Journal of the American Heart Association

ORIGINAL RESEARCH

Association of Circulating Phenylacetylglutamine With Multi-Vessel Coronary Disease Severity and Outcomes in ST-Segment–Elevation Myocardial Infarction

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BACKGROUND: There is a lack of evidence regarding the association between plasma phenylacetylglutamine levels and lesion severity and clinical prognosis in patients with ST-segment elevation myocardial infarction (STEMI) with multivessel coronary disease (MVCD). This study aims to investigate the potential of phenylacetylglutamine as a biomarker for major adverse cardiovascular events (MACEs) of patients with STEMI and MVCD.

METHODS AND RESULTS: Clinical data and blood samples were collected from 631 patients with STEMI and MVCD, who underwent primary percutaneous coronary intervention. Quantitative coronary angiography analysis was performed using the QAngio XA 7.3 system. Plasma phenylacetylglutamine concentrations were measured by rapid resolution liquid chromatography quadrupole time-of-flight mass spectrometry. Among a total of 631 patients, median plasma phenylacetylglutamine level was 3.8 (2.1–6.8) μmol/L and the cumulative MACE rate at follow-up was 12%. Plasma phenylacetylglutamine levels of patients with MACE were significantly higher than patients without MACE. We employed restricted cubic spline, Kaplan–Meier curves, and Cox proportional hazard models to explore the association between plasma phenylacetylglutamine and prognosis of patients with STEMI and MVCD. Per SD, an increment in phenylacetylglutamine was associated with a 24% higher risk of complexity lesion. Higher phenylacetylglutamine level was an independent predictor of MACEs (hazard ratio [HR], 2.76 [95% CI, 1.62–4.72]). A novel prognostic scoring system was established by combining phenylacetylglutamine levels with the synergy between percutaneous coronary intervention with Taxus and cardiac surgery score, with higher scores significantly increasing the risk of MACEs (HR, 4.01 [95% CI, 2.04–7.89]).

CONCLUSIONS: Phenylacetylglutamine levels were associated with lesion complexity and prognosis, may serve as a novel biomarker in patients with STEMI and MVCD.

Key Words: biomarkers ■ gut microbiota ■ multivessel coronary disease ■ phenylacetylglutamine ■ ST-segment–elevation myocardial infarction

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This article was sent to Krishnaraj S. Rathod, MBBS, BMedSci, MRCP, Assistant Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.038175

For Sources of Funding and Disclosures, see page 9.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- High phenylacetylglutamine levels significantly increased the risk of major adverse cardiovascular events in patients with ST-segment–elevation myocardial infarction and multi-vessel coronary disease
- There was a strong association between a higher hazard score, established by combining phenylacetylglutamine with synergy between percutaneous coronary intervention with Taxus and cardiac surgery score, and worse risk of major adverse cardiovascular events in patients with ST-segment–elevation myocardial infarction and multivessel coronary disease.

What Are the Clinical Implications?

- Our findings highlight the possibility of phenylacetylglutamine as a novel biomarker in patients with ST-segment–elevation myocardial infarction and multivessel coronary disease, providing new insights into biomarker-guided clinical strategies.

Nonstandard Abbreviations and Acronyms

MACE	major adverse cardiovascular event
MVCD	multivessel coronary disease

ST-segment–elevation myocardial infarction (STEMI) is the most acute manifestation of coronary artery disease (CAD), primarily caused by occlusive luminal thrombosis.[1] Multivessel coronary disease (MVCD) presents in up to half of patients with STEMI and is associated with an adverse prognosis.[2–4] Despite advances in myocardial revascularization and pharmacotherapy, patients with STEMI and MVCD still experience high short- and long-term mortality.[3,5] Biomarker-guided therapeutic strategies have opened up new possibilities for MVCD management.[6,7] Accurate prognosis estimation for these patients can facilitate appropriate decisions by physicians.[6,8]

An increasing number of studies have suggested that gut microbiota-generated metabolites can affect host physiology and cardiovascular disease.[9] Among these, phenylacetylglutamine, a novel gut microbiota-dependent metabolite, has attracted increasing interest.[10] In humans, the primary source of phenylacetylglutamine is dietary protein-derived phenylalanine, which is converted to phenylacetic acid by gut microbiota with a series of enzymes and finally conjugated to glutamine in the host liver and kidney.[11] A previous study demonstrated that phenylacetylglutamine is associated with an increased risk of future CAD.[12] Additionally, Liu et al. demonstrated that higher plasma phenylacetylglutamine levels correlated with increased coronary lesion complexity and severe atherosclerotic plaque burden in patients with suspected CAD.[13] Recent findings have shown that phenylacetylglutamine levels were associated with major adverse cardiovascular events (MACEs) and poorer survival in 2 large longitudinal cohorts.[14] Although the importance of phenylacetylglutamine in the clinical management of patients with STEMI has previously been established,[15] and the related pathophysiological processes of phenylacetylglutamine have been being explored, the association between plasma phenylacetylglutamine levels and clinical prognosis of patients with MVCD has not been systematically investigated.

Primary percutaneous coronary intervention is the preferred reperfusion strategy for patients with STEMI, but the clinical management of MVCD remains challenging.[1,16] The synergy between percutaneous coronary intervention with Taxus and cardiac surgery (SYNTAX) score is used to quantify CAD complexity and is strongly correlated with clinical outcomes.[13,15,17] We investigated the correlation among plasma phenylacetylglutamine levels, CAD complexity, and prognosis in patients with STEMI and MVCD using the SYNTAX score. The study aimed to identify a new biomarker and establish a novel scoring system to evaluate the prognosis of patients with STEMI and MVCD, revealing potential targets for intervention and improving patient survival.

METHODS

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

This was a single-center prospective study involving 631 eligible patients, aged ≥18 years, and diagnosed with STEMI and MVCD, who underwent percutaneous coronary intervention between January 2017 and June 2019 at the Second Affiliated Hospital of Harbin Medical University (Harbin, China). STEMI was defined as persistent chest pain lasting >20 minutes, ST-segment elevation of more than 0.1 mV in >two adjacent leads of 18-lead electrocardiogram, or new-onset left bundle-branch block accompanied by elevated troponin T and TnI (troponin I).[18] MVCD was defined as stenosis of 50% in at least 2 major epicardial arteries and their main branches on coronary angiography.[3] All

participants were followed up with until April 30, 2020, or death. The inclusion and exclusion criteria are presented (Figure S1).

The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University. In accordance with the ethical guidelines, written informed consent was obtained from all patients.

Clinical Data Collection

All baseline clinical data were collected from electronic medical records by trained investigators who were unaware of the purpose of the study. The clinical data encompassed a range of variables. Age, sex, current smoking, past medical history, such as hypertension, diabetes. Laboratory data, such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, fasting plasma glucose, hs-CRP (high-sensitivity C-reactive protein), NT-proBNP (N-terminal pro-brain-type natriuretic peptide), TnI, and creatinine at admission were measured. Medication history before hospitalization, such as statins, aspirin, P2Y12 receptor inhibitors, β-blockers, and angiotensin-converting enzyme indicators or angiotensin II receptor blockers, body mass index, and estimated glomerular filtration rate (eGFR) were calculated.

Plasma Phenylacetylglutamine Levels

Peripheral whole blood was collected from all eligible patients. The blood was centrifuged at 1000*g* for 10 minutes, followed by plasma separation and storage at −80 °C until analysis. The gut microbiota-derived phenylacetylglutamine was analyzed in plasma samples using rapid resolution liquid chromatography quadrupole time-of-flight mass spectrometry, with d5-phenylacetylglutamine serving as the internal standard. Mass spectrometry identified the mass-to-charge ratios (m/z) of plasma phenylacetylglutamine and d5-phenylacetylglutamine as 270 and 265, respectively. Chromatographic separation was performed using a Poroshell 120 HILIC-Z column (Rapid Resolution HT, 2.1 mm × 100 mm, 2.7 μm). The system operated with a mobile phase of 5 mmol/L ammonium acetate buffer containing 0.2% formic acid (solvent A) and acetonitrile containing 0.2% formic acid (solvent B). A linear mobile phase gradient was used as follows: 0 to 5 minutes (15%–50% A), 5 to 7 minutes (50% A), 7 to 8 minutes (50%–15% A), and 8 to 12 minutes (15% A) with a flow rate of 0.3 mL/min and an injection volume of 10 μL. Detailed parameters of the targeted mass spectrometry are presented in Table S1.

A favorable linear pattern was observed for phenylacetylglutamine in the range of 0.01 to 20 μmol/L, with a detection limit of 0.005 μmol/L. Both plasma and quality control samples were prepared as previously described.[19] To ensure consistency and accuracy, quality control samples were run after every 6 blood samples. Interbatch variations were quantified using the coefficient of variation, which in this study were all <10%.[20]

Coronary Angiography Analysis

Quantitative coronary angiography analysis was performed using the QAngio XA 7.3 system (Schuttersveld 9, 2316 XG Leiden, The Netherlands). The baseline SYNTAX score was calculated via www.syntaxscore.com. Two independent investigators, blinded to phenylacetylglutamine levels and patients' clinical information, measured the angiographic variables.

Ascertainment of Study End Points

The primary end point was MACEs, defined as a composite of nonfatal myocardial infarction, stroke, all-cause death, and heart failure (HF). The definitions of nonfatal myocardial infarction, stroke, and HF were based on a previous description.[15]

Statistical Analysis

Continuous variables were tested for normality and presented as mean±SD or medians (interquartile ranges), and categorical variables were presented as percentages. Continuous variables were analyzed using the independent Student's *t* test or nonparametric tests, and categorical variables were analyzed using the chi-square or Fisher's exact test, as appropriate. Spearman's correlation analysis and linear regression with restricted cubic spline were used to assess the correlation between phenylacetylglutamine levels and laboratory data.[21] To explore the association between phenylacetylglutamine and the high complexity of coronary lesions, multivariate linear regression and logistic regression were performed.

Phenylacetylglutamine levels were divided into 2 groups according to the median (3.84 μmol/L). Throughout the follow-up period, survival curves were plotted using the Kaplan–Meier method, and the log-rank test was used for comparisons between groups. To determine whether higher phenylacetylglutamine levels independently predicted clinical prognosis, multivariate Cox proportional hazard models were employed. Covariates were integrated to control for confounding in the 2 models. Model 1 adjusts for age and sex; model 2 additionally adjusts for current smoking, hypertension, diabetes, low-density lipoprotein cholesterol, NT-proBNP, TnI, hs-CRP, and e-GFR. The missing values ranged from 0.32% to 5.86% and

were coded with MissForest imputation[22] (Table S2). In addition, we depicted detailed descriptions of the dose–response curves among phenylacetylglutamine levels, MACEs, all-cause death, and postdischarge HF. Sensitivity and subgroup analyses were performed to validate our results.

Higher SYNTAX scores indicated more complex CAD, with scores >22 considered high risk and prognostically significant.[17,23] Based on the results of this study, we incorporated phenylacetylglutamine >3.84 μmol/L to build a novel hazard score for each patient. One point was assigned to each indicator above its respective threshold, and all scores were summed to categorize the patients as low (score 0), intermediate (score 1), or high (score 2) risk. Kaplan–Meier curves were used to illustrate the MACE rates within the groups. A Cox proportional hazards model was constructed to generate hazard ratios (HRs) and 95% CIs, with all adjusted covariates of age, sex, current smoking, hypertension, diabetes, low-density lipoprotein cholesterol, NT-proBNP, TnI, hs-CRP and e-GFR. A trend test was applied to compare graded differences in HRs between groups. The accuracy of phenylacetylglutamine, independently and in combination with the SYNTAX score, for predicting MACEs in patients with STEMI and MVCD was analyzed using receiver operating characteristic curves. The area under the curve was evaluated to determine its predictive value. Data analyses were performed using SPSS statistical software (version 25.0) and R software (version 4.2.1). A *P* value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

The baseline clinical characteristics of all patients are presented in Table 1. A total of 631 patients were included in this study and divided into 2 groups: those with MACEs (n=76, 12.0%) and those without MACEs (n=555, 88.0%). The mean participant age was 64.8±11.1 years, and 68.6% were male. Patients with MACEs were older than those without MACEs (*P*=0.002) and had a higher proportion of current smoking (*P*=0.027). Phenylacetylglutamine concentrations were significantly higher in patients with MACEs, particularly in cases of all-cause death (*P*<0.001). The mean SYNTAX score was significantly higher in patients with MACEs at 20.0±5.2 compared with those without MACEs (Table 1 and Figure 1). However, there were no significant differences in the proportions of American Heart Association (AHA) (B2/C), calcified lesions, and thrombus between the 2 groups. Laboratory results indicated that patients with MACEs tended to have worse renal and heart functions.

Association Between Phenylacetylglutamine Levels and Coronary Lesion Severity

Spearman's correlation analysis and linear regression with restricted cubic spline showed significant linear correlations between plasma phenylacetylglutamine levels and NT-proBNP, hs-CRP, eGFR, and SYNTAX scores (Figure S2 and Table S3). However, phenylacetylglutamine levels showed no significant correlations with other biochemical markers, except for age. Multivariate linear regression analysis confirmed that phenylacetylglutamine levels were independently associated with the SYNTAX score (HR, 1.18 [95% CI, 1.04–1.34]) (Table S4). Table 2 presented the results of logistic regression analysis, indicating that per SD, phenylacetylglutamine increased the risk of CAD complex lesions by 24% (HR, 1.24 [95% CI, 1.02–1.49]).

Primary Outcome

The patients were followed up with for a median of 639 (interquartile range, 360–720) days. Patients with high phenylacetylglutamine levels had higher rates of MACEs and all-cause death, whereas postdischarge HF did not show a significant difference (*P*=0.065). This indicated that high phenylacetylglutamine levels were associated with MACEs and all-cause death (Figure S3). Kaplan–Meier survival curves (Figure 2A and B) revealed that patients with higher phenylacetylglutamine levels were more likely to experience MACEs and all-cause death (both log-rank *P*<0.001).

Cox proportional hazard models were used to evaluate the impact of elevated phenylacetylglutamine levels on MACEs. After multivariable adjustment, high phenylacetylglutamine levels increased the risk of MACEs (HR, 2.76 [95% CI, 1.62–4.72]) and all-cause death (HR, 3.79 [95% CI, 1.35–10.66]). However, elevated phenylacetylglutamine levels did not significantly increase the risk of postdischarge HF (Figure 3A). Restricted cubic spline analysis showed a similar trend, showing a nearly flat HR for postdischarge HF (nonlinear *P*=0.195) (Figure 3B and C, Figure S4).

Patients with STEMI and 3-vessel CAD had higher phenylacetylglutamine levels (median: 4.0 nmol/L compared with 3.7 μmol/L), incidence of diabetes, low-density lipoprotein cholesterol levels, and coronary lesion severity compared with those with 2-vessel disease (Table S5). The difference between patients with 3-vessel disease with and without MACEs was similar to the results for all patients, with patients with MACEs exhibiting higher phenylacetylglutamine levels (median: 8.3 nmol/L compared with 3.7 μmol/L) (Table S6). After grouping by median phenylacetylglutamine levels, survival curves confirmed these findings, aligning with the trends observed in the overall patient cohort (Figure S5). Higher phenylacetylglutamine levels increased the risk

Table 1. Baseline Clinical Characteristics of Study Participants

Characteristic	Total (N=631)	MACEs (N=76)	Non-MACEs (N=555)
Phenylacetylglutamine, median (IQR), μmol/L	3.8 (2.1–6.8)	6.9 (3.8–10.4)	3.7 (1.9–6.3)
Male sex, No (%)	433 (68.6)	56 (73.7)	377 (67.9)
Age, y, mean±SD	64.8±11.1	68.4±9.6	64.3±11.2
Body mass index, kg/m^2, mean±SD	25.0±3.5	24.6±3.5	25.1±3.5
Risk factors			
Hypertension, No. (%)	348 (55.2)	47 (61.8)	301 (54.2)
Diabetes, No. (%)	146 (23.1)	16 (21.1)	130 (23.4)
Smoker, No. (%)	410 (65.0)	58 (76.3)	352 (63.4)
Laboratory finding			
Fasting plasma glucose, mean±SD, mmol/L	9.4±4.0	9.7±4.2	9.3±3.9
Total cholesterol, mean±SD, mmol/L	4.8±1.0	4.7±1.0	4.8±1.0
Low-density lipoprotein cholesterol, mean±SD, mmol/L	3.0±0.8	3.1±0.8	3.0±0.9
High-density lipoprotein cholesterol, mean±SD, mmol/L	1.3±0.3	1.2±0.3	1.3±0.3
Triglyceride, mean±SD, mg/dL	149.7±105.7	145.6±82.0	150.2±108.6
Estimated glomerular filtration rate, mean±SD, mL/min/1.73 m^2	77.9±27.4	71.5±21.6	78.8±28.0
High-sensitivity C-reactive protein, median (IQR), mg/L	5.1 (2.2–10.5)	8.1 (3.5–13.3)	4.8 (2.0–9.7)
Troponin I, median (IQR), μg/L	65.9 (27.3–128.3)	80.2 (36.9–169.3)	64.4 (26.2–119.4)
N-terminal pro-brain-type natriuretic peptide, median (IQR), pg/mL	952.0 (372.0–2283.0)	2018.0 (807.5–3531.0)	852.0 (335.0–2074.0)
CK, median (IQR), U/L	251.0 (117.0–920.0)	454.0 (151.3–1392)	241.0 (113.0–849.0)
CK-isoenzyme MB, median (IQR), ug/L	14.5 (2.5–96.2)	27.6 (4.4–133.3)	12.5 (2.3–85.9)
Medications			
Statins, No. (%)	93 (14.7)	16 (21.1)	77 (13.9)
Aspirin, No. (%)	312 (49.4)	42 (55.3)	270 (48.6)
β-blockers, No. (%)	73 (11.6)	12 (15.8)	61 (11.0)
Angiotensin-converting enzyme inhibitors/angiotensin-receptor blockers, No. (%)	44 (7.0)	7 (9.2)	37 (6.7)
P2Y12 receptor inhibitors, No. (%)	71 (11.3)	11 (14.5)	60 (10.8)
Coronary angiography features			
Synergy between percutaneous coronary intervention with Taxus and cardiac surgery score, mean±SD	17.6±6.3	20.0±5.2	17.3±6.3
AHA (B2/C), No. (%)	280 (44.4)	36 (47.4)	244 (44.0)
Calcified lesion, No. (%)	37 (5.9)	5 (6.6)	32 (5.8)
Thrombus, No. (%)	453 (71.8)	61 (80.3)	392 (70.6)
Left main disease, No. (%)	25 (4.0)	2 (0.3)	23 (3.6)

CK indicates creatine kinase; andMACEs, major adverse cardiovascular events.

of MACEs (HR, 3.42 [95% CI, 1.44–8.09]) but were not statistically associated with the risk of all-cause death (Figure S6). The receiver operating characteristic curve demonstrated that phenylacetylglutamine could be used as a prognostic predictor for patients with STEMI and MVCD, with an area under the curve of 0.699 (95% CI, 0.636–0.763) (Figure S7).

In the sensitivity analysis, the top and bottom 2.5% of phenylacetylglutamine levels were removed to minimize heterogeneity, but the results remained consistent (Figure S8). After excluding patients with renal dysfunction (eGFR ≤60 mL/min/1.73 m^2) and HF (ejection fraction ≤50%),[24] phenylacetylglutamine remained a risk factor for MACEs (Figure S9). Additionally, 4% of patients with left main artery lesion were included in the current study, and excluding them, higher phenylacetylglutamine levels still increased the risk of MACE (HR, 2.64 [95% CI, 1.54–4.51]) and all-cause death (HR, 3.59 [95% CI, 1.27–10.16]) (Table 1) (Figure S10). Subgroup analysis showed consistent results across different age and sex groups, there were no significant interactions between phenylacetylglutamine and variables (Table 3).

Hazard Scores and MACEs of Patients With STEMI and MVCD

Consistent with a previous study,[17] higher SYNTAX score increased the risk of MACEs (HR, 1.64 [95%

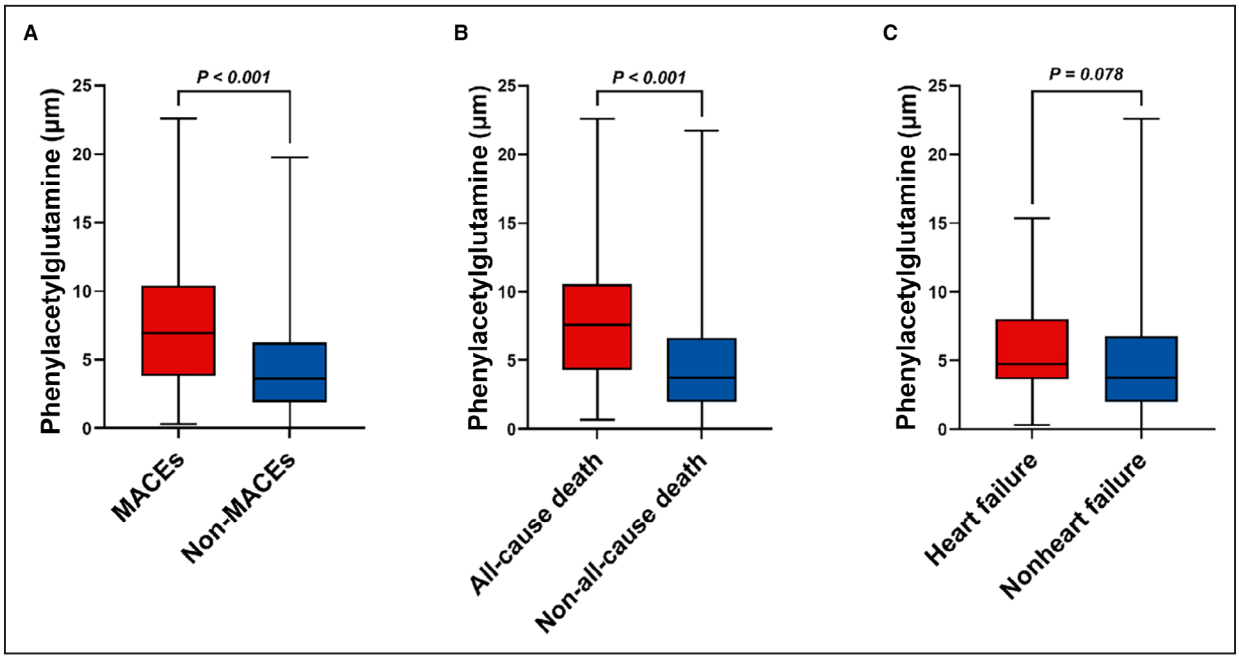

Figure 1. Comparison of plasma phenylacetylglutamine levels among patients with and without clinical end point.
A, The difference of plasma phenylacetylglutamine level between MACEs and non-MACEs groups; **B**, between all cause-death and non-all-cause death groups; **C**, between heart failure and non-heart failure groups.

CI, 1.00–2.66]) (Figure S11). Patients with a higher novel hazard score, calculated by combining the SYNTAX score and phenylacetylglutamine levels, had a higher rate of MACEs, increasing from 5.8% in patients with a low score to 27.8% in patients with a high score (P<0.001) (Figure S12). Kaplan–Meier survival curves showed a higher MACE risk in patients with a high hazard score (log-rank P<0.001) (Figure 4A). There was a significant graded association between increasing hazard score and the risk of subsequent MACEs (HR, 4.01 [95% CI, 2.04–7.89]) (Figure 4B). Additionally, combining the SYNTAX score with phenylacetylglutamine levels significantly enhanced the predictive value for MACEs (P=0.019) (Figure S7).

Table 2. Independent Correlation of Phenylacetylglutamine Levels With Lesion Complexity in Patients With STEMI and MVCD

	Mode	HR (95% CI)	*P* value
Phenylacetylglutamine	Unadjusted	1.26 (1.06–1.51)	0.010
	Model 1	1.27 (1.06–1.53)	0.009
	Model 2	1.24 (1.02–1.49)	0.026

Correlation between phenylacetylglutamine and lesion complexity was assessed using multivariate logistic regression analysis. Model 1: age and sex; Model 2: age, sex, hypertension, diabetes, current smoking, low-density lipoprotein cholesterol, N-terminal pro-brain-type natriuretic peptide, and troponin I. HR indicates hazard ratio; MVCD, multivessel coronary disease and STEMI, ST-segment–elevation myocardial infarction.

DISCUSSION

The present study focused on patients with STEMI and MVCD, demonstrating for the first time an association between phenylacetylglutamine levels and MACEs. Additionally, we developed a novel prognostic hazard score combining phenylacetylglutamine with the SYNTAX score. Given the poor prognosis and high mortality associated with MVCD, these findings provide valuable insights into the role of phenylacetylglutamine for clinical management.

Phenylacetylglutamine is known to be excreted through renal tubules, and its renal clearance is markedly reduced in patients with renal dysfunction, leading to its accumulation and poor prognosis.[25,26] Phenylacetylglutamine has also been strongly associated with the incidence and severity of HF and a higher risk of adverse events in patients with HF.[20,24] Our findings aligned with these observations, as Spearman's and restricted cubic spline analyses indicated significant associations among eGFR, NT-proBNP, and phenylacetylglutamine levels in our patients. Even after adjusting for NT-proBNP and eGFR levels, higher phenylacetylglutamine levels still increased the risk of MACEs and all-cause death in patients with MVCD. Importantly, when excluding subjects with HF and renal dysfunction from our analysis, higher phenylacetylglutamine levels still correlated with MACEs. Similarly, probably the low all-cause mortality in our study, higher phenylacetylglutamine levels in patients

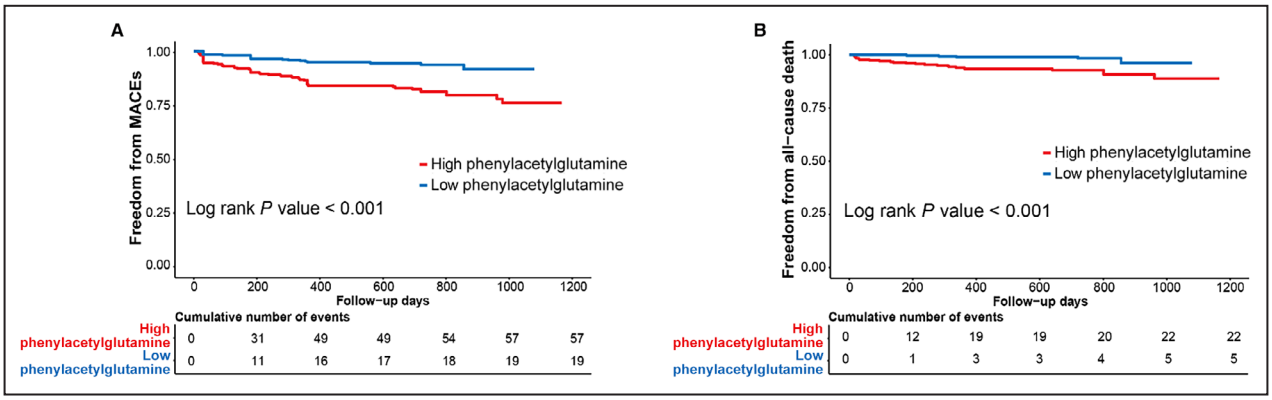

Figure 2. The Kaplan–Meier survival curves of plasma phenylacetylglutamine with MACEs and all-cause death.
A, Survival curve of plasma phenylacetylglutamine with MACEs; **B**, Survival curve with all-cause death.

with STEMI and 3-vessel CAD no longer increased the risk of all-cause death.

High systemic hs-CRP levels accelerate atherosclerotic plaque progression and are strong predictors of MACEs.[27,28] Our study observed significantly higher hs-CRP levels in patients with MVCD and MACEs. Phenylacetylglutamine has been implicated in the inflammatory response, exacerbating myocardial tissue inflammatory cell infiltration through the TLR4/AKT/mTOR (toll-like receptor 4/protein kinase B/mammalian target of rapamycin) signaling pathway.[29] In patients with CAD, phenylacetylglutamine levels are positively correlated with the neutrophil-to-lymphocyte ratio,[30] suggesting that phenylacetylglutamine may be involved in the pathological process through immune-inflammatory responses in patients with MVCD. After

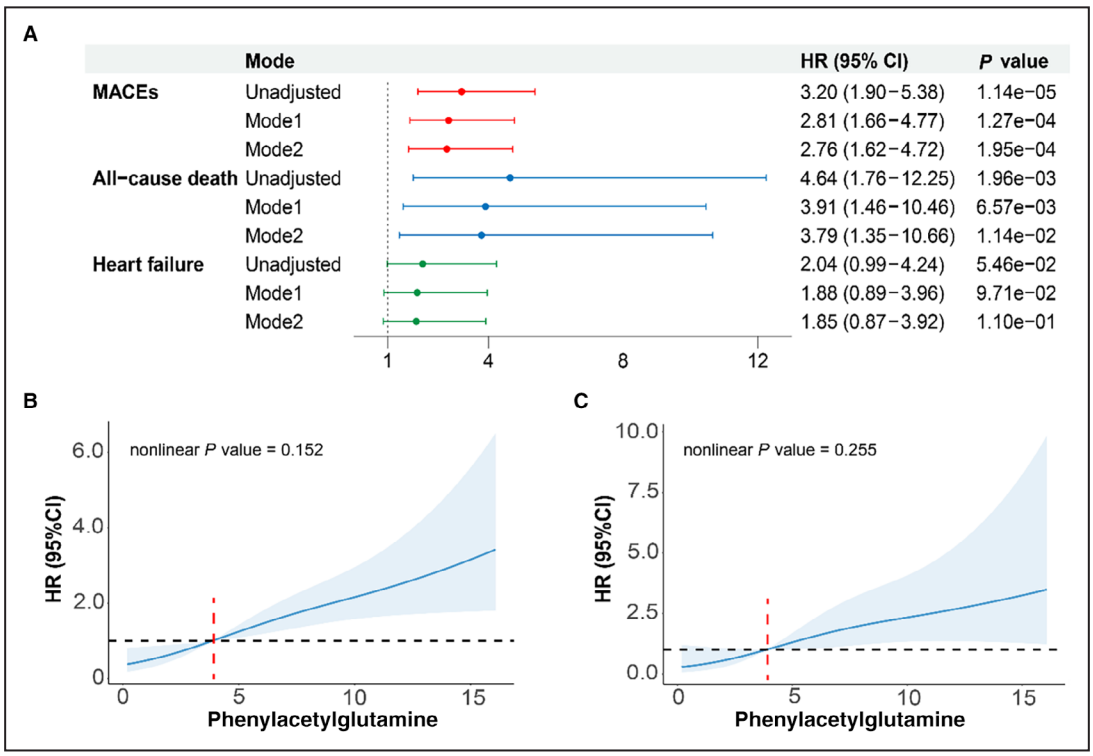

Figure 3. The association of plasma phenylacetylglutamine with MACEs and all-cause death.
A, Cox regression analysis for phenylacetylglutamine levels and end point; **B, C**, Restricted cubic spline regression analysis for phenylacetylglutamine levels and MACEs and all-cause death, respectively. Model 1 was adjusted for age and sex; Model 2 was adjusted for age, sex, current smoking, hypertension, diabetes, low-density lipoprotein cholesterol, N-terminal pro-brain-type natriuretic peptide, troponin I, high-sensitivity C-reactive protein, and estimated glomerular filtration rate. HR indicates hazard ratio; and MACEs, major adverse cardiovascular events.

Table 3. Stratified Analyses of the Associations Between Phenylacetylglutamine and MACEs

Subgroup	HR (95% CI)	*P* value	*P* interaction
Sex			
Male	2.20 (1.18–4.08)	0.013	0.282
Female	4.67 (1.51–14.42)	0.007	
Age, y			
>65	2.45 (1.21–4.94)	0.012	0.577
≤65	3.32 (1.42–7.75)	0.005	

Adjusted covariates included age, sex, current smoking, hypertension, diabetes, low-density lipoprotein cholesterol, N-terminal pro-brain-type natriuretic peptide, troponin I, high-sensitivity C-reactive protein, and estimated glomerular filtration rate. HR indicates hazard ratio; and MACEs, major adverse cardiovascular events.

adjusting for hs-CRP, elevated phenylacetylglutamine still increased the risk of MACEs, and Table S7 showed no interaction between hs-CRP and phenylacetylglutamine, indicating that the unknown biological effects of phenylacetylglutamine have yet to be fully elucidated.

Phenylacetylglutamine has also been associated with in-stent stenosis and hyperplasia in patients with CAD.[30] Although percutaneous coronary intervention therapy significantly improves clinical outcomes in acute coronary syndromes and MVCD, some patients still experience in-stent restenosis.[2,31] Therefore, we hypothesized that the increased MACE risk in patients with MVCD due to phenylacetylglutamine may partly be attributed to ischemia resulting from in-stent hyperplasia, exacerbated by elevated phenylacetylglutamine levels. Studies in animal models and human platelets have demonstrated that phenylacetylglutamine may enhance platelet activation-related phenotypes and thrombogenic potential.[10] Platelets express the NLRP3 (NOD-like receptor protein 3) inflammasome, which mediates caspase-1 activation, promoting IL-1β (interleukin-1β) synthesis and inflammatory responses. Inflammatory cells drive intravascular thrombin formation and platelet coagulation, and phenylacetylglutamine may act as an inflammatory trigger involved in thrombosis.[32] Notably, phenylacetylglutamine mediates cellular events through AdRs (adrenergic receptors), including α2A, α2B, and β2 receptors.[10] Compared with wild-type mice, β2 AdR-overexpressing mice are more susceptible to ischemic injury. Conversely, pretreatment with an AdR blocker can inhibit the prothrombotic phenotype in mice.[10,33,34] Carvedilol, a highly effective β-blocker for improving survival after myocardial infarction, similarly reverses the prothrombotic effects of phenylacetylglutamine.[10,35] Exploring ways to effectively reduce plasma phenylacetylglutamine levels in patients with STEMI and MVCD may offer novel strategies to improve prognosis and prolong survival. In summary, phenylacetylglutamine may affect the prognosis of patients with STEMI and MVCD through multiple pathophysiological processes such as inflammation, platelet activation, thrombosis, and cell receptor response.

Phenylacetylglutamine levels are influenced by various factors. A high-protein diet, a significant source of phenylalanine, tends to promote atherosclerosis, highlighting the necessity for healthy dietary management in patients with MVCD.[36,37] Alterations in gut microbiota structure and abundance affect genes related to phenylacetylglutamine synthesis, increasing circulating phenylacetylglutamine levels.[30,38] Specifically, the microbial *porA* gene in *Clostridium sporogenes* oxidatively metabolizes phenylalanine to phenylacetic acid,[10] with phenylpyruvate ferredoxin oxidoreductase and phenylpyruvate decarboxylase also involved in phenylacetylglutamine

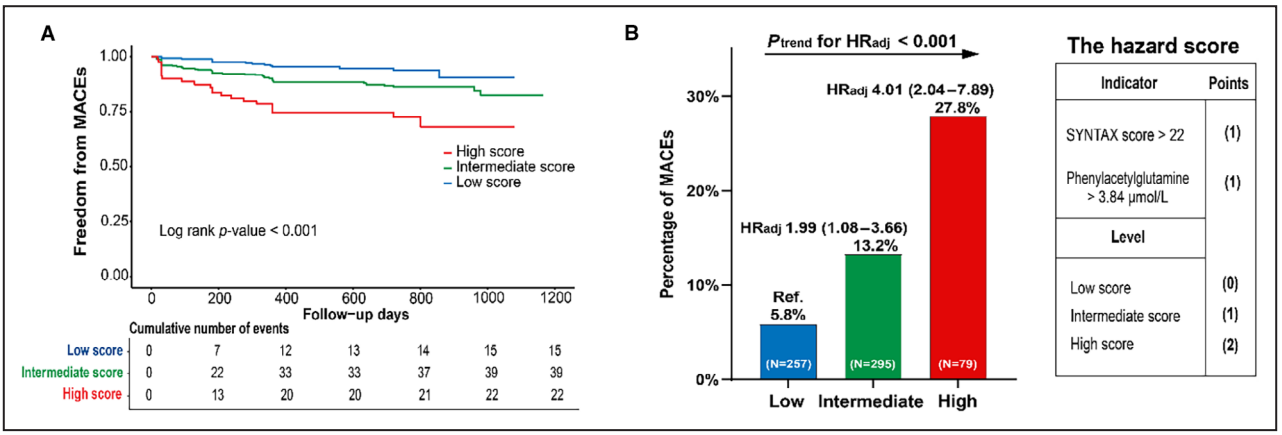

Figure 4. Association of hazard score with MACEs.
A, Kaplan–Meier survival curves for the hazard score with MACEs; **B**, Adjusted association of the hazard score with MACEs. Adjusted covariates included age, sex, current smoking, hypertension, diabetes, low-density lipoprotein cholesterol, N-terminal pro-brain-type natriuretic peptide, troponin I, high-sensitivity C-reactive protein, and estimated glomerular filtration rate. HR indicates hazard ratio; MACEs, major adverse cardiovascular events; and SYNTAX, synergy between percutaneous coronary intervention with Taxus and cardiac surgery.

synthesis.[11] Phenylacetylglutamine mediates the effects of *Escherichia coli* and *Desulfovibrio piger* on aging.[38] Stratified analysis showed minimal differences between age groups, but higher phenylacetylglutamine levels posed a greater risk of MACEs in female patients with MVCD compared with males. This could be due to sex differences in vascular remodeling, lipid metabolism, and endothelial dysfunction, with phenylacetylglutamine linked to underlying genetic and molecular mechanisms.[39]

Of particular note, we established a novel scoring system combining phenylacetylglutamine levels with the SYNTAX scores, with high scores significantly increasing the risk of MACEs, reinforcing the potential of phenylacetylglutamine as a biomarker for patients with STEMI and MVCD. The novel hazard score to identify high-risk patients compensates for the poor prognosis of patients assessed only from a single perspective of coronary lesion severity, offering a comprehensive assessment of a patient's hemodynamic, inflammatory, and coronary complexity status, and has the potential to integrate clinical risk. Potential therapies targeting gut microbiota metabolites are the current foci of attention. In order to reduce phenylacetylglutamine level in patients with STEMI and MVCD, dietary structure could be adjusted, probiotics and prebiotics supplementation and fecal transplantation could be used.[40] Early identification of phenylacetylglutamine level in patients with STEMI and MVCD and early effective risk stratification is helpful for accurate prevention and improvement of poor prognosis.

Several limitations of the study should be noted. First, despite controlling for several confounders (including demographic characteristics and common risk factors), the possibility of unknown confounders cannot be entirely excluded. Second, our analysis focused on the correlation and prognostic prediction rather than on causation between phenylacetylglutamine levels and coronary lesions. Finally, humans and their gut microbiota evolved in parallel, and some strains can exhibit remarkable population specificity across continents. *P. copri, E. rectale*, and *B. longum* are known to function differently according to population. The population specificity of microbiota diversity affected phenylacetylglutamine level to some extent.[30,38,41] Therefore, the subjects in this study were from a single center. For improving the generalizability of our results, it is essential to expand the sample size and conduct validation across multiple centers.

CONCLUSIONS

In patients with STEMI and MVCD, higher phenylacetylglutamine levels were associated with increased coronary lesion severity and risk of MACEs. The novel hazard score, constructed by combining phenylacetylglutamine levels with the SYNTAX score, proved useful for prognostic assessment.

ARTICLE INFORMATION

Received August 7, 2024; accepted December 20, 2024.

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Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this article.

Sources of Funding

This work was supported by the National Natural Science Foundation of China (U21A20391, 82370343 and 82000409), the Natural Science Foundation of Heilongjiang Province of China (ZD2023H005). Noncommunicable Chronic Diseases-National Science and Technology Major Project (2023ZD0503100). The open competition mechanism to select the best candidates for key research projects of Ningxia Medical University (XJKF230108). The Postdoctoral Scientific Research Developmental Fund of Heilongjiang Province (LBHQ21120) and the Fund of Key Laboratory of Myocardial Ischemia, Ministry of Education (KF201609).

Disclosures

None.

Supplemental Material

Tables S1–S7
Figures S1–S12

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