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Original Research

Alterations in the fecal microbiota and serum metabolome in unstable angina pectoris patients

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Abstract

Background: Unstable angina pectoris (UAP) is a type of Coronary artery disease (CAD) characterized by a series of angina symptoms. Insulin-like growth factor 1 (IGF-1) system may be related to CAD. However, the correlation between the IGF-1 system, metabolism, and gut microbiota has not been studied. In the present study, we investigated the alterations of serum IGF-1 system, metabolomics, and gut microbiota in patients with UAP. **Methods**: Serum and stool samples from healthy volunteers and UAP patients were collected. Serum metabolomics, PAPP-A, IGF-1, IGFBP-4, STC2, hs-CRP, TNF-α, and IL-6 were detected in serum samples by LC-MS, and commercial ELISA kits, respectively. Fecal short-chain fatty acids (SCFAs) were measured by gas chromatography. 16S rDNA was used to measure the changes of the gut microbiota. The correlation of the above indicators was analyzed. **Results**: There were 24 upregulated and 31 downregulated metabolites in the serum of UAP patients compared to those in the controls. Pathway analysis showed that these metabolites were enriched in pathways including linoleic acid metabolism, amino acid metabolism, starch metabolism, sucrose metabolism, and citrate cycle (TCA cycle), *etc*. Additionally, the UAP patients had lower fecal levels of 2-hydroxyisobutyric acid and succinic acid. 16S rDNA sequencing results showed that the relative abundances of *Bacteroidetes*, *Synergistetes*, *Lactobacillaceae*, *Burkholderiaceae*, Synergistaceae, and *Subdoligranulum* were significantly higher in the UAP patients than the healthy subjects. Moreover, the UAP patients had lower serum IGF-1, IGFBP-4, and STC2 and higher serum inflammatory cytokines (hs-CRP, TNF-α, and IL-6) levels than the healthy controls. Furthermore, there was a strong correlation between serum amino acids and IL-6, which played an important role in the development of UAP. **Conclusions**: These results indicated that the UAP patients had decreased serum IGF-1 level and imbalanced amino acids metabolism, which may be caused by the altered gut microbiota. It may provide a new therapeutic strategy for unstable angina pectoris.

Keywords: insulin-like growth factor 1 (IGF-1); serum metabolomics; gut microbiota; short-chain fatty acids (SCFAs); unstable angina pectoris (UAP)

1. Introduction

Coronary artery disease (CAD), one of the major cardiovascular diseases, has been proved to be the leading cause of global mortality. Unstable angina pectoris (UAP) is a type of CAD characterized by a series of angina symptoms that belong to ischemic cardiovascular and cerebrovascular diseases [1]. Insulin-like growth factor 1 (IGF-1) has been identified as a valuable biomarker and an even therapeutic target of CAD. Clinical trials have shown that a low circulating level of IGF-1 is highly associated with a high incidence of CAD [2,3]. IGF-1 exhibits various beneficial effects, including anti-inflammation, anti-apoptosis, and stimulation of angiogenesis, which are all related to vascular function and atherosclerosis [4]. The bioavailability of IGF-1 is strongly regulated by IGF binding proteins (IGFBPs), which can form a binary complex with IGF-1. Among the IGFBPs, IGFBP-4 is the predominant IGFBP, which is produced by vascular smooth muscle cells. In addition, Pregnancy Associated Plasma Protein-A (PAPP-A) is able to cleave IGFBP-4 and then promotes the release of free IGF-1, which collectively induces the activation of IGF [5–7]. Evidence from clinical experiments has shown a positive relationship between the circulating IGFBP-4 and PAPP-A levels and the incidence of cardiovascular diseases [6,8]. Furthermore, it has been reported that stanniocalcin-2 (STC2) acts as an inhibitor of PAPP-A [9].

Recent studies have highlighted the role of gut microbiota between the incidence and development of CAD [10]. For example, a clinical study showed that CAD patients had a lower abundance of *Bacteroidetes* and a higher abundance of *Firmicutes* than healthy individuals [11]. Additionally, a study using multi-omic analyses revealed the intricated interaction of gut microbiota, circulating metabolites, and the severity of CAD [10]. It has been reported that the gut microbiota involves in regulating IGF-1-related signaling and then contributes to the development of UAP [12,13]. Despite the mechanism of IGF-1 induction of gut microbiota is still unclear, gut microbial metabolites, such as short-chain fatty acids (SCFAs), which may contribute to the increased IGF-1 production [13].

In view of the intricate relationship among the gut microbiota, IGF-1, and CAD, we hypothesized that the gut

microbiota was associated with the IGF-1-related signaling pathway in the patients with UAP, which is mediated by the microbial metabolites. In the present study, we evaluated the serum IGF-1, IGFBP-4, PAPP-A, STC-2 levels, serum metabolomics, and fecal microbiota and its metabolites in patients with UAP and healthy controls. We further explored whether the gut microbiota contributed to the development of UAP and whether microbial metabolites were a remarkable mechanism.

2. Materials and methods

2.1 Patients and study design

In this study, we enrolled 10 UAP patients and 10 healthy controls at Xiangya Hospital Central South University in accordance with the established inclusion and exclusion criteria. All participates were from southern China and had the same dietary style. The inclusion criteria included UAP patients confirmed by a cardiologist. UAP was defined by chest pain at rest or angina equivalent, transient ST-T segment depression but without increased cardiac enzymes in the serum. The exclusion criteria included patients with: (1) History of chronic disease, congenital heart diseases, aortic aneurysm, cardiac valve diseases, connective tissue diseases, and cancer; (2) History of organic digestive system or digestive tract surgery; (3) History of smoking or alcohol abuse; (4) Use of an antibiotic or antibiotic within 1 month [14]. All the general information were collected within 12 hours after the participants were admitted. Blood samples were collected by venipuncture after a 12-hour fast. After 30 min of coagulation at room temperature, the blood samples were centrifuged at 3000 g for 15 min under 4 °C. Serum was removed and stored at –80 °C until the test. The clinical information of the participants was shown in Table 1.

Table 1. Baseline characteristics of CAD patients and healthy controls.

Variable	Controls (n = 10)	CAD patients (n = 10)	*p*-value
Age (years)	65.50 ± 2.83	65.70 ± 2.11	0.893
Sex (male, %)	70.00	70.00	
BMI (kg/m^2)	22.91 ± 0.48	22.55 ± 0.83	0.701
SBP (mmHg)	127.50 ± 3.90	139.20 ± 7.14	0.199
DBP (mmHg)	74.5 ± 4.11	82.40 ± 3.58	0.165
ALT (U/L)	28.44 ± 8.25	27.71 ± 4.05	0.935
CRE (μmol/L)	76.38 ± 4.41	81.45 ± 8.88	0.643
FBG (μmol/L)	5.76 ± 0.53	6.02 ± 0.51	0.731
TC (μmol/L)	5.67 ± 0.35	5.07 ± 0.37	0.007*
TG (μmol/L)	2.74 ± 1.09	1.09 ± 0.16	0.178
HDL (μmol/L)	1.26 ± 0.10	1.11 ± 0.05	0.039*
LDL (μmol/L)	3.36 ± 0.46	2.83 ± 0.33	0.012*

Data were expressed as the mean ± SEM. BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; ALT, Alanine transaminase; CRE, Creatinine; FBG, Fasting Blood Glucose; TC, Total cholesterol; TG, Triacylglycerol. HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.

2.2 Measurement of serum metabolomics

100 μL serum samples were accurately weighted and the metabolites were extracted with 400 μL internal standards (0.02 mg/mL L-2-chlorophenylalanin in 80% methanol solution). Then the samples were placed at –20 °C for 30 min to precipitate proteins. After centrifugation at 13,000 g for 15 min at 4 °C, the supernatants were carefully transferred to new sample vials for LC-MS/MS analysis. Chromatographic separation was performed using a Thermo UHPLC system equipped with an ACQUITY UPLC HSS T3 (100 mm × 2.1 mm i.d., 1.8 μm; Waters, Milford, USA). After preparation, the data were collected using a Thermo UHPLC-Q Extractive HF-X Mass Spectrometer equipped with and electrospray ionization source operating in either positive or negative ion mode. The raw data were first imported into the Progenesis QI 2.3 for peak detection and alignment. After normalization, orthogonal partial least squares discriminate analysis (OPLS-DA) performed to visualized the metabolic difference between groups. Variable importance in the projection (VIP) were calculated in OPLS-DA model. The different metabolites were defined with VIP value >1 and p value < 0.05.

2.3 Measurement of fecal SCFAs

Fresh fecal samples were collected and snap frozen in liquid nitrogen and then stored at –80 °C prior to analysis. Stool samples were mixed with ddH_2O, homogenized, centrifuged, and then filtered with a 0.22 μm filter. The extraction of SCFAs was performed as previously described [12]. Briefly, the volatile compounds, including d7-isobutyric acid (the internal standard), were acidified by adding to HCl, extracted with diethylether, and then analyzed using Agilent5977B gas chromatography (Agilent Tecnologies, Inc, Palo Alto, CA, USA). The SCFAs concentrations were calculated with internal standard method. The standard solution was prepared on the day of analysis. The correlation coefficients of the calibration curves were 0.9923–0.9976. Recovery tests were conducted and ranged from 99.38% to 100.36%.

2.4 Fecal sample collection and 16S rDNA gene sequencing

Fresh fecal samples were collected and snap frozen in liquid nitrogen and then stored at –80 °C prior to analysis. DNA was extracted from fecal material using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). DNA concentration and purity were monitored on 1% agarose gels. The V3-V4 region of the 16S rRNA gene were amplified. Paired-end reads were then performed using an Illumina MiSeq instrument according to the instructions (Illumina, San Diego, CA, USA). The paired-end sequences

with 97% similarity were then assigned to the same operational taxonomic units (OTUs). Chimeric sequences were identified and removed using UCHIME and Silva 16S rDNA database. α-Diversity was estimated to evaluate the complexity and diversity of samples . β-Diversity was performed using unweighted UniFrac and principle component analysis (PCA), which was based on a Bray-Curtis dissimilarity matrix.

2.5 Measurement of PAPP-A, STC2, IGFBP-4, IGF-1 and inflammatory cytokines in serum

The levels of PAPP-A, STC2, IGFBP-4, IGF-1, IL-6, hs-CRP, and TNF-α in serum were detected using commercial ELISA kits (Wuhan Huamei Bioengineering Co., Ltd, Wuhan, China), according to the standard protocols.

2.6 Statistical analysis

The normality of the data was checked by using the Shapiro-Wilk test and the results showed that variables were normally distributed. Therefore, student's *t*-test (SPSS 21 version, IBM Corp., Chicago, IL, USA) was used for comparing the data between the UAP patients and healthy controls according to a previous study [15]. Pearson correlation analysis between the altered fecal microbiota and serum PAPP-A, STC2, IGFBP-4, and IGF-1 were conducted using GraphPad Prism 7.0 (GraphPad software, San Diego, CA). Data were expressed as the mean $\pm$ SEM. A *p*-value less than 0.05 was considered significant.

3. Results

3.1 Baseline characteristics of participants

The baseline characteristics of the participants were shown in Table 1. The proportions of males in the UAP patients and healthy subjects were both 70%, with no statistical significance ($p > 0.05$). In addition, there was no significant difference in the baseline characteristics between the healthy controls and the UAP patients, such as BMI, ALT, CRE, FBG, TG, and cTnI levels ($p > 0.05$) (Table 1). There was significant difference in the TC, HDL, and LDL levels between the healthy controls and the UAP patients ($p < 0.05$).

3.2 Serum metabolomics

To identify the altered metabolites in patients with UAP, serum samples were examined for the initial untargeted metabolomics analysis. PCA showed that metabolic profiles of patients with UAP clustered distinctly from profiles among the healthy controls (Fig. 1A). As shown in Fig. 1B, 55 differential metabolites in the UAP patients were changed compared with the healthy individuals. Moreover, 24 out of the 55 metabolites were significantly increased, and 31 out of the 55 metabolites were significantly decreased in UAP patients (Fig. 1B). The data illustrated that the different metabolites involved in various pathways including linoleic acid metabolism, taurine and hypotaurine metabolism, D-glutamine and D-glutamate metabolism, arginine biosynthesis, histidine metabolism, alanine and aspartate and glutamate metabolism, arginine and proline metabolism, starch and sucrose metabolism, and citrate cycle (TCA cycle) (Fig. 1C).

3.3 UAP patients had lower fecal SCFAs

We further tested the gut microbiota-derived SCFAs levels in the stool. Due to the deficient samples, we could not add the test of fecal acetic acid concentration. A clinical study showed that the plasma acetic acid level in the patients with CAD was similar to the healthy controls, but the propionate tended to increase in patients with CAD [16]. Another study showed that the serum butyric acid level was significantly lower in the patients with CAD than the healthy controls [17]. Thus, we mainly focused on the fecal concentrations of butyric acid, propionate, and other SCFAs instead of acetic acid. As shown in Fig. 2, there was no significant difference in the concentrations of fecal propionic acid, butyrate acid, 3-hydrxytyric acid, propandioic acid, and crotonic acid ($p > 0.05$). However, the UAP patients had significantly lower levels of fecal 2-hydroxyisobutyric acid and succinic acid ($p < 0.05$).

3.4 UAP patients had altered fecal microbiota

The fecal microbiota was analyzed to study the relationship between the fecal microbiota composition and the development of UAP. α-Diversity were examined to detect the difference in microbial richness of the UAP patients and healthy controls. As shown in Fig. 3A, there was no significant difference in the α-diversity between the two groups ($p > 0.05$). The Venn diagram showed that there are 350 common OTUs between the healthy controls and UAP patients. Meantime, the healthy controls and UAP patients contained individual 267 and 198 OTUs, respectively (Fig. 3B). Furthermore, the result of PCA indicated that that the microbial community structure in the UAP patients was different from that in the healthy controls (Fig. 3C).

The overall microbial composition in the healthy controls and UAP patients differed at different levels. Fig. 4A showed that, at the phylum level, UAP patients had markedly increased relative abundances of *Bacteroidetes* ($p = 0.028$) and *Synergistetes* ($p = 0.005$). At the family level, the relative abundances of *Lactobacillaceae* ($p = 0.008$), *Burkholderiaceae* ($p = 0.032$), and *Synergistaceae* ($p = 0.036$) were significantly increased in the UAP patients than that in the control group (Fig. 4B). At the genus level, UAP patients had a significantly higher relative abundance of *Subdoligranulum* ($p = 0.015$) (Fig. 4C). Additionally, compared with control individuals, the relative abundances of *Bacteroides* ($p = 0.075$) and *Lactobacillus* ($p = 0.057$) tended to increase in the patients with UAP (Fig. 4C).

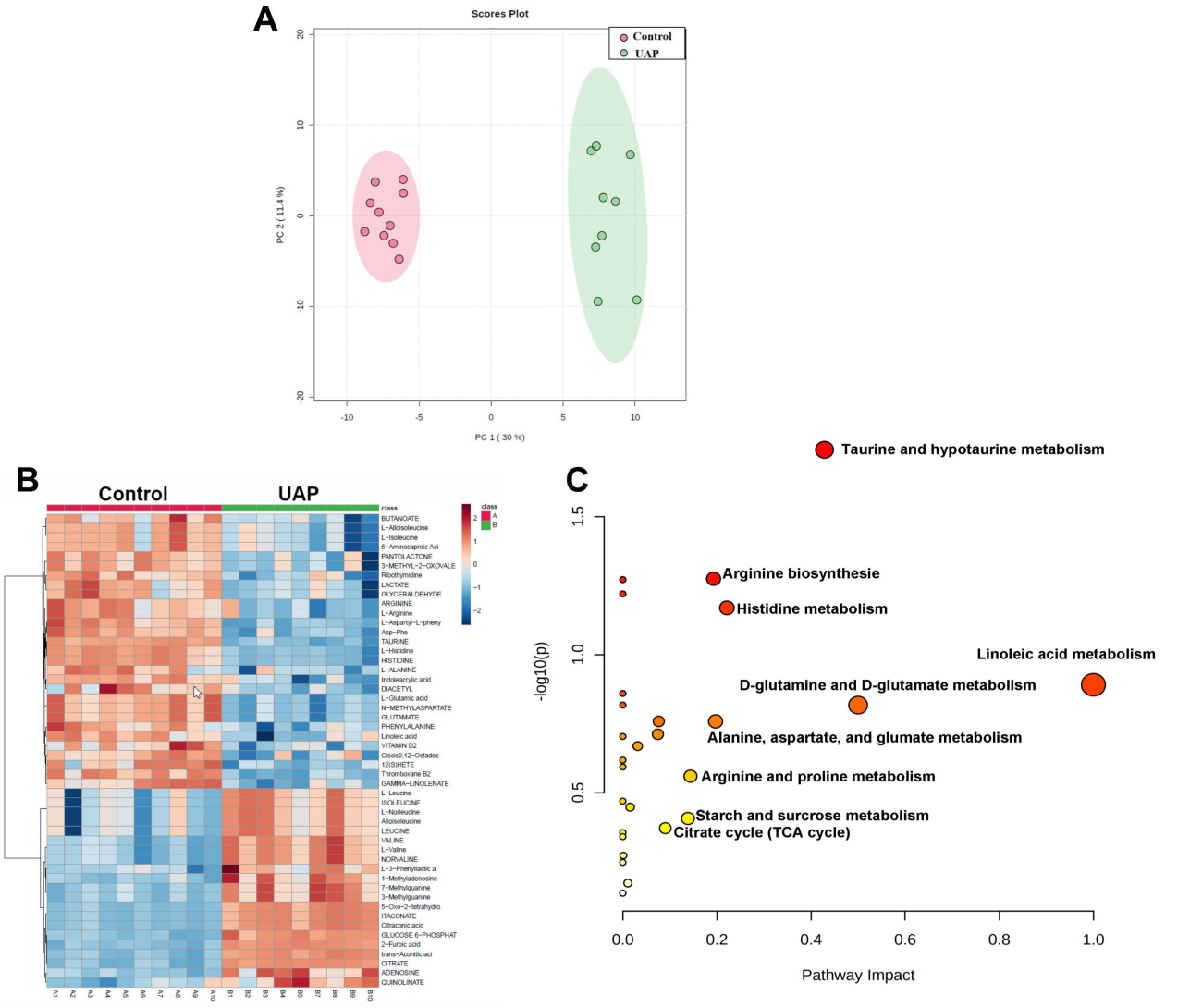

Fig. 1. Serum metabolomics changes. (A) PCA analysis on metabolites in serum recealed distinct metabolite composition between control samples and CAD patients. (B) Heat-map of the intensity of 55 significantly different metabolites showing significantly different metabolic profiles between control samples (n = 10) and CAD patients (n = 10). Each row represents data for a specific metabolite, and each column represents an individual (healthy control or CAD). Different colors correspond to the different intensity levels of metabolites: red represents high levels, and blue represents low levels. (C) The disturbed metabolic pathways showed differential metabolites changed in the CAD patients as compared with the healthy controls by MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Node radius was based on pathway impact values. Node color was based on *p* value.

3.5 UAP patients had lower serum IGF-1 level

The levels of serum PAPP-A, STC2, IGFBP-4, and IGF-1 were tested using ELISA assay. The serum PAPP-A level tended to decrease in the UAP patients when compared to the healthy controls, although the difference was not significant ($p = 0.072$) (Fig. 5A). However, the UAP patients had lower levels of serum STC2 ($p = 0.021$), IGFBP-4 ($p = 0.048$), and IGF-1 than the healthy controls ($p = 0.045$) (Fig. 5B–D).

3.6 UAP patients had higher serum levels of inflammatory cytokines

Serum hs-CRP has been shown to be associated with the incidence of CAD. Thus, in this study, we also tested the serum levels of IL-6, hs-CRP, and TNF-α. As shown in Fig. 6A–C, UAP patients had higher serum IL-6 ($p = 0.800$), hs-CRP ($p = 0.728$), and TNF-α ($p = 0.088$) concentrations than the healthy controls (Fig. 6C).

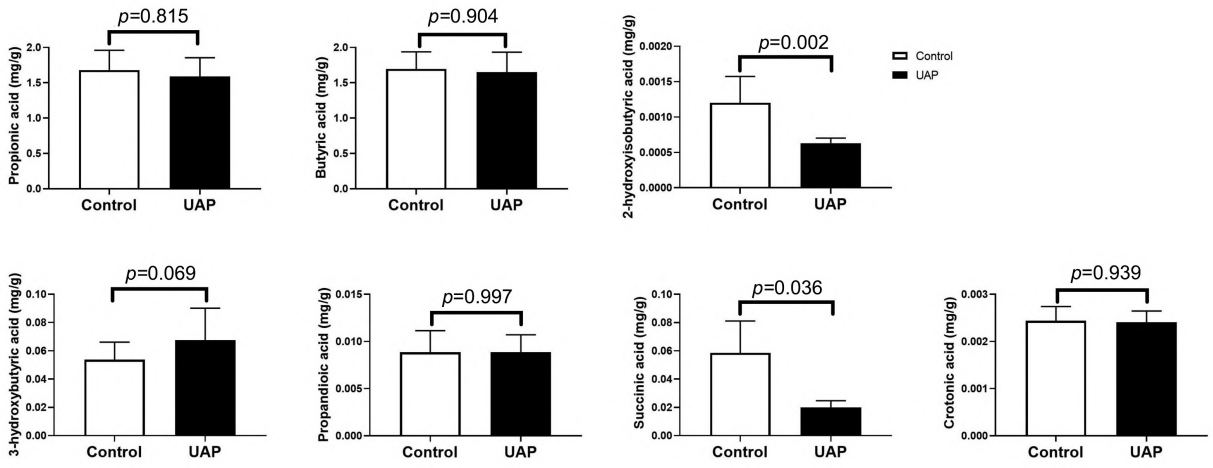

Fig. 2. Fecal SCFAs concentration. Data were expressed as the mean ± SEM.

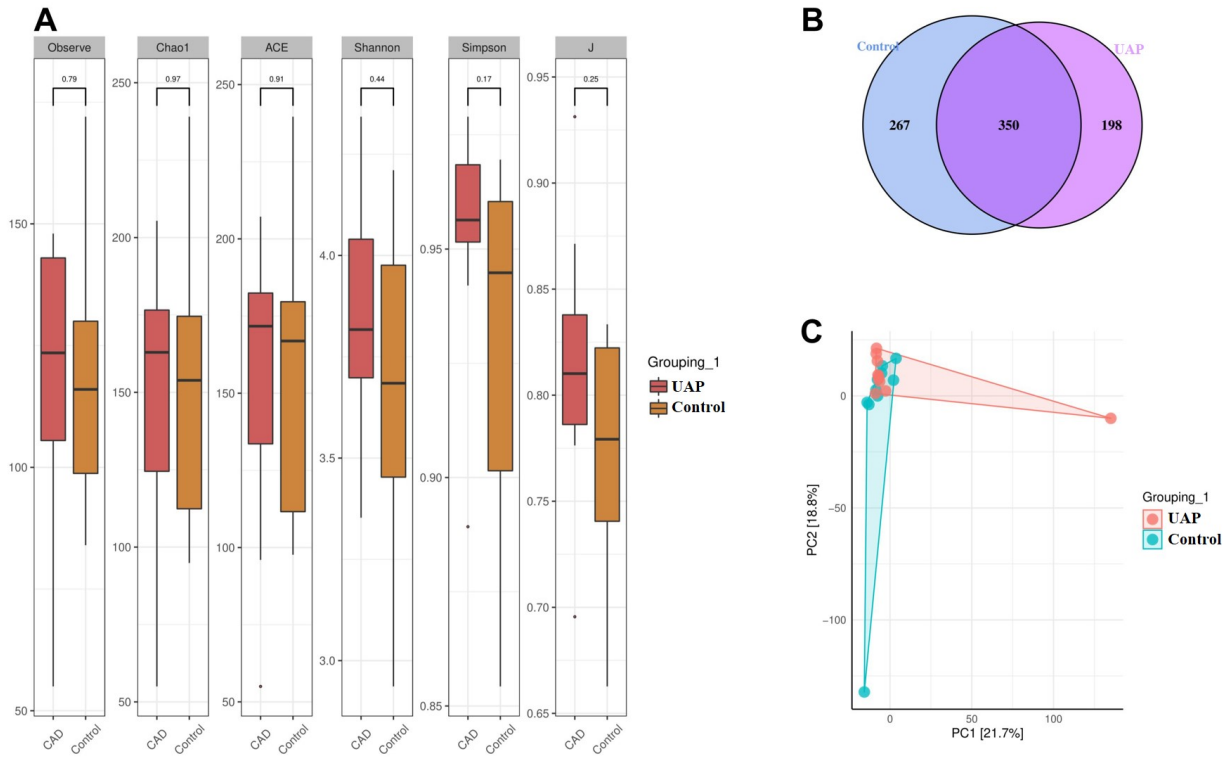

Fig. 3. Fecal microbial diversity. (A) α-diversity. (B) Venn diagram. (C) PCoA. Data were expressed as the mean ± SEM.

3.7 The association analysis between altered fecal microbiota, fecal SCFAs, serum parameters, and serum metabolites

To identify the association between fecal microbiota and serum IGF-1 system, we performed correlation analysis using the altered fecal microbiota and serum PAPP-A, STC2, IGFBP-4, and IGF-1 levels. As shown in Fig. 7A, there was a negative correlation between the relative abundance of *Bacteroidetes* and serum IGFBP-4 level ($p = 0.01$). Additionally, there was a negative correlation between the relative abundance of *Lactobacillaceae* and *Lactobacillus* and serum levels of histidine ($p < 0.05$). Fig. 7B showed that serum IGF-1was negatively correlated with serum valine, but positively correlated with serum glutamate, arginine, histidine, and succinic acid ($p < 0.05$). There was also a positive correlation between serum IL-6 and valine ($p < 0.05$) (Fig. 7C).

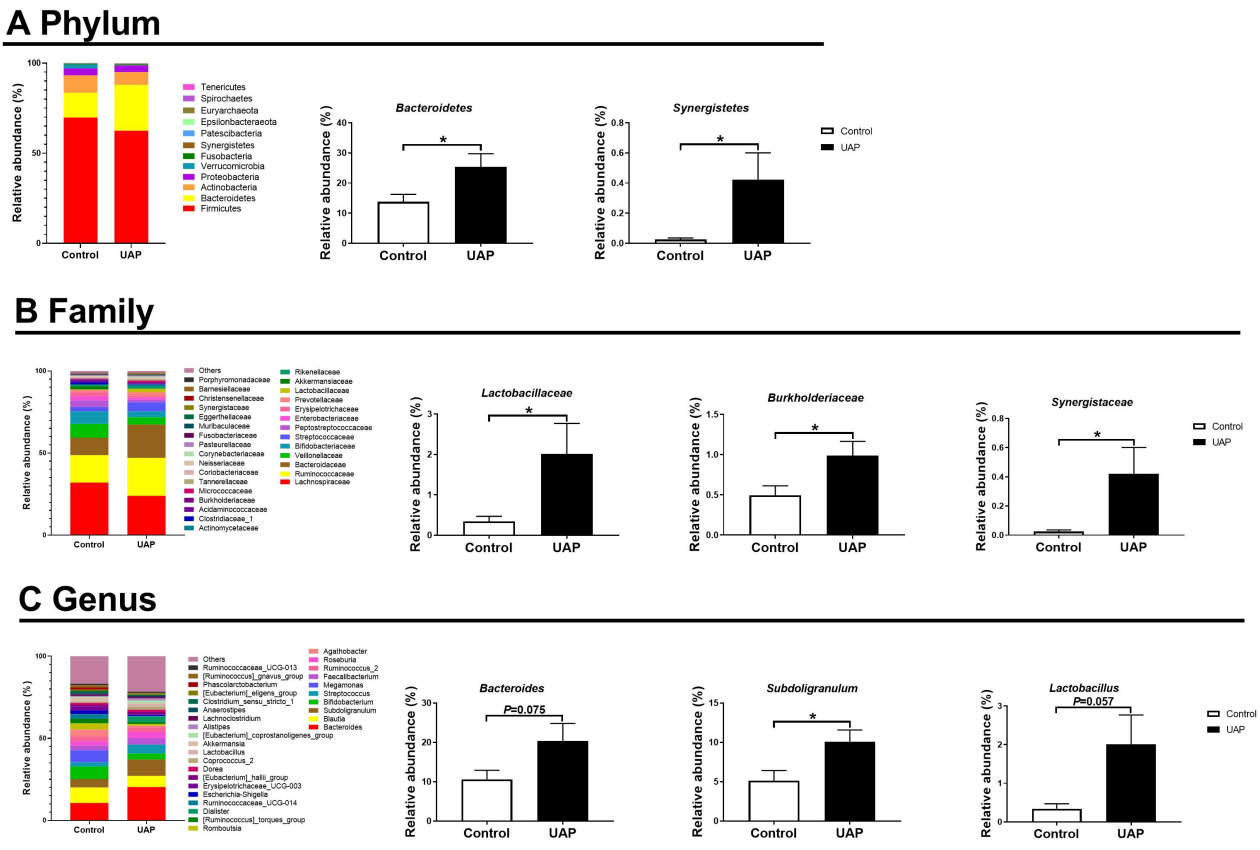

Fig. 4. Fecal microbial composition. (A) Phylum level. (B) Family level. (C) Genus level. Data were expressed as the mean ± SEM. *$p < 0.05$.

4. Discussion

It has been reported that CAD, a major cause of mortality and morbidity, has increased prevalence worldwide. UAP is a major type of CAD, which contains a series of acute coronary syndrome (ACS) and may cause acute myocardial infraction (AMI) and even sudden death [1]. Thus, it is urgent to identify the factors that contribute to the incidence and development of UAP in order to find efficient therapeutic targets for preventing and treating UAP. In this study, we have demonstrated that UAP patients have inhibited circulating IGF-1 level and altered serum metabolomics, which may be associated with the imbalanced serum metabolome induced by gut microbial dysbiosis.

Serum metabolomics provides a promising method to find cardiovascular biomarkers. However, little is known about the relationship between the metabolomic profiles and UAP development. In this study, we found that the serum metabolites involved in amino acids metabolism (glutamate, arginine, and histidine) were decreased in the UAP patients. In contrast, patients with UAP had increased levels of serum metabolites involved in starch and sucrose (glucose 6-phosphate) and the TCA cycle (citrate), which might be associated with the damaged mitochondrial energy metabolism [18,19]. Studies have shown that amino acids are precursors of glucose and fatty acids metabolism and are involved in the TCA cycle, which are associated with cardiovascular diseases [20]. For example, arginine exhibits antiatherogenic effects through modulating endothelial cell homeostasis in the development of cardiovascular diseases [21]. In line with this, in this study, we found that CAD patients had lower serum level of arginine. We hypothesized that, in this study, the decreased level of arginine might be due to the lower circulating glutamate level in the CAD patients, since arginine is produced by glutamate metabolism. Additionally, in this study, we found that CAD patients had lower serum levels of histidine. Glutamate exhibits various physiological functions, such as alleviating obesity and cardiovascular disease [22]. Studies have shown that patients with cardiovascular diseases have lower levels of glutamate and histidine [23,24]. However, the studies demonstrating the association between the glutamate level and cardiovascular diseases were inconsistent [25,26]. It has been shown that glutamate is a nonessential amino acid and synthesized by branch-chain amino acids (BCAAs), including valine, leucine, and isoleucine. In this study, the circulating levels of BCAAs were increased in the CAD patients, which may because of lower production

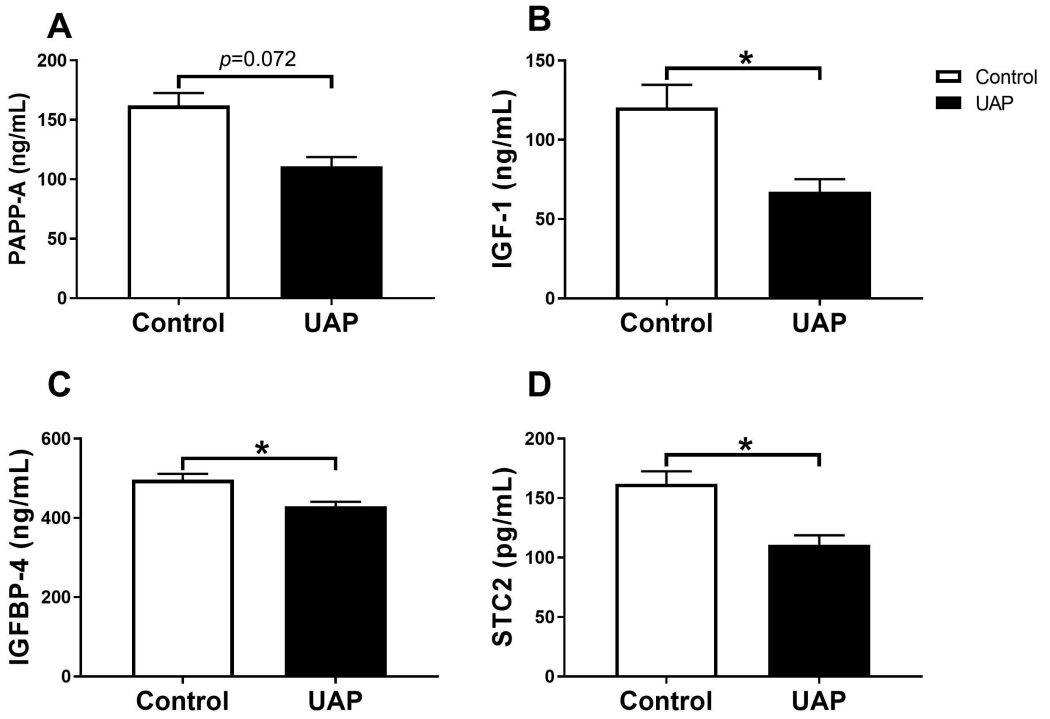

Fig. 5. The levels of serum PAPP-A (A), IGF-1 (B), IGFBP-4 (C), and STC2 (D). Data were expressed as the mean ± SEM. *$p <$ 0.05.

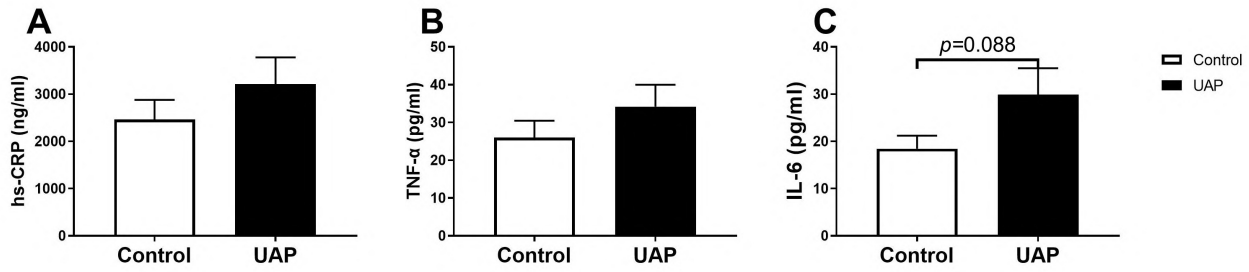

Fig. 6. The levels of serum hs-CRP (A), TNF-α (B), and IL-6 (C). Data were expressed as the mean ± SEM.

of glutamate. Consistently, clinical studies also showed that there was a positive relationship between the circulating levels of BCAAs and the incidence of CAD [20,24]. As for the mechanism, Excessive BCAAs can cause insulin resistance and lead to the development of diabetes, which strongly contribute to the incidence of CAD [25]. Collectively, these data demonstrated that CAD patients had imbalanced amino acids metabolism. Moreover, these conflicting results call for further precise studies to explore the association between amino acids metabolism and the risk of CAD. In addition to amino acids metabolism, studies also revealed a positive relationship between the incidence of cardiovascular diseases and elevated glucose metabolism and TCA cycle [18]. The TCA cycle plays a central role in regulating mitochondrial energy metabolism [26,27]. In this study, we also found the CAD patients had higher level of serum citrate, an important intermediates of TCA cycle. In the CAD, the absence of oxygen can inhibit the TCA cycle. Thus, we speculated that the elevation of citrate may be due to the down-regulation of enzymes related to the TCAS cycle, such as aconitase that involve in the transformation of citrate, however, this needs further more investigations.

Amounting studies have shown that SCFAs, the metabolites derived from gut microbiota, not only play an important role in providing energy but also act as signaling molecules to modulate host physiological function. Furthermore, there is a strong association between SCFAs and cardiovascular diseases [27]. Additionally, a study found that treatment with SCFAs increased serum IGF-1 level in antibiotic-exposed mice, demonstrating the role of SCFAs

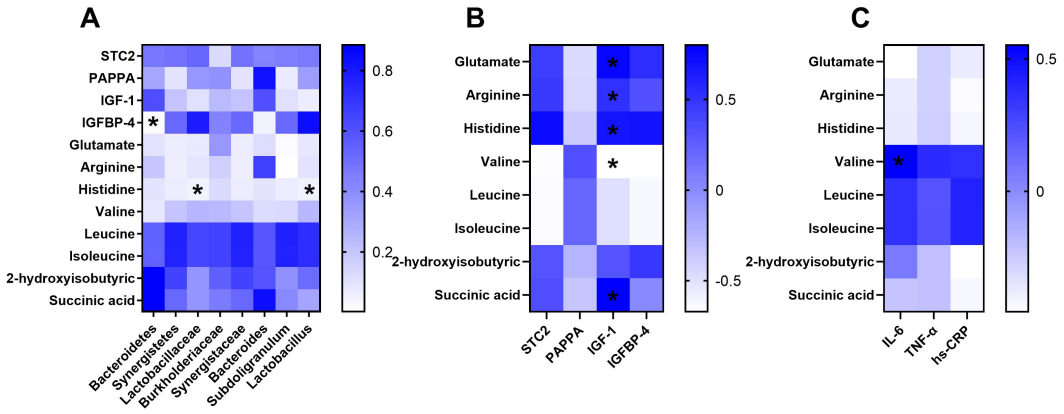

Fig. 7. Pearson correlation analyses between the altered fecal microbiota, fecal SCFAs, serum parameters, and serum metabolites. *$p < 0.05$. (A) A negative correlation between the relative abundance of Bacteroidetes and serum IGFBP-4 level ($p = 0.01$). Additionally, there was a negative correlation between the relative abundance of Lactobacillaceae and Lactobacillus and serum levels of histidine ($p < 0.05$). (B) Serum IGF-1 was negatively correlated with serum valine, but positively correlated with serum glutamate, arginine, histidine, and succinic acid ($p < 0.05$). (C) There was also a positive correlation between serum IL-6 and valine ($p < 0.05$).

in the IGF-1 signaling pathway [12,13]. In this study, the results showed that the UAP patients had lower levels of fecal 2-hydroxyisobutyric acid and succinic acid. Similarly, another study showed that the CAD patients exhibited lower serum concentrations of fatty acids, such as 2-OH-butyric acid and 3-OH-butyric acid [17]. Thus, we speculated that gut-derived SCFAs might be involved in gut microbiota-mediated UAP development.

The gut microbiota and its metabolites have been reported to be associated with various cardiovascular diseases, including CAD and UAP [28–32]. In this study, we found that the relative abundance of *Bacteroidetes* increased in the UAP patients, which was consistent with a recent study [33]. It has been shown that some bacteria belonging to *Bacteroidetes* are involved in the biosynthesis and degradation of amino acids [34], suggesting that the imbalanced amino acids metabolism in the UAP patience might be because of the higher abundance of *Bacteroidetes*. Additionally, the Pearson correlation analysis showed that there was a negative correlation between the relative abundance of *Bacteroidetes* and serum IGFBP-4 level, suggesting that the *Bacteroidetes* associated with the IGF-1system and even the development of UAP. Besides, in this study, we found that the UAP patients had increased relative abundance of *Synergistetes,* which has been shown to be related to periodontal diseases. Like the fact that patients with periodontal diseases are at a high risk of developing cardiovascular diseases, it is reasonable to hypothesize that the elevated abundance of *Synergistetes* is associated with the development of UAP. Additionally, patients with UAP had higher abundance of *Lactobacillaceae*, which was significantly positively correlated with the serum histidine. This may be because *Lactobacillaceae* was involved in the metabolism and absorption of histidine [35,36]. Similarly, another clinical study also demonstrated that the CAD patients had increased *Lactobacillales* abundance [37]. Moreover, patients with UAP had increased levels of *Burkholderiaceae* and *Subdoligranulum*. Consistently, previous studies also found that *Subdoligranulum* genus is strongly associated with the host energy metabolism and increases the incidence of metabolism-related diseases including cardiovascular diseases [38–41]. However, another study showed that there was a negative association between the abundance of *Burkholderiaceae* and the risk of atherosclerosis [42]. Collectively, alterations of gut microbiota found in the present study were strongly associated with the host energy and amino acids metabolism, which may collectively contribute to the development of UAP.

Numerous clinical studies have shown that CAD patients have decreased circulating IGF-1 levels [2,3]. Additionally, IGFBP-4 can inhibit the activation of IGF-1 by binding with IGF-1. Inconsistent with this, our study found that the serum IGF-1 and IGFBP-4 levels were lower in the UAP patients than the healthy controls. Nevertheless, amounting studies have shown that the effects of IGFBP-4 have inconsistent or even contrary effects on CAD development [43–45]. Thus, the intricate relationship between the circulating levels of IGFBP-4 and IGF-1 and UAP needs further investigation. Moreover, STC2 acts as an inhibitor of PAPP-A and thus inhibits IGF-1-related signaling. However, in this study, we found that the UAP patients had lower serum STC2 levels, which was consistent with another study showing that increased STCT2 inhibited the development of atherosclerotic lesions [45]. Despite the conflicting results, this study adds to mounting evidence linking the imbalanced IGF-1 system to UAP. Moreover, inflammation is involved in acute syndromes [46]. It has been recently reported that the UAP patients have higher

serum levels of hs-CRP [47]. In this study, the UAP patients had higher serum levels of IL-6, hs-CRP, and TNF-α than the healthy controls. Consistently, another clinical study also showed that the serum IL-6 level was higher in the UAP patients [48]. These data confirmed that UAP patients had higher levels of serum inflammatory cytokines. Furthermore, there were significant correlations between the serum levels of amino acids and serum parameters related to the IGF-1 system and IL-6, suggesting that the imbalanced amino acids metabolism was strongly associated with the development of UAP.

5. Conclusions

In conclusion, the results of the current study suggested that the UAP patients had decreased serum IGF-1 level, increased serum inflammatory cytokines, imbalanced amino acids metabolism, and altered gut microbiota. Additionally, the imbalanced amino acids metabolism may be associated with the altered gut microbiota in the UAP patients. Thus, we hypothesized that the related gut microbiota and serum metabolites may have the potential for the prediction of UAP. Meawhile, the identified gut microbiota and serum metabolites in this study may provide novel potential strategies for alleviating UAP in clinical settings.

However, there were several limitations to this study. First, the samples of UAP patients were only obtained from 10 individuals, which is too small to minimize the experimental bias and make a subgroup comparison. Second, the study only analyzed the fecal microbial composition, which may not be completely representative of the entire gastrointestinal microbiome. Third, untargeted metabolomics is less accurate to annotate serum metabolomics. Fourth, the causal relationship between UAP, gut microbiota, and serum metabolites remains unclear and need further investigation. Given the limitations, studies with more individuals are needed to confirm the results of this study.

Author contributions

LL conducted the designed, experiments, data analysis, and drafted the manuscript; FL contributed to conception, design, and critically revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethic Committee of Xiangya Hospital Central South University (201803209). The research was conducted according to the World Medical Association Declaration of Helsinki. All the information about the study will be fully explained to the subjects by the researchers. All the participants provided informed consent before sampling.

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Conflict of interest

The authors declare no conflict of interest.

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