ORIGINAL ARTICLE

Phenylacetylglutamine as a risk factor and prognostic indicator of heart failure

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Abstract

Aims   To explore the associations between serum phenylacetylglutamine (PAGln) and chronic heart failure (HF).
Methods and results   Totally 956 subjects were enrolled consecutively from the Department of Cardiovascular Medicine, Ruijin Hospital. Baseline data were obtained from all participants, and 471 stable chronic HF subjects were followed up. Serum PAGln was analysed by liquid chromatography–tandem mass spectrometry. The association between PAGln and basic renal indicators was assessed by simple correlation analysis. Logistic regression analysis was conducted to measure the association between PAGln and HF risk. Event-free survival was determined by Kaplan–Meier curves, and differences in survival were assessed using log-rank tests. Cox proportional hazards analysis was used to assess the prognostic value of PAGln in HF. Serum PAGln levels were increased in patients with chronic HF (3.322 ± 8.220 μM vs. 1.249 ± 1.168 μM, *P* < 0.001) and were associated with HF after full adjustment [odds ratio (OR), 1.507; 95% confidence interval (CI): 1.213–1.873; *P* < 0.001]. PAGln levels were correlated with the levels of basic renal indicators. High PAGln levels indicated a high risk of renal dysfunction in HF (OR: 1.853; 95% CI: 1.344–2.556; *P* < 0.001), and elevated PAGln levels were associated with a high risk of cardiovascular death in patients with chronic HF (HR: 2.049; 95% CI: 1.042–4.029; *P* = 0.038).
Conclusions   Elevated PAGln levels are an independent risk factor for HF and are associated with a higher risk of cardiovascular death. High PAGln levels could indicate renal dysfunction in HF patients. PAGln can be a valuable indicator of HF.

Introduction

Heart failure (HF) is the detrimental end stage of the course of cardiovascular disease. Despite advances in medications and treatment strategies for HF, it continues to affect 23 million worldwide and is considered a major public health issue.[1] The pathogenesis of HF is very complex, at least including metabolic abnormalities, inflammation, immune system activation, and gut microbiota imbalance.[2–5] Biomarker-guided therapy strategies have brought a new dimension to HF management. However, existing HF biomarkers including *N*-terminal pro-B-type natriuretic peptide (NT-proBNP) and soluble ST2 as well as galectin-3 are inadequate in clinical practice to manage HF patients.[6] Therefore,

new indicators in different pathophysiologic processes need to be identified.

As an important pathophysiology change in HF, gut microbiota is a potential therapeutic target for HF.[7,8] By generating bioactive metabolites, gut microbiota functions like an endocrine organ that affects host physiology directly or indirectly.[7] Recent studies on the gut–heart axis described the links between gut microbiota metabolites and cardiovascular diseases.[9–14] One of the promising metabolites that have the potential to play an important role in cardiovascular diseases is phenylacetylglutamine (PAGln). PAGln derived from the phenylalanine metabolism through gut microbiota and human liver.[15] Studies have identified it as a cardiovascular disease-related molecule[15–19] and suggest that it functions

by activating adrenergic receptors.[15] A previous study demonstrated that PAGln was an independent risk factor for incident HF in African Americans,[13] whereas another study found it useful for identifying patients at high risk of HF-related events after acute uncompensated HF.[14]

To date, the association between PAGln and HF has not been fully clarified. We designed this study to further investigate the relationship between PAGln and chronic HF, searching for a potential biomarker of gut microbiota dysfunction in HF.

Methods

Study design and population

Two separate sets of analyses were designed in this study. The cross-sectional analysis was conducted on all 956 participants to examine the association of serum PAGln levels and the presence of HF, whereas prospective analysis was performed on 471 stable chronic HF patients to access the prognostic value of PAGln in HF.

Overall, 956 inpatients aged 18 years or older were consecutively enrolled in this study from the Department of Cardiovascular Medicine, Shanghai Jiao Tong University-Affiliated Ruijin Hospital (Shanghai, China). Those with infections, malignant tumours, acute myocardial infarction and autoimmune diseases, those who had undergone renal replacement therapy, those who had used antibiotics within 4 weeks before enrolment and those who had undergone open-heart surgery within 4 weeks before enrolment were excluded. Because PAGln levels can be elevated by phenylketonuria,[20] our patients were also excluded from this condition. HF was defined as cardiac systolic dysfunction with left ventricular ejection fraction (LVEF) $\leq$ 50%. Renal dysfunction was defined as estimated glomerular filtration rate (eGFR) $\leq$ 60 mL/min/1.73 m^2. eGFR was calculated by age, sex and creatine.[21]

This study was approved by the institutional review committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine (Shanghai, China) and was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all participants before enrolment.

Follow-up and clinical endpoints

Of the 956 participants enrolled, 471 were stable chronic HF patients and were followed up by telephonic interviews to collect data on survival, HF rehospitalizations and other adverse events. The mean follow-up time was 1.98 ± 1.09 years. Subsequently, six patients were lost to follow-up.

The primary endpoint of this study was a composite of cardiovascular death and the first HF rehospitalization.

Data collection

Baseline data were recorded via face-to-face interviews with an experienced physician. Laboratory tests were routinely performed during the subjects' hospitalization. Peripheral intravenous blood samples were collected in pre-chilled tubes. Serum was separated via centrifugation at 1500 *g* for 30 min at 4°C and stored at −80°C before testing.

Echocardiography

All enrolled subjects underwent transthoracic echocardiography, which was conducted by an experienced ultrasonographer with the patient in the left decubitus position. The ultrasound probe was placed at the left margin of the sternum in the anterior region of the heart between the 2nd and 5th rib. By M-mode echocardiography, the left ventricular end-diastolic diameter, left atrial diameter, aortic dimensions, interventricular septal thickness and left ventricular posterior wall thickness were measured. Simpson's biplane method in two-dimensional apical four-chamber views was used to measure the LVEF. Echocardiography was conducted within a week following the collection of venous blood samples.

Statistical analysis

Continuous variables are presented as means ± SD if normally distributed, whereas log transformations were performed to normalize non-normally distributed variables. Categorical data were summarized as proportions and frequencies. Independent Student's *t*-test or one-way analysis of variance (ANOVA) was conducted to compare means between or among groups. Chi-squared tests were used to compare categorical variables. Simple correlation analysis was used to evaluate the association between PAGln levels and traditional renal indicators. Logistic regression analysis was conducted to measure odds ratios (ORs) with 95% confidence intervals (CIs) between PAGln levels and the risk of HF. Log-transformed PAGln was analysed as a continuous variable, whereas the PAGln tertiles were analysed as classification variables. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, body mass index, presence of hypertension and diabetes mellitus and levels of haemoglobin, albumin, low-density lipoprotein cholesterol, HbA1c, high-sensitivity C-reactive protein and NT-proBNP.

Event-free survival was determined using Kaplan–Meier curves, and differences in survival across PAGln tertiles were

assessed using the log-rank test. Cox proportional hazards analysis was used to estimate hazard ratios (HRs) with 95% CIs and to assess whether PAGln is a prognostic indicator of HF. The area under the receiver operating characteristic (ROC) curve was applied to determine the predictive value of PAGln for adverse events in HF, and the cut-off value was determined by the highest Youden index in the ROC curve.

All statistical analyses were performed using SPSS software (Version 22.0; SPSS, Inc., Chicago, IL, USA). A two-tailed *P*-value of <0.05 was considered statistically significant for all the models.

Detection of serum PAGln

PAGln levels were detected using liquid chromatography–tandem mass spectrometry (LC-MS). Isotopically labelled phenylacetylglutamine-d5 (PAGln-d5, HY-W050026s, MedChemExpress, Monmouth Jn, NJ, USA) was used as internal standard (IS). Before testing, 50 μL of serum and 400 μL of IS diluent (PAGln-d5 25 ng/mL) were added to each well of the 96-well plate, which was swirled and mixed for 15 min followed by centrifugation at 4°C at 2500 *g* for 15 min; then, 150 μL of supernatant from each well was added to a new 96-well plate. Mass spectrometry analysis identified serum

Table 1 Baseline characteristics of all subjects according to tertiles of plasma PAGln

	PAGln < 0.79 μM (n = 318)	0.79 μM ≤ PAGln<1.87 μM (n = 319)	PAGln ≥ 1.87 μM (n = 319)	P value
Demographic characteristics				
Age (years)	57.040 ± 10.594	61.450 ± 9.734	65.580 ± 10.785	<0.001
Male	182 (57.2)	193 (60.5)	225 (70.5)	0.001
Current smoking	85 (26.7)	106 (33.2)	123 (38.6)	0.006
Current drinking	67 (21.1)	72 (22.6)	72 (22.6)	0.870
Body mass index (kg/m²)	25.050 ± 3.527	24.909 ± 3.673	24.522 ± 3.695	0.164
Systolic blood pressure (mmHg)	131.840 ± 19.316	130.220 ± 20.695	131.610 ± 19.447	0.536
Diastolic blood pressure (mmHg)	77.100 ± 11.581	76.450 ± 13.248	75.270 ± 13.160	0.184
Heart rate (beats/min)	79.980 ± 12.653	79.150 ± 14.200	79.250 ± 13.265	0.694
Family history	42 (13.2)	53 (16.6)	43 (13.5)	0.396
Medical history				
Hypertension	163 (51.3)	169 (53.0)	198 (62.1)	0.013
Diabetes mellitus	57 (17.9)	69 (21.6)	114 (35.7)	<0.001
Dyslipidaemia	44 (13.8)	57 (17.9)	46 (14.4)	0.313
Renal dysfunction	23 (7.2)	30 (9.4)	100 (31.4)	<0.001
Stroke	20 (6.3)	22 (6.9)	42 (13.2)	0.003
Lab. examination				
WBC (*10⁹/L)	6.348 ± 1.967	6.427 ± 1.944	6.445 ± 2.059	0.806
Haemoglobin (g/L)	139.469 ± 15.667	137.561 ± 15.184	132.680 ± 18.733	<0.001
Platelet (*109/L)	191.199 ± 55.211	185.574 ± 48.112	178.947 ± 54.718	0.014
HbA1c (%)	5.996 ± 1.021	6.096 ± 0.988	6.411 ± 1.151	<0.001
ALT (IU/L)	33.673 ± 74.906	34.279 ± 71.419	27.003 ± 37.568	0.277
Albumin (g/L)	39.572 ± 3.827	38.746 ± 3.806	37.840 ± 4.457	<0.001
Creatinine (μmol/L)	75.912 ± 27.772	80.119 ± 31.896	114.151 ± 118.663	<0.001
Uric acid (μmol/L)	354.975 ± 110.506	357.426 ± 107.902	387.088 ± 121.558	<0.001
eGFR (mL/min/1.73 m²)	87.211 ± 19.442	81.057 ± 16.729	70.144 ± 24.793	<0.001
Triglyceride (mmol/L)	1.675 ± 1.375	1.463 ± 0.765	1.532 ± 1.061	0.046
Total cholesterol (mmol/L)	4.332 ± 1.807	4.293 ± 1.532	3.946 ± 1.125	0.002
LDL-C (mmol/L)	2.523 ± 0.863	2.572 ± 0.923	2.362 ± 0.928	0.009
HDL-C (mmol/L)	1.190 ± 0.306	1.171 ± 0.307	1.116 ± 0.285	0.006
Troponin I (ng/mL)	0.667 ± 4.861	0.912 ± 6.161	1.525 ± 8.287	0.240
NT-proBNP (pg/mL)	891.990 ± 3131.548	1310.933 ± 3303.611	3418.032 ± 7345.832	<0.001
D-dimer (mg/L)	0.500 ± 0.958	0.657 ± 1.474	0.732 ± 1.421	0.075
LAD (mm)	39.170 ± 6.197	40.113 ± 7.484	42.332 ± 6.903	<0.001
LVEDD (mm)	52.890 ± 9.215	53.994 ± 9.547	56.599 ± 9.813	<0.001
LVESD (mm)	36.928 ± 11.651	38.745 ± 12.239	42.320 ± 11.728	<0.001
LVEF (%)	56.651 ± 15.990	53.483 ± 17.356	47.326 ± 15.700	<0.001
Medications				
ACEI/ARB/ARNI	152 (47.8)	170 (53.3)	204 (64.0)	<0.001
β-Blocker	183 (57.6)	189 (59.3)	228 (71.5)	<0.001
Spironolactone	62 (19.5)	80 (25.1)	126 (39.5)	<0.001
Statins	220 (69.2)	244 (76.5)	247 (77.4)	0.033
Hypoglycaemic drugs	43 (13.5)	52 (16.3)	90 (28.2)	<0.001

ACEI, angiotensin-converting enzyme inhibitors; ALT, glutamic-pyruvic transaminase; ARB, angiotensin receptor blockers; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; LAD, left atrial diameter; LDL-C, low-density lipoprotein cholesterol; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end systolic diameter; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; WBC, white blood cell.

PAGln at a retention time of 1.37 min and m/z = 265. The peak area of PAGln was corrected by the peak area of the IS, and the concentration of PAGln in the serum samples was calculated semi-quantitatively using the ratio of the peak area of each analyte to that of the IS compound.

Results

Cross-sectional study

Totally, 956 subjects were enrolled and divided into roughly equal three groups according to the tertiles of serum PAGln levels. The baseline data of the subjects are shown in *Table 1*. Of all the 956 subjects, 485 (50.7%) were not meet the diagnosis of HF, 600 (62.8%) were men, 530 (55.4%) had hypertension, 240 (25.1%) had diabetes mellitus, and 147 (15.4%) had dyslipidaemia. As the PAGln tertiles increased, the number of subjects who had hypertension, diabetes mellitus, renal dysfunction or stroke was significantly higher. The laboratory examination results showed that subjects in higher tertiles of PAGln levels tend to have worse renal function and heart function.

To further explore the relationship between PAGln and HF, subjects were grouped according to the presence of HF or not. We found that serum PAGln levels were significantly higher in the HF group than in the non-HF group (*Figure 1A*; 3.322 ± 8.220 vs. 1.249 ± 1.168 μM respectively, $P < 0.001$). Logistic regression analysis of the relationship between serum PAGln levels and the occurrence of HF showed that the risk of HF increased by 50.7% per 1-SD increase in PAGln levels after full adjustment (OR, 1.507; 95% CI: 1.213–1.873; $P < 0.001$). Compared with the lowest tertile, the risk of HF in the highest tertile increased by 126.2% (OR: 2.262; 95% CI: 1.413–3.620; $P = 0.001$) (*Table 2*). These results suggested that PAGln is an independent risk factor for HF.

Furthermore, in HF subjects, we found that serum PAGln levels were significantly increased in subjects with renal dysfunction than those without (*Figure 1B*; 6.892 ± 14.695 vs. 1.918 ± 1.626 μM, $P < 0.001$). As shown in *Figure 2*, PAGln levels showed a remarkable correlation with creatinine ($r = 0.765$, $P < 0.001$), blood urea nitrogen (BUN, $r = 0.478$, $P < 0.001$), eGFR ($r = 0.610$, $P < 0.001$) and cystatin C ($r = 0.661$, $P < 0.001$). We then conducted the logistic regression analysis of serum PAGln levels and the occurrence of renal dysfunction in patients with HF. After full adjustment, the risk of renal dysfunction in HF increased by 85.3% per 1-SD increase of PAGln (OR: 1.853; 95% CI: 1.344–2.556; $P < 0.001$). Subjects in the highest tertile had a 148.6% increased risk of renal insufficiency compared with those in the lowest tertile (OR: 2.486; 95% CI: 1.254–4.930; $P = 0.009$; *Table 3*).

Figure 1 Phenylacetylglutamine (PAGln) levels in different groups. (A) PAGln levels were increased in patients with heart failure (HF). (B) PAGln levels were increased in HF patients with renal dysfunction.
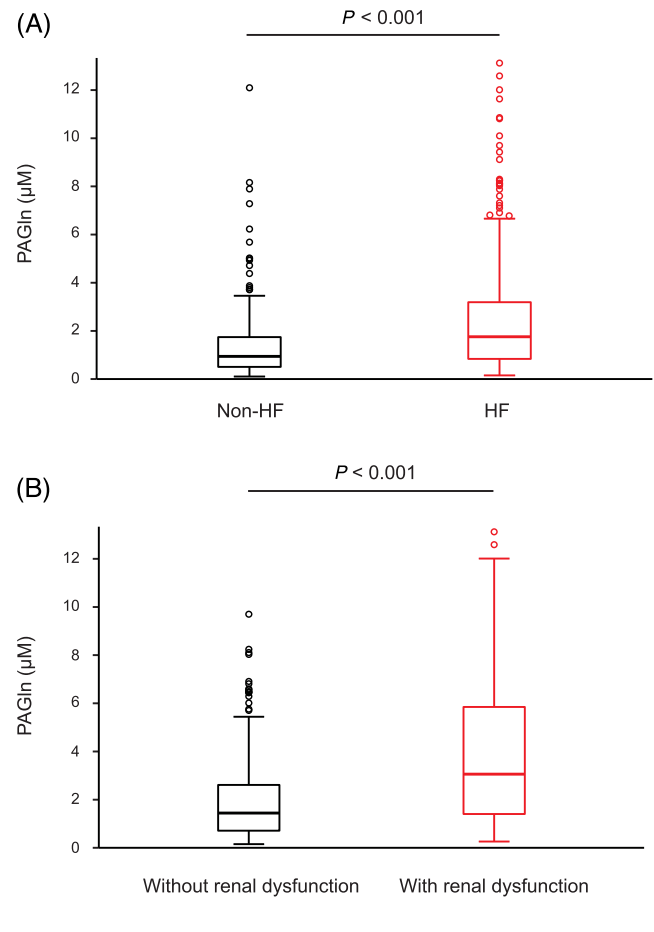
Because renal dysfunction is a common complication of HF and significantly increases PAGln levels,[22] we further performed the logistic regression analysis on patients with HF but without renal dysfunction to exclude the influence of renal dysfunction. After adjusted for age and sex, PAGln remained a strong risk factor for HF (OR: 1.815; 95% CI: 1.527–2.158; $P < 0.001$; Supporting Information, *Table S1*).

Prospective study

To explore the prognostic value of PAGln in HF, we followed up with the 471 stable chronic HF patients for a mean of 1.98 ± 1.09 years after their discharge. We chose a composite of cardiovascular death and the first HF rehospitalization as our primary endpoint. Of our 471 HF patients, 154 met the primary endpoint events, including 57 who had cardiovascular death and 111 who had HF rehospitalization. We found that patients who met the primary endpoint or only met

Table 2 Serum PAGln levels were associated with the presence of HF in all subjects
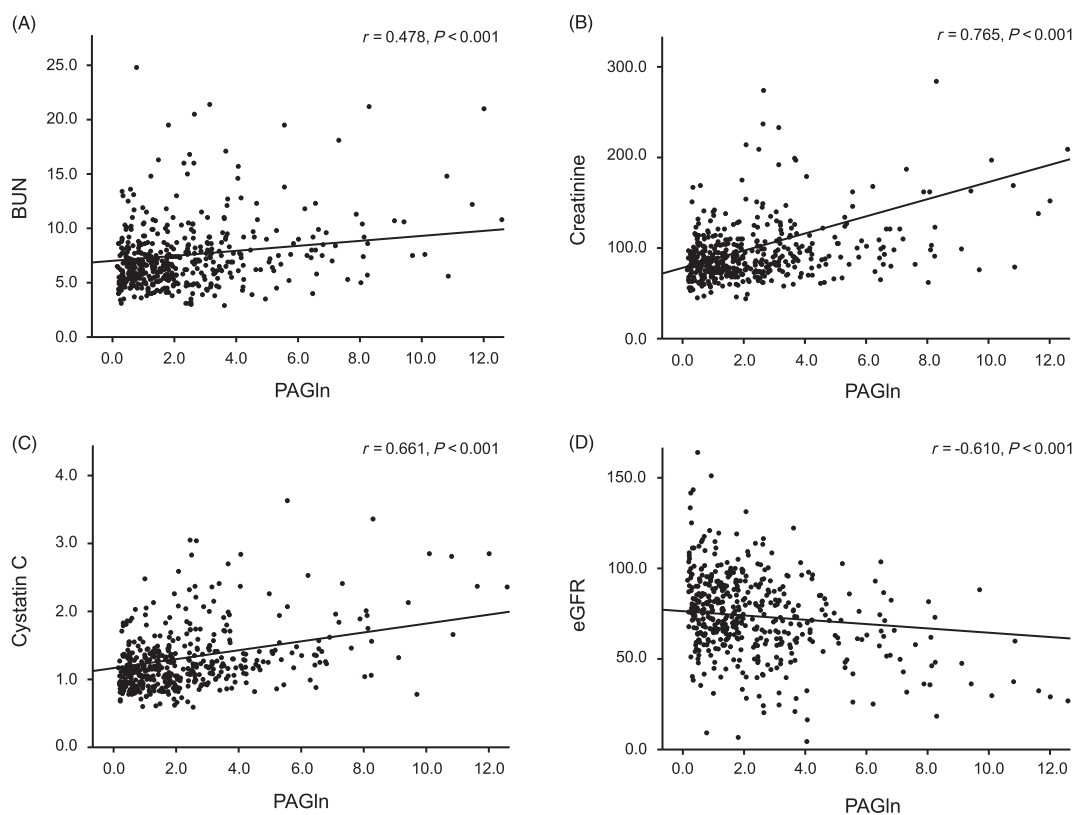
	Unadjusted OR	P value	Adjusted for Model 1 OR	P value	Adjusted for Model 2 OR	P value
log PAGln per SD	2.059 (1.769–2.395)	<0.001	1.978 (1.663–2.352)	<0.001	1.507 (1.213–1.873)	<0.001
PAGln tertiles	2.005 (1.700–2.364)	<0.001	1.825 (1.509–2.207)	<0.001	1.494 (1.181–1.890)	0.001
Tertile 1	1 (ref)		1 (ref)		1 (ref)	
Tertile 2	1.496 (1.087–2.059)	0.013	1.373 (0.962–1.960)	0.081	1.184 (0.766–1.830)	0.447
Tertile 3	4.025 (2.894–5.598)	<0.001	3.346 (2.285–4.898)	<0.001	2.262 (1.413–3.620)	0.001

Model 1: Adjusted for age and sex.
Model 2: Adjusted for age, sex, body mass index, hypertension, diabetes mellitus, haemoglobin, albumin, creatinine, low-density lipoprotein cholesterol, HbA1c and high sensitivity C reactive protein.
Continuous variables were entered per 1 SD.
HF, heart failure; OR, odds ratio; PAGln, phenylacetylglutamine; SD, standard deviation.

Figure 2 Phenylacetylglutamine (PAGln) were significantly correlated with several markers of renal dysfunction in patients with heart failure (HF). Simple analysis for PAGln and BUN (A), creatinine (B), cystatin C (C), and eGFR (D).

Table 3 Serum PAGln levels were associated with the presence of renal dysfunction in patients with HF

	Unadjusted OR	P value	Adjusted for Model 1 OR	P value	Adjusted for Model 2 OR	P value
log PAGln per SD	2.279 (1.799–2.885)	<0.001	2.275 (1.792–2.887)	<0.001	1.853 (1.344–2.556)	<0.001
PAGln tertiles	2.108 (1.615–2.750)	<0.001	1.966 (1.483–2.605)	<0.001	1.607 (1.142–2.263)	0.007
Tertile 1	1 (ref)		1 (ref)		1 (ref)	
Tertile 2	1.179 (0.671–2.072)	0.566	1.034 (0.580–1.841)	0.911	1.291 (0.621–2.682)	0.494
Tertile 3	4.004 (2.392–6.702)	<0.001	3.452 (2.002–5.953)	<0.001	2.486 (1.254–4.930)	0.009

Model 1: Adjusted for age and sex.
Model 2: Adjusted for age, sex, body mass index, hypertension, diabetes mellitus, haemoglobin, albumin, low-density lipoprotein cholesterol, HbA1c, high sensitivity C reactive protein and N-terminal pro-brain natriuretic peptide.
Continuous variables were entered per 1 SD.
HF, heart failure; OR, odds ratio; PAGln, phenylacetylglutamine; SD, standard deviation.

cardiovascular death had significantly higher PAGln levels at baseline than those who did not (*Figure 3A*), which indicates that high baseline PAGln levels were associated with a higher risk of cardiovascular death in HF. Next, we mapped the Kaplan–Meier survival curves to visualize the relationship between PAGln levels and outcomes. When grouped by PAGln tertiles, patients in the highest tertile were more likely to meet the primary endpoint events (*Figure 3B*), especially cardiovascular death (*Figure 3C*) compared with patients in the lower tertiles. Then we used Cox proportional hazards models to evaluate the cardiovascular risk resulting from increased PAGln levels. The results showed that the risks of

Figure 3 Follow-up data of heart failure (HF) subjects and prognosis analysis. (A) HF patients who met the primary endpoint or cardiovascular death had higher levels of phenylacetylglutamine (PAGln) at baseline. PAGln levels did not show any difference in those who met or did not meet HF rehospitalization during follow-up. (B) Kaplan–Meier (KM) curves and log-rank analysis for the primary endpoint according to PAGln tertiles. (C) KM curves and log-rank analysis for cardiovascular death according to PAGln tertiles. (D) Cox regression analysis for primary endpoint and cardiovascular death, respectively.
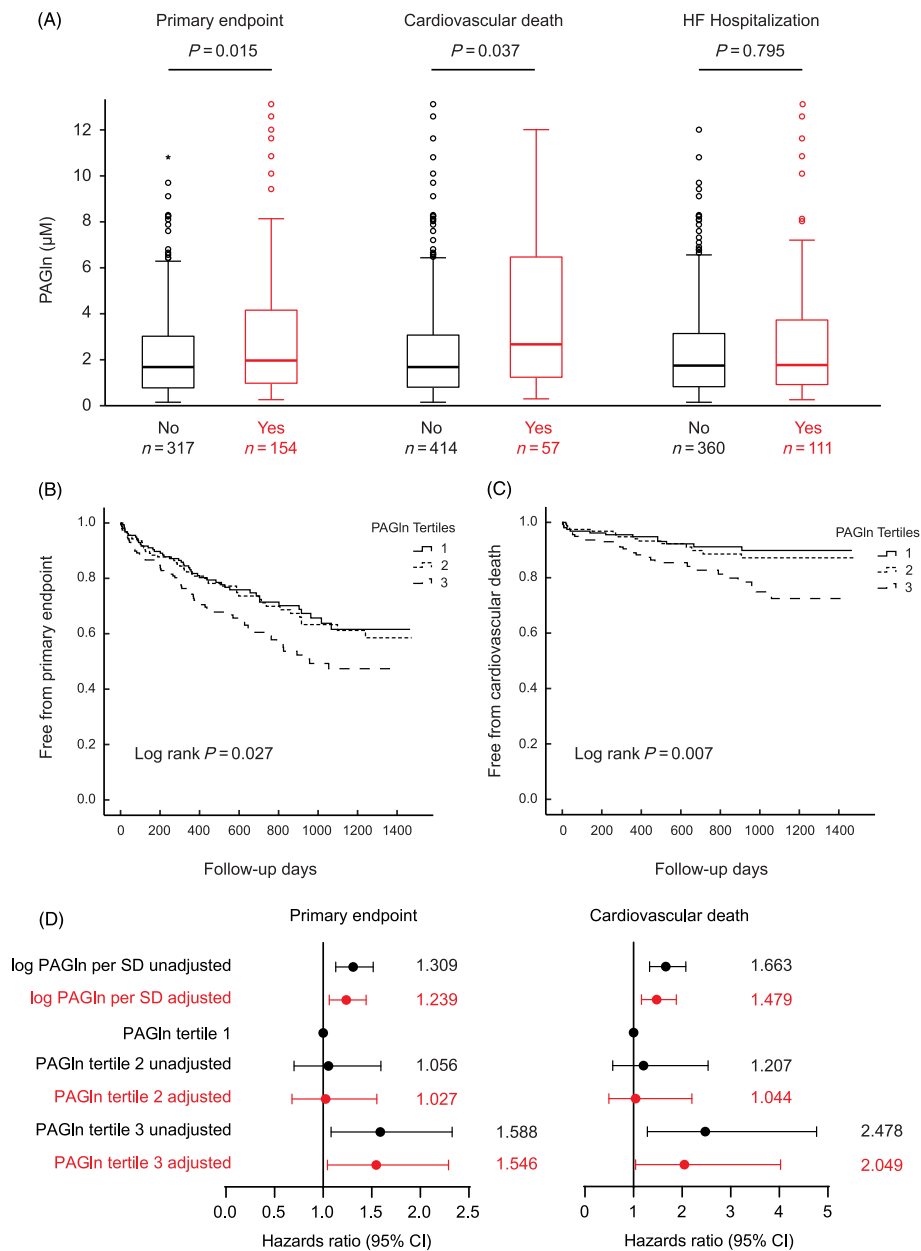
meeting the primary endpoint or cardiovascular death increased by 23.9% or 47.9%, respectively, per 1-SD increase after adjusted. The risks of meeting the primary endpoint or cardiovascular death in the highest tertile increased by 54.6% or 104.9%, respectively, compared with the lowest tertile after adjusted (*Figure 3D*). The ROC curve demonstrated an area under curve (AUC) of 0.576 (95% CI: 0.520–0.0.632, $P = 0.029$) for PAGln's ability to predict adverse outcomes in HF (Supporting Information, *Figure S1*). The predictive cut-off value of PAGln for adverse outcomes was 3.9 μM.

Discussion

In the present study, three major findings were reported. First, we found that PAGln is an independent risk factor for the presence of HF. Second, in HF patients, high levels of PAGln could indicate a worse renal function. Last, high PAGln levels are associated with a higher risk of cardiovascular death in stable chronic HF patients. Though it is not the first evidence to prove the associations between PAGln and HF, the present study still has its unique value: (1) The associations between PAGln and HF previously reported[13,14] were found through untargeted metabolomics, and this is the first proof to support this association using targeted isotopically labelled internal standard calibrated LC-MS; (2) we explored the relationship between PAGln levels and adverse events in stable chronic HF; (3) we verified the association between PAGln and the occurrence of HF on Chinese populations because gut microbiota and metabolites can be influenced by races, diets, living environments and metabolic levels.[23,24] These findings provide new evidence on the relationship between gut microbiota metabolite and HF.

Gut bacteria and their metabolic activities have a significant impact on human health.[5,25,26] Investigating the role of gut microbiota in cardiovascular disease can help to better understand the development of HF; for example, the gut hypothesis of HF explains that reduced cardiac output and systemic congestion in the organ systems induce intestinal ischaemia and oedema, resulting in intestinal bacterial translocation and increased circulating hazardous substances levels, which synergistically induce the production of inflammation-related cytokines. The activated cytokines can, in turn, promote inflammation and induce fibrosis and microvascular and myocardial dysfunction, thereby exacerbating HF.[27,28] The gut hypothesis is not fully validated, but evidence now is increasing. For example, gut microbiota profile on 16S rRNA gene sequencing has revealed that patients with HF had a significantly decreased diversity in the gut microbiota, indicating that altered composition of gut microbiota might be a potential player in the pathogenesis and progression of HF.[29] A double-blind, placebo-controlled study of HF has proved that the administration of probiotics could significantly improve the ejection fraction of HF patients.[30] Although the study had a limited sample size, it sheds light on the intestinal treatment of HF. In addition, the first randomized controlled clinical trial targeting gut microbiome therapy for HF, the Gut-Heart study,[31] is currently underway, and the publication of its results will provide direct evidence of the effectiveness of gut therapy for HF. If intestinal therapy for HF is validated, the current biomarkers of gut–heart axis, trimethylamine-*N*-oxide (TMAO), PAGln and other biomarkers to be discovered in the future, will become important tools for assessing intestinal therapy for HF.

The strong association between PAGln and cardiovascular death was the main finding from our prognostic analysis. A previous study has reported the association between PAGln and mortality in up to 27 diseases,[18] so it is likely that PAGln is not specific to HF but is a broad-spectrum mortality-associated molecule. However, this does not negate the fact that PAGln can be useful in the management of HF. The wide range of confidence intervals for the association with the risk of cardiovascular death (HR: 2.049; 95% CI: 1.042–4.029; $P = 0.038$) is mainly due to the limited sample size in the study and the short follow-up period. Notably, we did not find an association between PAGln and HF rehospitalization alone. There are two possible explanations for the lack of association in HF rehospitalization; one can be attributed to the short follow-up period and the resistance of patients to hospitalization during the pandemic, and the other is the elevated PAGln may be related to some kind of death signal, which is not known yet, for example, sudden cardiac death, arrhythmia and multiple organ complications. The association between PAGln and emergency department visit for HF will also be explored in future studies.

The relationship between PAGln and renal dysfunction was not a new discovery.[13,32] According to our current study, serum PAGln levels correlate well with renal biomarkers (creatinine, BUN, eGFR and cystatin C). PAGln levels increased dramatically in subjects with impaired renal function. The folds increase of serum PAGln levels in renal dysfunction subjects is probably because PAGln is excreted mainly by kidney.[33] However, the rise of serum PAGln in HF patients without renal dysfunction probably results from the increased PAGln biosynthesis from the disorder of gut microbiota in HF patients. Due to the limited dietary availability of phenylalanine as raw material for PAGln synthesis, the rise in serum PAGln levels caused by increased synthesis is not as dramatic as that caused by excretion disorders.

We believe that PAGln is not just a sign of imbalanced gut microbiota, but also a role in the course of HF, based on the present findings. The overactivated sympathetic nervous system is a salient feature of HF, which helps to maintain cardiac performance in the short term, but worsens HF in the long run.[34] It is clear that PAGln can function through activating G-protein-coupled receptors, including α2A, α2B

and β2-adrenergic receptors.[15] Though not the main type of β-adrenergic in the heart, β2-adrenergic receptor comprises 20–25% of cardiac β-adrenergic receptors.[35] PAGln, to some extent, may contribute to the overactivation of the sympathetic nervous system, thus exacerbating HF.

Additionally, it seems that NT-proBNP, the classic HF indicator, and PAGln are similar in that both increase during the progression of HF and are greatly affected by renal function. However, NT-proBNP and PAGln represent different pathophysiological pathways. NT-proBNP is mainly provoked by atrial and ventricular distension, whereas PAGln is induced by the dysbiosis of gut microbiota. Therefore, PAGln has a unique value in understanding the risk of HF, showing prognostic and therapeutic value in the future.

Study limitations

Although this study revealed clinical connections between PAGln and HF, whether PAGln contributes directly to HF or only reflects the deterioration of intestinal ecology induced by HF needs to be elucidated via further research. Second, serum levels of PAGln were not obtained during our follow-up; thus, whether the improvement of HF could decrease the levels of serum PAGln was not described in this study. Third, our HF patients enrolled were those with reduced ejection fraction. Thus, whether the indicative value of PAGln still exists in HF patients with preserved ejection fraction remains unknown. Moreover, further studies should focus on combining these HF-associated indicators to build a more comprehensive assessment tool to manage HF patients.

Conclusions

PAGln levels are an independent risk factor for HF and are linked to a higher risk of cardiovascular death. High PAGln levels could indicate renal dysfunction in HF patients. PAGln can be a valuable indicator of HF.

Acknowledgements

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 ROC curve for predicting the adverse events of HF. **Table S1.** PAGln levels in subjects with or without the presence of HF or renal dysfunction and logistic regression analysis for the presence of HF in subjects with or without renal dysfunction.

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