated the causal relationships of both *B. fragilis* and *Clostridium sp.CAG.226* on DBP variation.

Conclusion: Multi-omics datasets integration has the potential to capture complementary effect and their interactions for BP variation, revealed the potential pathogenesis of BP variation and may be useful for studying other complex diseases/traits.

Keywords: blood pressure, metabolomics, metagenomics, short-chain fatty acid

Abbreviations: BP, blood pressure; CIT, causal inference test; GWAS, genome-wide association study; IVW, inverse variance weighted; PAG, phenylacetylglutamine; PLS, partial least squares; SCFAs, short-chain fatty acids; SD, standard deviation; VIP, variable importance in projection; WGCNA, weighted gene co-expression network analysis

INTRODUCTION

Hypertension is a global public health concern impacting 1.4 billion people worldwide, accounts for 10 million deaths/year [1]. As a complex disease, hypertension is affected by a myriad of environmental and genetic factors [2,3], and the precise cause of this morbidity has not been well elucidated to date. Many lines of seminal evidence suggested the crucial roles of host-metabolites, short-chain fatty acids (SCFAs) and gut microbiota in blood pressure (BP) variation [4–7]. Numerous metabolites associated with hypertension incidence had been discovered in metabolomics studies [5,6]. Wang *et al.* [5] suggested that disorders of amino acid metabolism (glycine, lysine and cystine) may play an important role in predisposing young men to developing hypertension. In addition, Zhong *et al.* illustrated that patients with hypertension possessed a much lower quantity of amino acids (valine, alanine, pyroracemic acid, inose, *p*-hydroxyphenylalanine and methylhistidine), and a significant increase in very-low-density lipoprotein, low-density lipoprotein, lactic acid, and acetone compared with healthy individuals [6]. As known, SCFAs were produced exclusively by gut

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microbiota in host colon [8], and subsequently absorbed into the bloodstream of the host [9], function as critical signaling molecules between the host and gut microbiota [8]. A previous study reported that SCFAs (acetic, propionic and butyric acid) could modulate vasodilatation and regulate the colon resistance arteries [7]. Most recently, the direct effects of gut microbiota on BP variation of the host were demonstrated by fecal transplantation from hypertensive human donors to germ-free mice [4]. This study also identified that the metabolism changes of the host with hypertension was closely linked to gut microbiota [4], and a disease classifier based on gut microbiota, and host serum metabolites were constructed, which could discriminate pre-hypertensive or hypertensive individuals from controls accurately [4]. These studies implied strong correlations of gut microbiota, host metabolites and SCFAs in BP variation.

Menopause is an endocrine-related transition that induces a number of physiological and potentially pathological changes in middle-aged and elderly women. Many cross-sectional and longitudinal studies consistently reported a higher prevalence of hypertension in postmenopausal women than in premenopausal women, even after adjustment for age [10–13]. Therefore, significant gut bacterial species and serum metabolites associated with BP variation in early postmenopausal women may serve as biomarkers for early diagnosis, prevention and treatment of hypertension and their interaction may provide new perspectives on the pathogenesis of hypertension in postmenopausal women. To address the questions above, we performed a novel systematic integrative multi-omics analysis of gut microbiota metagenomic sequencing, host serum metabolomics (untargeted host serum metabolomics and targeted metabolomics for SCFAs) and human whole genome sequencing from 402 healthy early postmenopausal Chinese women to illustrate the crucial roles of gut bacterial species, serum metabolites and their interaction in influencing BP variation.

METHODS

Study cohort and patient characteristics

Totally, 402 early postmenopausal Chinese women were randomly recruited from Guangzhou City, China. The inclusion criteria included aged 40 years or older, being in early postmenopausal stage, and have lived in GuangZhou City for at least 3 months. Menopause time means time length from last menstruation to the time of the questionnaire done. Early postmenopausal stage means menopause time more than 1 year and less than 6 years [10]. Individuals who had used antibiotics, estrogens, anticonvulsant drugs and antihypertensive drugs in the past 3 months, had any chronic conditions (such as diabetes mellitus, chronic renal failure, chronic liver failure, significant chronic lung disease, chronic obstructive pulmonary disease, rheumatoid arthritis and chronic gastrointestinal disease) or had any serious diseases (such as serious cerebral vascular disease, liver transplant and cirrhosis) were excluded from the study. All the participants had signed informed consent form before they were recruited, and this study was approved by the Third Affiliated Hospital of Southern Medical University (Guangzhou City, China) institutional review board.

All the questionnaire data were recorded by face-to-face interview. Trained research staff administered a standardized questionnaire to collect information on demographic characteristics and menopause time. Body weight (kg) and height (cm) were measured twice in light indoor clothes without shoes to the nearest 0.1 kg and 0.1 cm, respectively. BMI was calculated as weight in kilograms divided by height in squared meters. BP was measured by nurses or physicians after a 15 min rest in a sitting position. Three readings were recorded at 5 min intervals with a random-zero mercury column sphygmomanometer, and the average was taken as the final measurement.

Biological sample collection and DNA extraction

Blood and stool samples were freshly collected after an overnight fast for more than 8 h from all participates and transported to the laboratory with ice pack and immediately frozen at −80 °C within 30 min. The whole blood samples were separated into serum by centrifugation and used for DNA extraction with the SolPure DNA Kit (Magen, Guangzhou, China). Stool samples were used for gut microbiota DNA extraction with the E.Z.N.A. Stool DNA Kit (Omega, Norcross, Georgia, USA).

Serum, blood and gut microbiota DNA samples were stored the at −80 °C until further analyses.

Multi-omics data

A detailed description of metagenomics (bacterial species), metagenomics function [*Kyoto Encyclopedia of Genes and Genomes* (KEGG) modules], untargeted metabolomcis, targeted metabolomics (SCFAs) and human whole genome sequencing data acquisition can be found in the Supplementary materials, http://links.lww.com/HJH/B595.

Genome-wide association study

Quality control of genotype data with the following criteria applied: individual missingness less than 10%, SNP call rate above 90%, minor allele frequency (MAF) greater than 1%, and Hardy–Weinberg equilibrium (HWE) P value greater than 1.0×10^{-5}. GWAS was conducted to test for associations between phenotypes (BP and omics-variables) and genotyped SNPs, respectively. Potential confounding factors, including age, BMI and menopause time were adjusted in GWAS. Quality control of genotype data and GWAS were implemented with Plink 1.9 software [14].

Clustering of co-abundant metagenomics and untargeted metabolomics

Clusters of co-abundant gut bacterial species and serum untargeted metabolites were identified using R package weighted gene co-expression network analysis (WGCNA) (Fig. 1) [15]. Normalization and autoscaling were conducted for metagenomics, and metabolomics abundance before WGCNA [15,16]. Gut bacterial species and serum untargeted metabolites were clustered separately. Signed and weighted co-abundance correlation networks were calculated across all examined individuals. A scale-free topology criterion was used to choose the soft threshold $\beta = 12$ for the gut bacterial species and $\beta = 10$ for the serum untargeted metabolites.

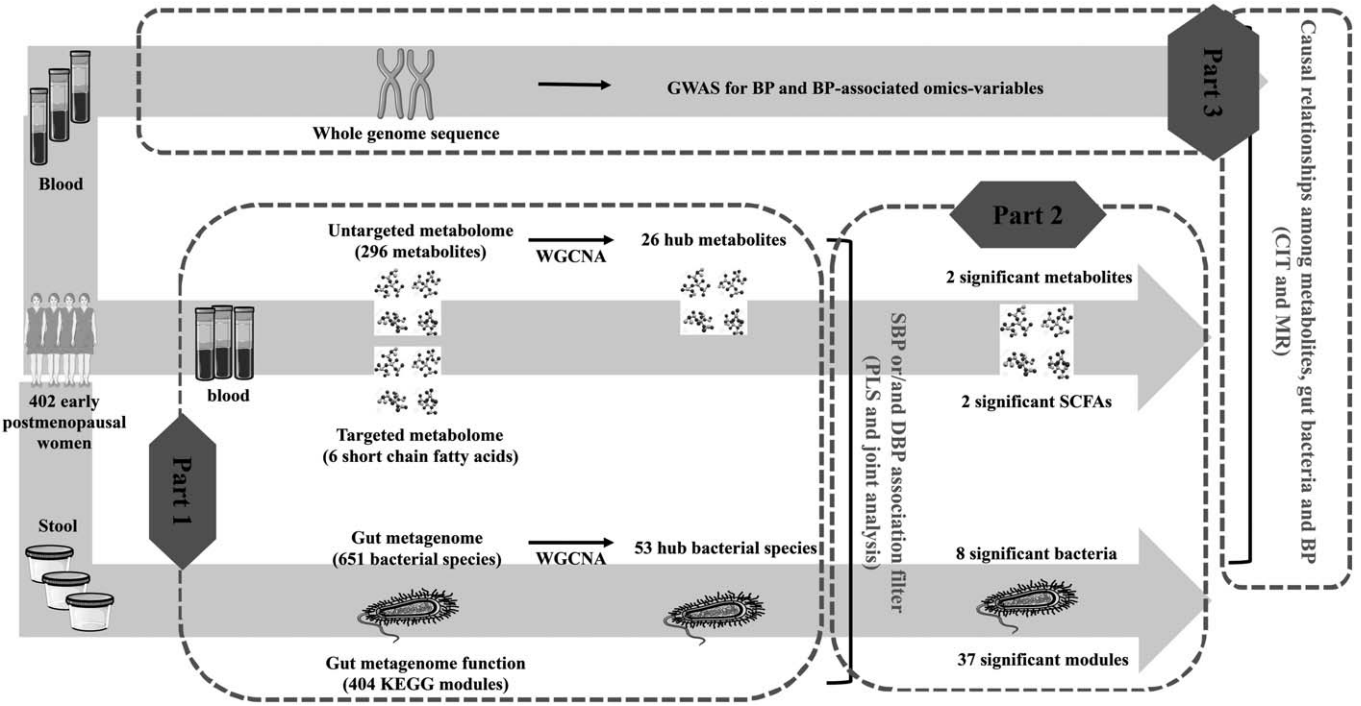


FIGURE 1 Schematic representation of structural analysis. The structural analysis of our study was divided into three parts. Part 1: multi-omics data acquisition, using WGCNA to cluster the metagenomics and untargeted metabolomics independently and find out the hub variables in the clusters; part 2: associations among bacterial species, metabolites and BP; part 3: causal relationship among bacterial species, metabolites and BP. BP, blood pressure; CIT, causal inference test; GWAS, genome-wide association study; KEGG, Kyoto Encyclopedia of Genes and Genomes; MR, Mendelian randomization; PLS, partial least square; VIP, variable importance in projection; WGCNA, weighted gene co-expression network analysis.

Clusters were identified with the dynamic hybrid tree-cutting algorithm, using a minimum cluster size of 3 for both gut bacterial species clusters and serum untargeted metabolites clusters. Gut bacterial species and serum untargeted metabolites that did not fit the clustering criteria were removed from further analysis. The clustered gut bacterial species and serum untargeted metabolites were collectively termed metagenomic clusters (labeled MG01–MG53) and metabolomics clusters (labeled MB01–MB26). The profile of each cluster was summarized by the cluster eigenvector (that is, the first principal component of a given cluster). Then, module membership was assessed by the correlation between omics-variables and clusters eigenvectors for a given cluster. Hub gut bacterial species and hub metabolites were selected by the highest module membership each module.

Association analysis between omics variables and blood pressure

To decrease false positives accumulated by the multiple tests, we simultaneously conducted partial least squares (PLS) analysis and joint analysis [17] of multivariate phenotypes to examine the associations of omics-variables (hub bacterial species, hub serum untargeted metabolites, SCFAs and KEGG modules) with both SBP and DBP (Fig. 1). PLS provides a variable importance in projection (VIP) value for the descriptor data set X (omics-variables) that best describe the response data set Y (BP), as it maximizes the covariance expressing the common structures between X (omics variables) and Y (BP) [18]. Meanwhile, the seemingly unrelated regression of 'systemfit' [19] was used to estimate the coefficients of SBP and DBP with the omics-variables, and the *F*-statistics tests were performed to test the null hypothesis. The null hypothesis (H_0) of the joint analysis was that none of the traits (SBP and DBP) was related to the tested omics-variables. At least one trait (SBP or DBP) associated with the omics-variables would reject the null hypothesis. Potential confounding factors, including age, menopause time and BMI were adjusted in the PLS model and joint analysis. Finally, results with both VIP greater than 1.5 in PLS model and *P* value less than 0.05 in joint analysis were considered statistically significant.

Explained variance of blood pressure in prediction models

In this study, we constructed the prediction models for BP to estimate the explained variance (adjusted coefficient of determination, R^2) improved by significant omics-variables associated with both SBP and DBP. In prediction model 1, we included the age, BMI and menopause time as the independent variables. In prediction model 2, we included the age, BMI, menopause time and significant bacterial species as the independent variables. In prediction model 3, we included the age, BMI, menopause time and significant metabolites as the independent variables. In prediction model 4, we included the age, BMI, menopause time, significant bacterial species and significant metabolites as the independent variables. In all the prediction models, SBP and DBP were included as dependent variables, respectively.

The interactions of microbe–host in blood pressure variation

Omics-variables (bacterial species, serum untargeted metabolites and SCFAs) significantly associated with both SBP and DBP would be enrolled in Pearson correlation analysis to evaluate the interactions of microbe–host for host blood pressure variation (Fig. 1).

Causal relationships among gut microbiota, metabolites and blood pressure variation

We simultaneously conducted one-sample Mendelian randomization analysis [20] and causal inference test (CIT) [21,22] to examine causal relationships among gut microbiota (bacterial species associated with both SBP and DBP), metabolites (serum untargeted metabolites and SCFAs associated with both SBP and DBP) and BP variation (Fig. 1). As previous studies confirmed the causal effect of gut microbiota on BP variation [4], and significant metabolites we detected in the present study were gut microbiota-driven metabolites (detailed description was in the Results section) [23–25], one-sample Mendelian randomization was performed to test the causal effects of bacterial species on BP variation, metabolites on BP variation and bacterial species on metabolites, respectively. Inverse variance-weighted (IVW), simple median and weighted median were used for one-sample Mendelian randomization analysis, respectively [20]. Mendelian randomization–Egger regression was performed to assess the horizontal pleiotropic pathway between genetic variants and outcomes (metabolites and BP) [26]. Intercept with P value greater than 0.05 indicates no horizontal pleiotropic exists in Mendelian randomization–Egger regression. Result with P values less than 0.05 in all the three Mendelian randomization analysis methods was considered statistically significant. Additionally, CIT was conducted to estimate whether the causal relationship of bacterial species on BP variation might be mediated by metabolites [21,22]. CIT is a statistical framework resulting in a P value, to quantify uncertainty in a causal inference pertaining to a measured factor [21,22]. Potential confounding factors, including age, BMI and menopause time were adjusted in CIT, and the result with P value less than 0.05 was considered statistically significant. All the statistical analyses were performed in the statistical computing language R 4.0.2 (https://www.r-project.org/).

RESULTS

Characteristics description of the samples

A total of 402 early postmenopausal Chinese women were recruited in this study, age ranging from 41.47 to 63.80 years. The mean (standard deviation, SD) of menopause time was 2.22 (0.81) years, ranging from 1 to 5 years. Generally, the participants had normal BMI levels. The mean (SD) of SBP and DBP of study participants was 123.19 (16.27) mmHg and 74.80 (10.61) mmHg, respectively. According to 2018 Chinese hypertension classification criteria, 41% of the individuals had normal BP (SBP $<$ 120 mmHg and DBP $<$ 80 mmHg), 40% of the subjects had high normal BP (120 $\leq$ SBP $<$ 140 mmHg and/or 80 $\leq$ DBP $<$ 90 mmHg) and 19% hypertension (SBP $\geq$ 140 mmHg and/or DBP $\geq$ 90 mmHg). Characteristics description of the samples was provided in Table 1.

Blood pressure-associated bacterial species and functional capacity

We obtained an average of 49 891 245 clean reads after removing low-quality reads. The clean reads of each sample had high Q20 (98.98%) and Q30 (96.74%), which showed high sequencing quality. On average, we identified 88 505 contigs in all individuals. In total, we identified 10 303 taxa at the species level by the taxonomic annotation. Among them, 651 species were nonrare bacterial species whose relative abundance, each exceeded 0.01%. These nonrare bacterial species were binned into 53 co-abundance clusters (labeled MG01—MG53) by WGCNA, and then 53 hub bacterial species were selected with the highest module membership across all participants (Supplementary Table 1, http://links.lww.com/HJH/B596). By PLS regression and joint analysis, we detected eight of the 53 hub bacterial species were significantly associated with SBP or DBP (P values $<$0.05, Fig. 2). It is worth mentioning that *Acinetobacter radioresistens* (positive), *Bacteroides fragilis* (positive), *Clostridium sp.CAG.226* (negative) and *Coprococcus sp.CAG.131* (negative) were significantly associated with both SBP and DBP, and association directions were consistent (Fig. 2).

All the unigenes were aligned to the KEGG database, and the resulting proteins were assigned to the KEGG orthology. KEGG orthologies were further mapped to gut microbiota-associated KEGG modules. We totally detected 704 KEGG modules by functional annotation. Among them, 404 modules were non-rare modules whose relative abundance each exceeded 0.01%. Furthermore, by PLS analysis and joint analysis, we totally identified 37 modules associated with SBP (29 modules) and/or DBP (30 modules) (P values$<$0.05, Supplementary Table 2, http://links.lww.com/HJH/B597). Among these 37 modules, 25 modules were significantly associated with both SBP and DBP. The 25 modules were mainly involved in metabolic functions

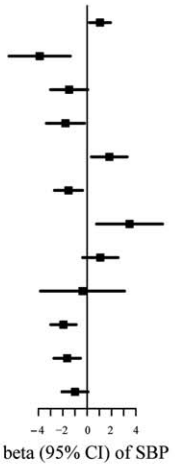
TABLE 1. Characteristics of the study population

Variables	*N*	Mean	SD	Minimum	Maximum
Menopause time (years)	402	2.22	0.81	1.00	4.90
Age (years)	402	52.96	2.88	41.47	63.80
Height (cm)	402	157.85	5.12	142.00	172.00
Weight (kg)	402	57.19	7.61	40.00	82.00
BMI (kg/m^2)	402	22.94	2.82	16.42	33.73
SBP (mmHg)	402	123.19	16.27	80.00	170.00
DBP (mmHg)	402	74.80	10.61	50.00	107.00

SD, standard deviation.

(a)

Omics−variables	N	F−statistic	*p*−value of F−statistucs	beta (95% CI)	*p*−value	VIP
Acetic acid *	402	3.198	0.041	1.05 (0.19, 1.92)	0.018	1.71
Caproic acid *	402	4.809	0.008	−3.9 (−6.42, −1.38)	0.003	2.18
Chrysin	402	4.564	0.011	−1.48 (−3.01, 0.05)	0.059	1.38
PAG *	402	3.777	0.023	−1.78 (−3.35, −0.21)	0.027	1.59
Acinetobacter radioresistens *	402	3.273	0.038	1.82 (0.34, 3.29)	0.016	1.82
Alistipes sp.AL.1	402	3.887	0.021	−1.53 (−2.69, −0.38)	0.01	1.94
Bacteroides fragilis *	402	3.483	0.031	3.48 (0.77, 6.19)	0.012	1.89
Bacteroides sp.CAG.875	402	3.108	0.045	1.08 (−0.37, 2.54)	0.146	1.08
Clostridium sp.ATCC.BAA.442	402	3.404	0.034	−0.38 (−3.84, 3.07)	0.829	0.15
Clostridium sp.CAG.226 *	402	6.819	0.001	−1.95 (−2.99, −0.9)	0	2.72
Coprococcus sp.CAG.131 *	402	4.362	0.013	−1.64 (−2.73, −0.55)	0.003	2.19
Ruminococcus sp.CAG.579	402	4.474	0.012	−1 (−2.07, 0.07)	0.067	1.38



(b)

Omics−variables	N	F−statistic	*p*−value of F−statistucs	beta (95% CI)	*p*−value	VIP
Acetic acid *	402	3.198	0.041	0.65 (0.08, 1.22)	0.025	1.56
Caproic acid *	402	4.809	0.008	−2.12 (−3.77, −0.47)	0.012	1.75
Chrysin	402	4.564	0.011	−1.53 (−2.52, −0.53)	0.003	2.07
PAG *	402	3.777	0.023	−1.41 (−2.43, −0.38)	0.007	1.83
Acinetobacter radioresistens *	402	3.273	0.038	1.12 (0.15, 2.08)	0.024	1.81
Alistipes sp.AL.1	402	3.887	0.021	−0.4 (−1.16, 0.36)	0.302	0.81
Bacteroides fragilis *	402	3.483	0.031	2.08 (0.3, 3.85)	0.022	1.82
Bacteroides sp.CAG.875	402	3.108	0.045	1.19 (0.25, 2.14)	0.014	1.96
Clostridium sp.ATCC.BAA.442	402	3.404	0.034	−2.34 (−4.58, −0.09)	0.042	1.6
Clostridium sp.CAG.226 *	402	6.819	0.001	−1.01 (−1.7, −0.33)	0.004	2.3
Coprococcus sp.CAG.131 *	402	4.362	0.013	−0.8 (−1.52, −0.09)	0.029	1.73
Ruminococcus sp.CAG.579	402	4.474	0.012	−1.05 (−1.74, −0.36)	0.003	2.36

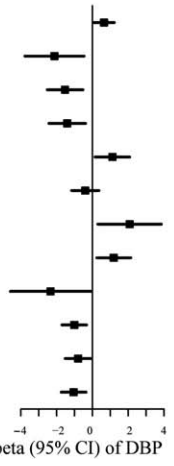


FIGURE 2 Omics variables associated with blood pressure. Omics variables significantly associated with SBP (a) or/and DBP (b) were selected by both joint analysis (*P* value of *F* statistics <0.05) and PLS analysis (VIP >1). Asterisk (*) indicates that omics variables are significantly associated with both SBP and DBP. Beta (95% CI) and *P* value were estimated by joint analysis, *P* value less than 0.05 means statistically significant. *N* means the number of participants. BP, blood pressure; PAG, phenylacetylglutamine; VIP, variable importance in projection.

essential for the host, such as 'Cysteine and methionine metabolism', 'Pyrimidine metabolism', 'Lipopolysaccharide metabolism', 'Lipid metabolism' and 'Saccharide, polyol, and lipid transport system', and so forth. The results suggested that imbalance of gut microbiota might evoke a disease-linked state through interference of host physiological metabolic functions.

Blood pressure-associated serum metabolites

Untargeted serum metabolomics and targeted serum metabolomics for SCFA profiles provided information about 296 known metabolites and 6 SCFAs, respectively. The 296 known metabolites were binned into 26 co-abundance clusters (labeled MB01−MB26) by WGCNA, then 26 hub metabolites were selected by the highest module membership across all individuals (Supplementary Table 3, http://links.lww.com/HJH/B598). Furthermore, by PLS analysis and joint analysis, we found chrysin and phenylacetylglutamine (PAG) were significantly associated with BP (*P* values <0.05, Fig. 2). Notably, PAG was negatively associated with both SBP (beta = −1.782, VIP = 1.588) and DBP (beta = −1.406, VIP = 1.832). Moreover, two of the six SCFAs (acetic acid and caproic acid) were significantly associated with both SBP and DBP (*P* values <0.05, Fig. 2). Specifically, acetic acid was positively associated with BP (SBP: beta = 1.053, VIP = 1.711; DBP: beta = 0.649, VIP = 1.557, *P* values <0.05, Fig. 2), whereas caproic acid was negatively associated with BP (SBP: beta = −3.902, VIP = 2.182; DBP: beta = −2,121, VIP = 1.749, *P* values <0.05, Fig. 2).

Explained variance of blood pressure in prediction models

The overall explained variance (adjusted R^2) of prediction model 1, prediction model 2, prediction model 3 and prediction model 4 was 6.7, 9, 11.2 and 12.2% for SBP and 6.4, 8.7, 9.4 and 10.3% for DBP, respectively (Table 2). Compared with prediction model 1, significant bacterial species (*A. radioresistens*, *B. fragilis*, *Clostridium sp.CAG.226* and *Coprococcus sp.CAG.131*) in prediction model 2 could improve 34.3% explained variance for SBP and 35.9% explained variance for DBP. Compared with prediction model 1, significant metabolites (PAG, acetic acid and caproic acid) in prediction model 3 could

TABLE 2. Explained variance (adjusted R^2) of blood pressure in prediction models

Prediction models	Prediction factors	Adjusted R^2		Fold change of adjusted R^2 [a]	
		SBP	DBP	SBP	DBP
Prediction model 1	Menopause time + age + BMI	0.067	0.064	/	/
Prediction model 2	Menopause time + age + BMI + metabolites	0.090	0.087	1.343	1.359
Prediction model 3	Menopause time + age + BMI + bacterial species	0.112	0.094	1.672	1.469
Prediction model 4	Menopause time + age + BMI + metabolites + bacterial species	0.122	0.103	1.821	1.609

Bacterial species included *A. radioresistens*, *Bacteroides fragilis*, *Clostridium sp.CAG.226* and *Coprococcus sp.CAG.131*. Metabolites included phenylacetylglutamine (PAG), caproic acid and acetic acid. BP, blood pressure.
[a]Compared with prediction model 1, fold change of adjusted R^2.

improve 67.2% explained variance for SBP and 46.9% explained variance for DBP. Compared with prediction model 1, significant bacterial species and metabolites in prediction model 4 could totally improve 82.1% explained variance for SBP and 60.9% explained variance for DBP (Table 2).

The interactions of microbe–host in blood pressure variation

By metagenomic function (KEGG modules) analysis, we found that gut microbiota would play important roles in host metabolic activity. Therefore, we performed Pearson correlation analysis to assess the relationship between gut microbiota and metabolic activity based on bacterial species and serum metabolites associated with both SBP and DBP. By Pearson correlation analysis, we identified that *A. radioresistens* and *B. fragilis* were negatively correlated to PAG, whereas *Clostridium sp.CAG.226* was positively related to PAG (P values <0.05, Fig. 3). Furthermore, *B. fragilis* was positively related to acetic acid, whereas *Clostridium sp.CAG.226* was negatively related to acetic acid (P values <0.05, Fig. 3). Moreover, *B. fragilis* showed negative correlation to caproic acid, whereas *Clostridium sp.CAG.226* showed positive correlation to caproic acid (P values <0.05, Fig. 3). However, we did not detect a significant correlation between *Coprococcus sp.CAG.131* and any of the significant metabolites.

Causal relationships among gut microbiota, metabolites and blood pressure

As previous studies confirmed the causal effects of gut microbiota on BP variation [4], and three BP-associated serum metabolites (PAG, caproic acid and acetic acid), we detected in present study were gut microbiota-driven metabolites [23–25], we simultaneously performed one-sample Mendelian randomization analysis and CIT to assess the potential causal relationships among gut bacteria species (*B. fragilis, Clostridium sp.CAG.226, Coprococcus sp.CAG.131, A. radioresistens*), serum metabolites (PAG, caproic acid and acetic acid) and BP variation. One sample Mendelian randomization results (Supplementary Table 4A–C, http://links.lww.com/HJH/B599**)** indicated that *B. fragilis* had causal effects on acetic acid (positive), caproic acid (negative) and PAG (negative). In addition, *Clostridium sp.CAG.226* had causal effects on acetic acid (negative), caproic acid (positive) and PAG (positive). Moreover, all the four bacterial species (*B. fragilis*: positive, *A. radioresistens*: positive, *Clostridium sp.CAG.226*: negative, *Coprococcus sp.CAG.131*: negative) and three metabolites (PAG: negative, caproic acid: negative and acetic acid: positive) were causally related to both SBP and DBP, and the association directions were consistent. In summary, one-sample Mendelian randomization results revealed that rising *B. fragilis* abundance would elevate BP via increased acetic acid or decreased caproic acid and PAG levels in serum, and rising *Clostridium sp.CAG.226* abundance would lower BP via decreased acetic acid and increased caproic acid and PAG levels in serum. Significant causal effects detected in one-sample Mendelian randomization analysis were those with P values less than 0.05 in all the three Mendelian randomization analysis methods (IVW, simple median and weighted median) and without detected pleiotropy bias (P values of Mendelian randomization–Egger intercept >0.05) (Supplementary Table 4A–C, http://links.lww.com/HJH/B599**)**. Meanwhile, by CIT, we detected that increased *B. fragilis* may elevate BP via decreased caproic acid (SBP: P value of CIT = 0.013 and DBP: P value of CIT = 0.029), and PAG may mediate the causal effect of both *B. fragilis* and *Clostridium sp.CAG.226* on DBP (P values of CIT <0.05, Supplementary Table 5, http://links.lww.com/HJH/B600). Taken one-sample Mendelian randomization results and CIT results together, our study revealed that *B. fragilis* would elevate BP via decreased caproic acid, and PAG may mediate the causal effect of both *B. fragilis* and *Clostridium sp.CAG.226* on DBP (Fig. 4).

DISCUSSION

We collected the largest comprehensive multi-omics data and totally identified eight gut bacterial species, two host serum metabolites and two SCFAs that were significantly associated with BP variation in early postmenopausal Chinese women. Specially, four gut bacterial species (*A. radioresistens, B. fragilis, Clostridium sp.CAG.226 and Coprococcus sp.CAG.131*) and three serum metabolites (PAG, caproic acid and acetic acid) can serve as the candidate target biomarkers for future BP-related biological experiments for eventual clinical interventions as they were associated with both DBP and SBP variations. Additionally, one-sample Mendelian randomization and CIT results both indicated that increased *B. fragilis* would elevate BP via decreased caproic acid, and PAG may mediate the causal relationship of both *B. fragilis* and *Clostridium sp.CAG.226* on DBP variation. In general, based on gut microbiota metagenomics sequencing, metagenomics function analyses (KEGG modules), untargeted metabolomics, targeted metabolomics for SCFAs datasets and human whole

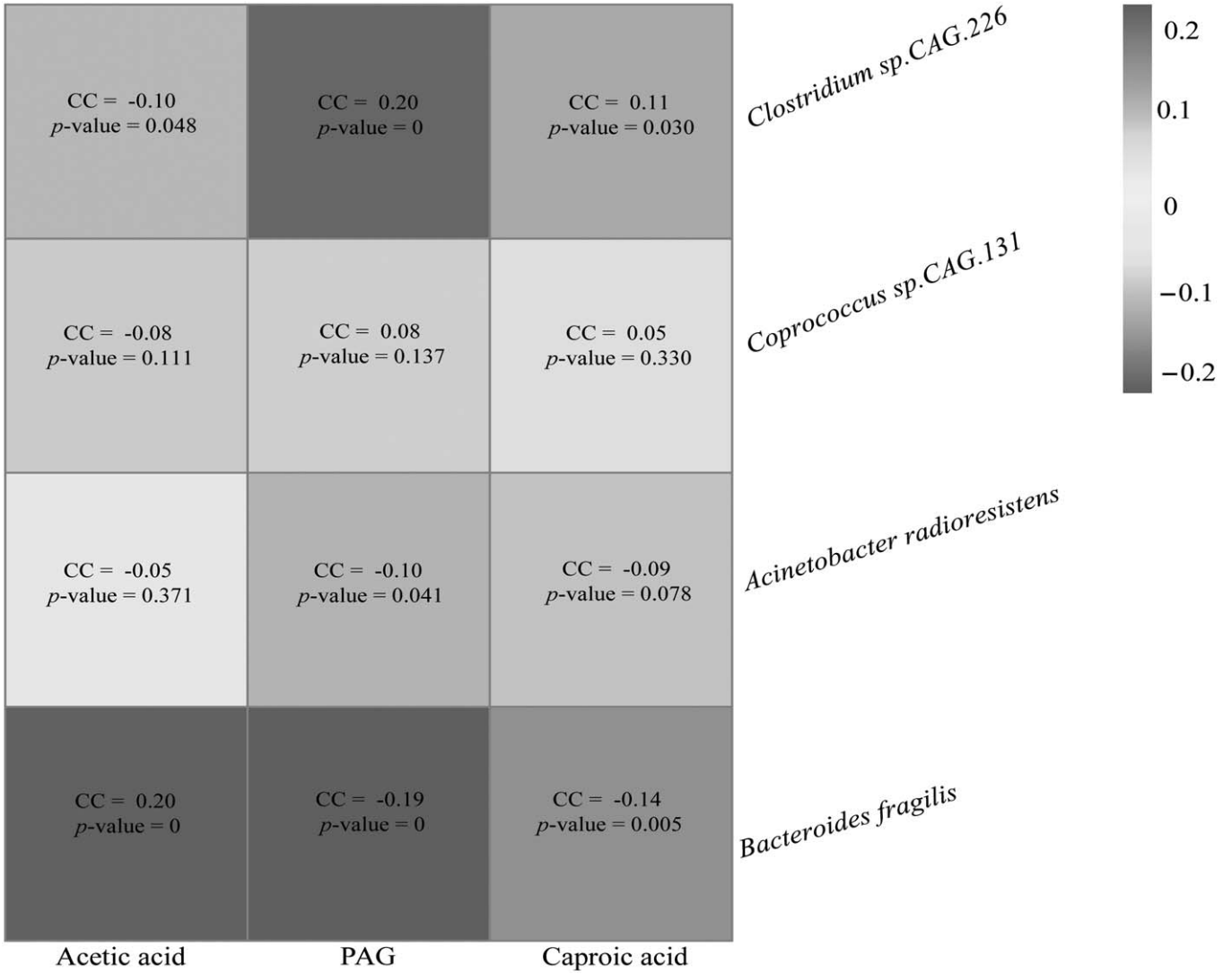


FIGURE 3 Partial Pearson correlation of bacterial species and metabolites ($N = 402$). Partial Pearson correlation analysis was performed to estimate correlation between significant bacterial species and metabolites associated with both SBP and DBP in 402 postmenopausal Chinese women. The magnitude of the correlation is denoted with a color, whereby red indicates a positive correlation and blue indicates a negative correlation. *P* value less than 0.05 means statistically significant. *N* means the number of participants. CC, correlation coefficient; PAG, phenylacetylglutamine.

genome sequencing, we provided a list of multi-omics biomarkers for BP variation in early postmenopausal women, which could be considered further as potential biomarkers for early diagnosis, prevention, intervention and treatment of hypertension in postmenopausal women. Our results also revealed the causal effects among dysbacteriosis, metabolic activity and BP variation, underlying the potential pathogenesis of hypertension in postmenopausal women.

A growing body of evidence supported the role of the gut microbiota in the regulation of BP [4,27,28], and the direct influence of gut microbiota on BP of the host was demonstrated by fecal transplantation from hypertensive human donors to germ-free mice [4]. In the present study, we identified eight gut bacterial species significantly associated with BP variation. Among these eight gut bacterial species, four bacterial species (*A. radioresistens, B. fragilis, Clostridium sp.CAG.226 and Coprococcus sp.CAG.131*) were significantly associated with both SBP and DBP variations. A previous study had confirmed that *A. radioresistens* was positively but weakly associated with expression of high-sensitivity C-reactive protein in postmenopausal breast cancer women [29]. C-reactive protein, as one of the most widely known biomarkers of systemic inflammation, was closely related to hypertension, and vascular diseases [30]. Therefore, the positive relationship between *A. radioresistens* and BP detected in this present study may be linked by systemic inflammation. We also detected a positive relationship between *B. fragilis* and BP variation, whereas previous studies had revealed a conflicting result of their relationship [31–33]. O'Donnell *et al.* [32] showed a similar result to this present research that an acute infusion of *B. fragilis* into a rabbit model gave rise to a transitory lowering of mean arterial pressure. However, a recent study confirmed that *B. fragilis* could increase intestinal-derived corticosterone production and corticosterone levels in serum and intestine, thereby promoting BP elevation in rat model [31]. No significant changes had been

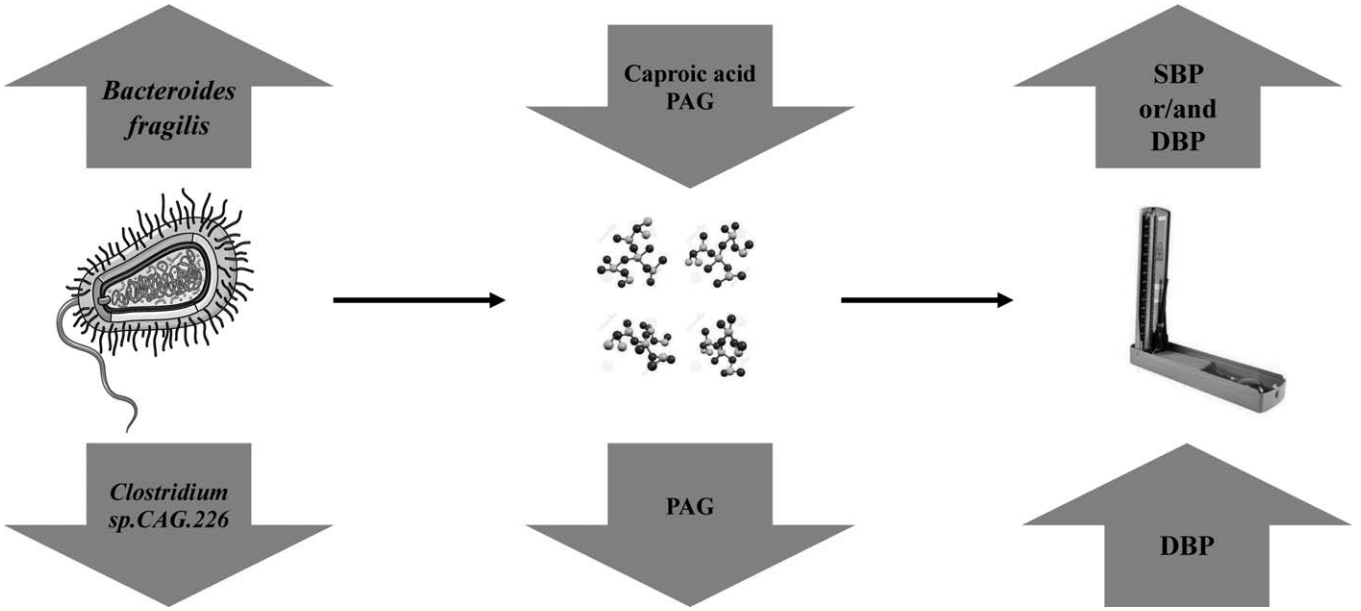


FIGURE 4 Causal relationships among significant omics variables and blood pressure variation. Taken one-sample MR results (*P* value <0.05 in all three MR methods) and CIT results (*P* value of CIT <0.05) together, this present study indicated that *Bacteroides fragilis* would elevate BP via decreased caproic acid, and PAG may mediate the causal effect of both *B. fragilis* and *Clostridium sp.CAG.226* on DBP. Upward arrows indicate increases, and downward arrows indicate decreases. CIT, causal inference test; MR, Mendelian randomization; PAG, phenylacetylglutamine.

detected in mean arterial pressure following *B. fragilis* infusion in subhuman primates [33]. We speculate that the conflicting relationship between *B. fragilis* and BP variation in previous studies may be influenced by the different species studied [31–33], and, to our knowledge, no study had been conducted on the topic *in vivo* in humans as in this study. In the present study, our result indicated that increased abundance of *B. fragilis* could elevate BP via decreased caproic acid and elevate DBP via decreased PAG in early postmenopausal Chinese women. Particularly, we for the first time identified *Clostridium sp.CAG.226* and *Coprococcus sp.CAG.131* that were both negatively associated with BP variation in early postmenopausal Chinese women in this study.

Two serum metabolites were identified to be significantly associated with BP variation in this study, and PAG was the only metabolite detected to be associated with both SBP and DBP variations. PAG as glutamine conjugates of phenylacetic acid, is a well known gut microbial-driven metabolite [34,35]. Menni *et al.* [36] reported that PAG showed the negative correlation to SBP in 1797 European female twins (age range: 18–84 years). More recently, another study also found that urinary PAG levels were negatively related to BP in a randomized controlled study from the Baltimore and Boston areas [37]. These published results were broadly consistent with our findings that serum PAG was negatively associated with BP variation in early postmenopausal women. PAG as the bacterial degradation product of phenylalanine, had been reported to be inversely associated with BMI [38]. BMI was used as the index of general obesity, showed a positive correlation to BP [39]. Furthermore, PAG had been confirmed to be positively associated with the gene-expression levels of *CIDEC* [36], a gene closely related to energy metabolism and lipid droplet formation [40]. Another study also showed that hepatic expression of *CIDEC* was increased in obese individuals and was downregulated by weight loss [41]. Therefore, the potential underlying mechanisms linking serum PAG levels and BP variation may be obesity. Moreover, our results indicated that PAG was highly related to gut bacterial species, such as *B. fragilis*, *Clostridium sp.CAG.226* and *A. radioresistens*. As expected, our one-sample Mendelian randomization and CIT results supported that increased *B. fragilis* would elevate DBP via decreased PAG, and increased *Clostridium sp.CAG.226* would lower DBP via increased PAG. Therefore, the discovered causal relationship among gut microbiota species (special *B. fragilis* and *Clostridium sp.CAG.226*), serum PAG and BP variation merit further validation and elucidation.

SCFAs are produced exclusively by gut microbiota in host colon [8], and subsequently absorbed into the bloodstream of the host [9], function as critical signaling molecules between the host and gut microbiota [8]. A previous study reported that SCFAs (acetic, propionic and butyric acid) could modulate vasodilatation and regulate the colon resistance arteries [7]. Additionally, a recent review suggested that SCFAs could modulate T-cell polarization in local secondary lymph organs and distal effects in vascular cells, then regulate BP [42]. In this study, we for the first time discovered that acetic acid was positively associated with BP variation, whereas caproic acid was negatively associated with BP variation. Of particular interest, we also detected a causal relationship among *B. fragilis*, caproic acid and BP variation, indicating that increased abundance of *B. fragilis* would elevate BP via decreased serum caproic acid in early postmenopausal Chinese women. The novel discovery findings here, therefore, deserve more in-depth verification in future studies.

Our study has multiple strengths. Firstly, to our knowledge, this is currently the largest multi-omics (human genome, metagenomics, untargeted metabolomics and

targeted metabolomics for SCFAs) study directly testing associations among gut microbiota, metabolic activity and human BP variation, especially in postmenopausal women. Secondly, this study used early postmenopausal women instead of older postmenopausal women to study the omics biomarkers for postmenopausal BP variation, the results of which may render early prevention, diagnosis and treatment of postmenopausal hypertension. Thirdly, causal effects of dysbacteriosis, metabolic activity and BP variation were detected by conducting an innovative multi-omics approach, and this approach might be potentially useful for studying other complex diseases/traits. Despite these strengths, our study also had limitations. The major limitation was that, the current study mainly serves as discovery, further verification steps need to be carried out. Furthermore, previous study showed that the potential link between gut microbiome to hypertension is through inflammation [43]. However, in the present study, we could not provide information about associations between significant bacteria and inflammation as we did not collect any markers of inflammation. Therefore, the underlying mechanisms among dysbacteriosis, metabolic activity and BP variation discovered here waits for further validation and exploration.

Altogether, gut microbiota metagenomics sequencing, metagenomics function (KEGG modules), untargeted serum metabolomics, targeted metabolomics for SCFAs and human whole genome sequencing dataset integration has the potential to capture complementary effect and their interactions for BP variation. In the present study, we discovered a list of candidate omics-biomarkers for future BP-related biological experiments, which would be considered as potential biomarkers for early diagnosis, prevention, intervention and treatment of hypertension in older postmenopausal women. In addition, the application of systematic integrative multi-omics analysis detected the causal effects of gut microbiota, metabolic activity and BP variation, revealed the potential pathogenesis of BP variation and may be useful for studying other complex diseases/traits.

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Conflicts of interest

There are no conflicts of interest.

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