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Stress Hyperphenylalaninemia Is Associated With Mortality in Cardiac ICU: Clinical Factors, Genetic Variants, and Pteridines*

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OBJECTIVES: Hyperphenylalaninemia predicts poor outcomes in patients with cardiovascular disease. However, the prognostic value and factors associated with stress hyperphenylalaninemia (SHP) were unknown in critical patients in the cardiac ICU.

DESIGN: Prospective observational study.

SETTING: Single-center, cardiac ICU in Taiwan.

PATIENTS: Patients over 20 years old with Acute Physiology And Chronic Health Evaluation II scores greater than or equal to 15 and/or ventilatory support in the cardiac ICU.

INTERVENTIONS: We measured plasma phenylalanine levels serially during patients' stays in the ICU to investigate their prognostic value for 90-day mortality. Gene array was performed to identify genetic polymorphisms associated with SHP (phenylalanine level ≥ 11.2 μmol/dL) and to develop a Genetic Risk Score (GRS). We analyzed the associations between SHP and clinical factors and genetic variants and identified the correlation between pteridines and genetic variants.

MEASUREMENTS AND MAIN RESULTS: The study enrolled 497 patients. Increased phenylalanine concentration was independently associated with increased mortality risk. Patients with SHP had a higher mortality risk compared with those without SHP (log rank = 41.13; $p < 0.001$). SHP was associated with hepatic and renal dysfunction and with genetic polymorphisms on the pathway of tetrahydrobiopterin (BH4) synthesis (CBR1 and AKR1C3) and recycling (PCBD2). Higher GRSs were associated with lower BH4 bioavailability in response to stress ($p < 0.05$). In patients without SHP at baseline, those with GRSs gretaer than or equal to 2 had a higher frequency of developing SHP during the ICU stay (31.5% vs 16.1%; $p = 0.001$) and a higher mortality risk ($p = 0.004$) compared with those with GRSs less than 2. In patients with SHP at baseline, genetic variants did not provide additional prognostic value.

CONCLUSIONS: SHP in patients admitted to the ICU was associated with a worse prognosis. In patients without SHP, genetic polymorphisms associated with SHP measured using a GRS of greater than or equal to 2 was associated with the subsequent SHP and higher mortality risk.

KEY WORDS: critical care; genetic polymorphisms; mortality; phenylalanine; pteridine; tetrahydrobiopterin

Even with remarkable advances in therapeutic modalities and strategies, high mortality risk remains a major issue in patients with cardiovascular diseases receiving care in the ICU (1). Recently, the Southall and Brent Revisited study and the British Women's Health and Heart Study showed that higher phenylalanine levels are associated with increased cardiovascular risk

*See also p. 1670.

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(2). Delles et al (3) demonstrated that elevated phenylalanine levels predicted heart failure-related hospitalization in community cohorts at cardiovascular risk based on the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial and the Finland National FINRISK Health Survey cohort. Further investigations in patients with heart failure revealed that higher phenylalanine levels were associated with a higher 1-year event rate of rehospitalization or mortality (4, 5). All evidence suggests that hyperphenylalaninemia is a prognostic biomarker for poor outcomes, rather than just an essential amino acid in patients with cardiovascular diseases.

Hyperphenylalaninemia is well known in phenylketonuria, a congenital metabolic disorder caused by genetic defects in phenylalanine hydroxylase or its cofactor, 5,6,7,8-tetrahydrobiopterin (BH4), which belongs to the complex system of pteridine production and recycling (6). Currently, extensive genetic screening worldwide powerfully identifies patients with phenylketonuria at the neonatal stage. In the cardiac ICU, all patients are adults without phenylketonuria. Intriguingly, our recent study found mild-to-moderate hyperphenylalaninemia in patients with heart failure receiving care in the ICU and related it to a remarkable increase in mortality risk (7). However, the associated mechanisms were unknown.

Phenylalanine elevation was associated with impaired liver and kidney function, inflammation, and muscle breakdown in noncritical patients (8) but was not well explored in critical patients. On the other hand, Wannemacher et al (9) noted that phenylalanine levels increased in many, but not all, patients with acute myocardial infarction or sepsis. Recent studies have demonstrated that the prognostic value of phenylalanine is strong and independent of all traditional risk factors and risk stratification scores in different cohorts (2, 3, 5, 7, 10). These findings led to the hypotheses that genetic polymorphisms exist in the phenylalanine metabolism pathway and that the gene-associated hyperphenylalaninemia presents only in response to critical stress. Full exploration of all related links and mechanisms may help develop innovative strategies for lowering the mortality risk in critical care.

The aims of this study were as follows: 1) to investigate the prognostic value of stress hyperphenylalaninemia (SHP) in patients facing critical illness in the cardiac ICU; 2) to explore the associates of SHP, including clinical variables and genetic variants; 3) to assess the associations between genetic variants and dysregulation in the pteridine system and the changes in phenylalanine concentrations in response to stress; and 4) to propose the clinical implications of our findings in critical care.

METHODS

Patient Enrollment

From October 2017 to May 2021, patients with cardiovascular diseases were consecutively enrolled at the cardiac ICU of Chang Gung Memorial Hospital based on the following inclusion criteria: they 1) had Acute Physiology And Chronic Health Evaluation (APACHE II) scores greater than or equal to 15 or were intubated due to respiratory failure, 2) were needed to stay in the ICU greater than 48 hours, and 3) were older than 20 years old. The exclusion criteria were as follows: 1) patients with comorbid disorders other than the main cause for admission that might compromise their survival within 3 months, such as terminal stage cancer or 2) patients who died before baseline phenylalanine measurement. All patients provided informed consent. Ethical approval was granted by the institutional Review Board of Chang Gung Memorial Hospital (201507968B0, 201701750B0, 201801514B0, 202000831B0). Details are provided in the **Supplementary Methods** (http://links.lww.com/CCM/H178).

Study Design

This study consecutively enrolled 497 patients with plasma phenylalanine measured at baseline and twice a week (study flow diagram provided in **Supplementary Fig. 1**, http://links.lww.com/CCM/H178). In the 356 patients enrolled from October 2017 to March 2020, we performed gene array in 270 patients to explore genetic polymorphisms associated with SHP (phenylalanine level ≥ 11.2 μmol/dL, based on the cutoff value for at-risk status published in our previous ICU study) (7). As planned, these 270 participants included 130 patients with maximal phenylalanine levels (Pmax) greater than or equal to 11.2 μmol/dL and 140 patients with Pmax

less than 8.5 μmol/dL (based on the upper limit of the normal range: 4.87–8.54 μmol/dL; mean: 6.78 μmol/dL) (7). Patients receiving hemodialysis 24 hours before or during blood sample collection were excluded to avoid the effects of hemodialysis on phenylalanine concentrations in the gene study. After identifying the association between SHP and genetic polymorphisms at the pathway of BH4 production and recycling, we enrolled 141 consecutive patients from March 2020 to May 2021 to analyze the correlations between genetic polymorphisms and pteridine levels. For the whole cohort (n = 497), we analyzed the prognostic value of phenylalanine concentration and genetic polymorphisms in the ICU.

Analysis of Pteridines in Plasma

Concentrations of biopterin, 7,8-dihydrobiopterin (BH2), and BH4 were quantified by liquid chromatography tandem mass spectrometry (UPLC-MS/MS). For biopterin, BH2 and BH4, methods of oxidation by acid iodine and alkaline iodine (11, 12) and UPLC-MS/MS were modified from previous studies (13, 14).

Follow-up Program

Follow-up data were prospectively obtained from hospital records and personal communication with the patients' physicians. Patients were followed until death or a maximum of 90 days. The primary endpoint was death of all causes.

Statistical Analyses

We presented receiver operating characteristic curve, the area under the curve, hazard ratios (HRs), odds ratios (ORs), and 95% CIs. The Genetic Risk Score (GRS) (range, 0–5) was the sum of the values from 0 to 2 for dominant genes and from 0 to 1 for recessive genes, based on the number of risk alleles. We estimated the necessary sample size using the genetic association power calculator (15). A minimum sample size of 250 was required to achieve 80% power to detect the differences between two groups, with an effect size of 0.25, an OR of 1.5, and an alpha of 0.05.

Detailed methods are provided in the Supplementary Materials (http://links.lww.com/CCM/H178).

RESULTS

Baseline Characteristics of All Study Patients

The baseline characteristics for 497 patients are shown in **Table 1**. These patients were admitted to the ICU for the following conditions: 237 patients (47.7%) for cardiac reasons (e.g., coronary artery disease, myocardial infarction, heart failure, or other cardiovascular diseases); 104 patients (21%) for infection; 84 patients (16.9%) for pulmonary diseases; 38 patients (7.6%) for gastrointestinal bleeding; and 34 patients (6.8%) for other conditions. Phenylalanine concentrations ranged from 3.81 to 53.4 μmol/dL.

Factors Associated With Mortality

During the 90-day follow-up period, 156 patients (31.4%) died. Factors associated with death frequency older age, higher APACHE II and Sequential Organ Failure Assessment (SOFA) scores, higher frequency of noncardiac reason for admission in ICU, atrial fibrillation, and higher levels of C-reactive protein and phenylalanine, but lower estimated glomerular filtration rate (eGFR) and lower levels of cholesterol and albumin (Table 1). Each increase of phenylalanine by 1 μmol/dL was associated with an 8.7% relative increase in mortality risk (HR = 1.087; 95% CI = 1.068–1.106; $p < 0.001$) (**Supplementary Table 1**, http://links.lww.com/CCM/H178). In multivariable analysis, phenylalanine level predicted 90-day mortality independent of age, reason for admission, atrial fibrillation, C-reactive protein, cholesterol, albumin, eGFR, and SOFA score (model 1) and APACHE II score (model 2).

SHP at baseline was noted in 114 patients (22.9%). In **Figure 1*A*** (left and right panels), the Kaplan-Meier curves revealed that baseline or maximal phenylalanine greater than or equal to 11.2 μmol/dL was associated with a lower accumulative survival rate, compared with baseline or maximal phenylalanine less than 11.2 μmol/dL. In the following studies, we focused on the genetic variants associated with SHP.

Exploration of Genetic Polymorphisms in SHP

The baseline characteristics of the 270 patients are shown in **Supplementary Table 2** (http://links.lww.com/CCM/H178). In the gene array, we focused on genetic polymorphisms in the pathways of phenylalanine

TABLE 1.
Demographic and Laboratory Data

Variables	Whole Cohort *N* = 497	Death *N* = 156	SURVIVOR *N* = 341	*p*
Age (yr)	71.3 ± 13.2	73.8 ± 11.7	70.2 ± 13.7	0.006
Male, *n* (%)	313 (63)	175 (64.8)	100 (70.9)	0.226
Acute Physiology And Chronic Health Evaluation II score	18.3 ± 5.91	21.1 ± 6.19	16.9 ± 5.29	< 0.001
Sequential Organ Failure Assessment score	6.51 ± 3.25	8.26 ± 3.21	5.70 ± 2.95	< 0.001
Left ventricular ejection fraction (%)	56.0 ± 27.0	56.9 ± 29.8	55.6 ± 25.6	0.629
Body mass index (kg/m^2)	24.6 ± 5.0	24.7 ± 5.38	24.5 ± 4.8	0.779
Noncardiac reason, *n* (%)[a]	260 (52.3)	96 (61.5)	164 (48.1)	0.005
Comorbidity, *n* (%)				
Diabetes mellitus	234 (47.1)	77 (49.4)	157 (46.0)	0.492
Hypertension	324 (65.2)	106 (67.9)	218 (63.9)	0.383
Coronary disease	217 (43.7)	62 (39.7)	155 (45.5)	0.234
Atrial fibrillation	73 (14.7)	31 (19.9)	42 (12.3)	0.027
Chronic obstructive pulmonary disease	41 (8.2)	9 (5.8)	32 (9.4)	0.174
Ventilator use, *n* (%)	341 (68.6)	121 (77.6)	220 (64.5)	0.004
Inotropic agent use, *n* (%)	159 (32)	69 (44.2)	90 (26.4)	< 0.001
Days in ICU (d)	11.8 ± 9.51	14.0 ± 11.5	10.8 ± 8.30	0.002
Laboratory data				
Hemoglobin (g/dL)	11.2 ± 5.77	10.5 ± 4.45	11.4 ± 6.27	0.093
C-reactive protein (mg/L)	30.3 (8.4–84.6)	51.8 (16.8–112)	23.5 (6.4–73.6)	< 0.001
Cholesterol (mg/dL)	137 ± 54.4	117 ± 41.0	146 ± 57.3	< 0.001
Albumin (g/dL)	3.24 (2.80–3.68)	2.97 (2.61–3.40)	3.38 (2.98–3.77)	< 0.001
Estimated glomerular filtration rate (mL/min/1.73 m^2)	38.0 (13.3–73.6)	28.7 (10.6–56.0)	40.0 (16.3–79.5)	0.005
Alanine aminotransferase (U/L)	30.0 (17.0–63.0)	29.5 (17.3–80.0)	30.0 (17.0–58.8)	0.562
Bilirubin, total (mg/dL)	0.5 (0.3–0.9)	0.6 (0.4–1.0)	0.5 (0.3–0.9)	0.132
Creatine kinase (U/L)	74.6 (25.0–223)	85.9 (22.8–268)	71.0 (26.0–200)	0.195
Phenylalanine (μmol/dL)	9.72 ± 5.18	12.01 ± 7.65	8.67 ± 2.99	< 0.001
Tyrosine (μmol/dL)	8.19 ± 5.49	9.85 ± 7.25	7.39 ± 4.17	< 0.001

[a]Reasons for admission in ICU.

Data are expressed as the mean ± SD for variables with normal distribution, median (interquartile range) for variables with skewed distribution, and as *n* (percentage) for categorical variables.

metabolism and BH4 synthesis. According to the algorithm to separate patients with Pmax greater than or equal to 11.2 μmol/dL from those with less than 8.5 μmol/dL, 13 genetic polymorphisms were identified in eight genes (**Supplementary Fig. 2** and **Supplementary Table 3**, http://links.lww.com/CCM/H178). We finally selected three single-nucleotide polymorphisms located on the genes for BH4 production and recycling to construct the GRS, including rs20572, rs17395698, and rs319598 mapped on *CBR1*, *AKR1C3*, and *PCBD2* genes, respectively (**Fig. 1*B***) (described in the *Statistical Analyses* section and in the Supplementary Methods, http://links.lww.com/CCM/H178).

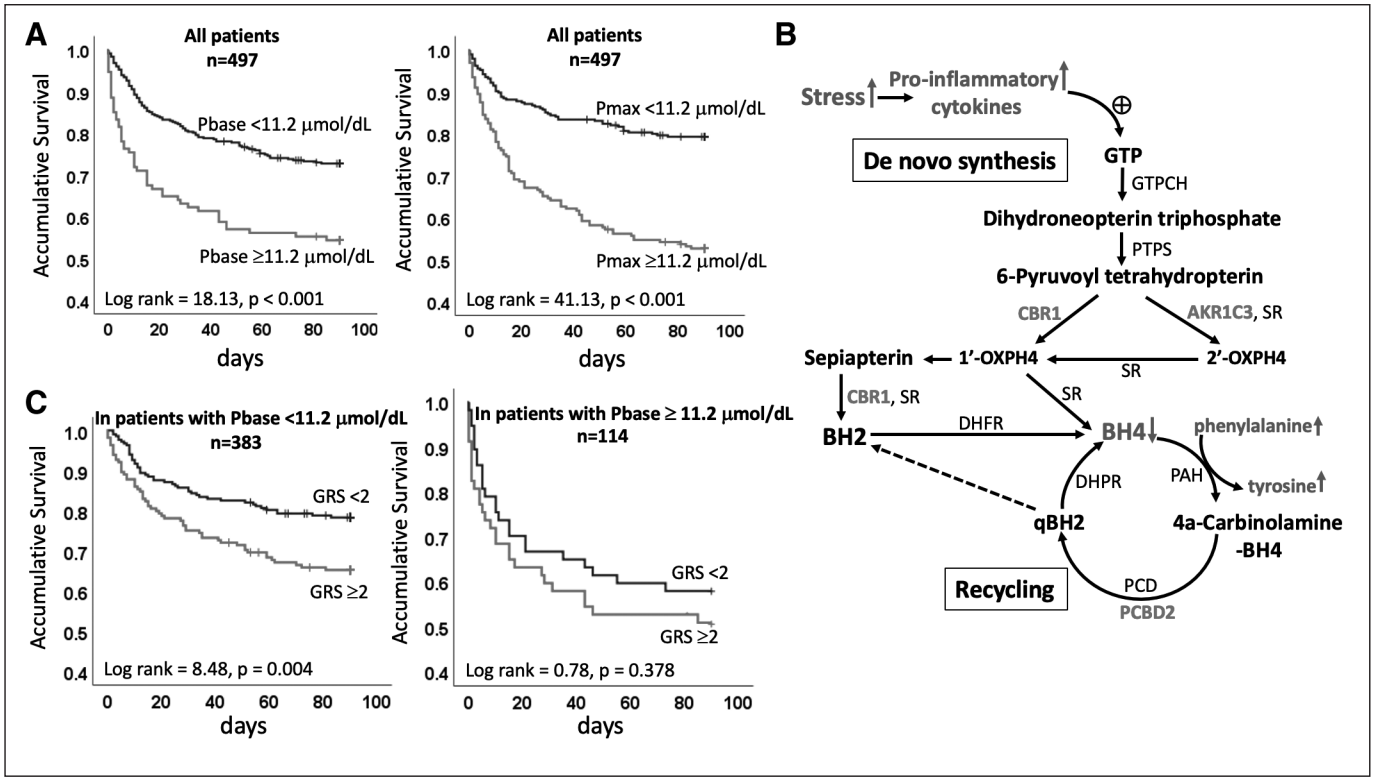

Figure 1. Prognostic value of phenotype and genotype. **A**, The Kaplan-Meier curves for patients with phenylalanine level at baseline (Pbase) greater than or equal to 11.2 µmol/dL versus Pbase less than 11.2 µmol/dL (left panel) and for patients with maximal phenylalanine level during the stay in the ICU (Pmax) greater than or equal to11.2 µmol/dL versus Pmax less than11.2 µmol/dL (right panel). **B**, Synthesis and recycling pathways of the tetrahydrobiopterin (BH4) and pteridine system. C, The Kaplan-Meier curves for patients with Genetic Risk Score (GRS) greater than or equal to 2 versus GRS less than 2 in patients with Pbase less than 11.2 µmol/dL (left panel) and in patients with Pbase greater than or equal to 11.2 µmol/dL (right panel). *Red* color indicates identified genetic variants. AKR = aldose reductase, BH2 = dihydrobiopterin, CBR = carbonyl reductase, DHFR = dihydrofolate reductase, DHPR = dihydropteridine reductase, GTP = guanosine triphosphate, GTPCH = GTP cyclohydrilase, PAH = phenylalanine hydroxylase, PCD = pterin-4a-carbinolamine dehydratase, PTPS = 6-pyruvoyl tetrahydropterin sunthase, qBH2 = quinonoid BH2, SR = sepiapterin reductase. 1'-OXPH4 = 6-(1'-oxo-2'-hydroxypropyl)-tetrahydropterin, 2'-OXPH4 = 6-(1'-hydroxy-2'-oxopropyl)-tetrahydropterin.

Genetic Polymorphisms and Clinical Factors Associated With SHP

In univariate analysis, factors with ability of discriminating patients with Pmax greater than or equal to11.2 µmol/dL from those with Pmax less than 85 µM included sex, eGFR, total bilirubin, creatine kinase, reason for admission, CBR1, PCBD2, AKR1C3, and GRS (**Table 2**). Multivariable analysis (model 1) revealed that CBR1, PCBD2, and AKR1C3 were able to discriminate these two patient groups after adjusting for sex, eGFR, total bilirubin, creatine kinase, and admission reasons. Model 2 showed that GRS, eGFR, total bilirubin, and admission reasons were independent factors related to Pmax. The area under the receiver operating characteristic of GRS was 0.71. Based on Youden's index, the cutoff value for GRS was set at 2 (OR = 3.53; 95% CI = 2.06–6.15; $p < 0.001$), with a sensitivity of 84% and a specificity of 48% for identifying Pmax greater than or equal to 11.2 µmol/dL.

Genetic Polymorphisms Associated Plasma Pteridines

After genetic variants for SHP were noted in the pathway of BH4 production and recycling but not on the genes for phenylalanine hydroxylase, the correlation between genetic variants and plasma pteridine levels was investigated in 141 patients. The baseline characteristics for these patients with different GRSs are shown in **Supplementary Table 4** (http://links.lww.com/CCM/H178). Along with the increase in GRS, we noted a significant trend of decrease in BH4, BH4/BH2, and BH4/total biopterin and increase in C-reactive protein and eGFR, but insignificant changes in BH2 (**Fig. 2*A*–*D***) (Supplementary Table 4,

TABLE 2.
Univariate and Multivariable Analysis of Clinical Variables and Single Nucleotide Polymorphism Associated With Phenylalanine Level Greater Than or Equal to 11.2 μmol/dL Versus < 8.5 μmol/dL (*N* = 270)

	Univariate		Multivariable (Model 1)		Multivariable (Model 2)	
Variables	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***	**OR (95% CI)**	***p***
Male	1.778 (1.070–2.955)	0.026	1.397 (0.764–2.553)	0.293	1.340 (0.741–2.422)	0.333
Noncardiac reason[a]	2.087 (1.200–3.630)	0.009	2.067 (1.603–5.848)	0.001	3.030 (1.590–5.747)	0.001
Estimated glomerular filtration rate (mL/min/1.73 m²)	0.994 (0.989–0.999)	0.023	0.991 (0.985–0.997)	0.002	0.991 (0.985–0.996)	0.02
Bilirubin, total (mg/dL)	1.378 (1.047–1.814)	0.022	1.413 (0.973–2.053)	0.069	1.454 (1.005–2.105)	0.047
Creatine kinase (log)	1.395 (1.035–1.880)	0.029	1.425 (0.974–2.085)	0.068	1.399 (0.965–2.027)	0.77
C-reactive Protein (log)	1.252 (0.875–1.793)	0.219				
AKR1C3	7.611 (1.683–34.42)	0.002	8.804 (1.850–46.29)	0.008		
PCBD2	1.995 (1.358–2.930)	0.001	2.500 (1.580–3.953)	<0.001		
CBR1	2.517 (1.597–3.965)	<0.001	3.243 (1.886–5.578)	<0.001		
Genetic Risk Score[b]	2.593 (1.864–3.608)	<0.001			2.940 (2.036–4.245)	<0.001

OR = odds ratio.

[a]Reason for admission to ICU.

[b]A score based on the count of risk alleles in three single nucleotide polymorphisms (SNPs) (AKR1C3, PCBD2, and CBR1); model 1, multivariable analysis of each SNP adjusting for all confounding factors; model 2, multivariable analysis of genetic risk score adjusting for all confounding factors.

http://links.lww.com/CCM/H178). Since previous studies showed that BH4/biopterin represents BH4 bioavailability better than BH4 alone (16), the correlation between GRS and BH4/total biopterin was analyzed. Linear regression analysis demonstrated that a higher GRS was associated with lower BH4/total biopterin (β = –0.22; p = 0.008). After adjusting for C-reactive protein and eGFR, GRS remained associated with BH4/total biopterin (β = –0.21; p = 0.016).

Prognostic Value of Genetic Variants in Patients Without SHP at Baseline

In the whole study cohort (n = 497), the differences between patients with and without SHP are shown in **Supplementary Table 5** (http://links.lww.com/CCM/H178). Of the 383 patients without SHP at baseline, 218 (56.9%) and 165 (43.1%) had GRS less than 2 and greater than or equal to 2, respectively (**Table 3**). No significant difference between these two subgroups was noted in baseline characteristics. However, in response to stress during the ICU stay, phenylalanine became greater than or equal to 11.2 μmol/dL in 52 patients with GRS greater than or equal to 2 and in 35 with GRS less than 2 (31.5% vs 16.1%, respectively; p = 0.001), supporting the association between genetic variants and phenylalanine elevation. In univariate analysis, GRS greater than or equal to 2 predicted a higher mortality risk (HR = 1.759; 95% CI = 1.196–2.589; p = 0.004). Multivariable analysis revealed that GRS greater than or equal to 2 independently predicted mortality after adjusting for APACHE II scores (HR = 1.741; 95% CI = 1.183–2.562; p = 0.005) or for age, reason for admission, atrial fibrillation, C-reactive protein, albumin, and eGFR (HR = 1.689; 95% CI = 1.142–2.497; p = 0.009). The Kaplan-Meier curves show that GRS greater than or equal to 2 was associated with a lower survival rate, compared with GRS less than 2 (log rank = 8.48; p = 0.004) (**Fig. 1C**, left panel). There was a significant trend of increasing mortality rates along with the increase of GRS from 0 to 4 (p for trend = 0.004) (**Supplementary Fig. 3**, http://links.lww.com/CCM/H178).

Of 114 patients with SHP at baseline, GRS greater than or equal to 2 was noted in 57 (50%) (Table 3). Compared with patients with GRS less than 2, patients

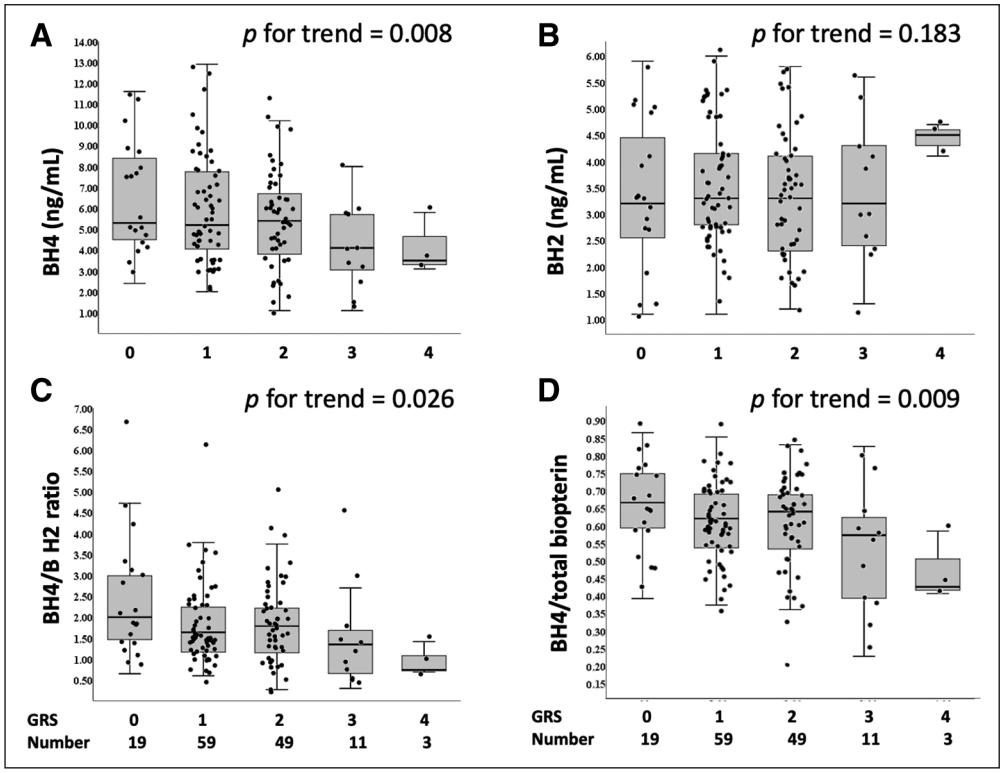

Figure 2. Genetic polymorphisms and plasma pteridines. The concentrations of tetrahydrobiopterin (BH4) (**A**), dihydrobiopterin (BH2) (**B**), ratio of BH4 to BH2 (**C**), and ratio of BH4 to (BH4+BH2) (**D**) in patients with different genetic risk scores.

with GRS greater than or equal to 2 had a lower frequency of inotropic agent use but higher albumin levels. Although patients with SHP had a higher mortality risk compared with those without SHP (Fig. 1*A*, left panel), GRS greater than or equal to 2 did not significantly provide additional prognostic value (Fig. 1*C*, right panel).

DISCUSSION

Our data showed that SHP predicted mortality in patients facing critical illness in the ICU, independent of traditional risk factors and scores. SHP was associated with genetic variants located at the pathway of BH4 production and recycling. Our study further unraveled the relationship between genetic variants and inadequate bioavailability of BH4 in response to stress. Although patients with SHP at baseline had a significantly increased mortality risk, in patients without SHP at baseline, genetic variants were associated with the development of SHP in response to stress during the ICU stay and increased mortality risk.

Hyperphenylalaninemia, Congenital, and Stress-Induced

Phenylketonuria is the most frequent inborn error of amino acid metabolism (6). More than 1,180 bi-allelic variants in the phenylalanine hydroxylase gene have been identified. In adults without phenylketonuria, blood phenylalanine concentrations increase due to liver and renal dysfunction (17, 18), systemic inflammation and stress (19), anabolic resistance (20), and increased tissue breakdown. Focusing on critically ill patients, the present study showed that the main factors associated with SHP included male, muscular breakdown (indicated by creatine kinase), dysfunctional liver and kidney, and genetic variants. However, in multivariable analysis, sex and creatine kinase became insignificant. These findings suggested that, although muscle mass and tissue breakdown were probably the source of phenylalanine, impaired ability of phenylalanine metabolism played a pivotal role in SHP.

In critical situations, tremendous oxidative stress, cytokine storms, nitric oxidase activation, and metabolism of phenylalanine to catecholamine may consume a substantial amount of BH4 and also activate the process of the BH4 production by de novo synthesis, BH4 regeneration from BH2, and recycling pathways (17, 21). The genetic polymorphisms in our patients with SHP were noted specifically on the pathway of BH4 recycling (PCBD2) and de novo synthesis (CBR1 and AKR1C3), but not on the genes for phenylalanine hydroxylase. The role of BH4 was further underlined by the findings that these genetic variants were correlated with decreased bioavailability of BH4 in response to stress, as indicated by low ratios of BH4 to BH2 and BH4 to biopterin. Different from congenital phenylketonuria, the deficiency of BH4 in our patients

TABLE 3.
Baseline Demographic and Laboratory Data in Patients With Different Baseline Phenylalanine Levels and Genetic Risk Scores (*N* = 497)

	Baseline Phenylalanine < 11.2 μmol/dL			Baseline Phenylalanine ≥ 11.2 μmol/dL		
	GRS < 2	GRS ≥ 2		GRS < 2	GRS ≥ 2	
Variables	*N* = 218	*N* = 165	*p*	*N* = 57	*N* = 57	*p*
Age (yr)	71.8 ± 13.3	72.9 ± 12.7	0.376	66.8 ± 12.2	69.6 ± 13.9	0.252
Male, *n* (%)	125 (57.3)	103 (62.4)	0.345	41 (71.9)	44 (77.2)	0.668
Acute Physiology And Chronic Health Evaluation II score	18.0 ± 5.62	18.4 ± 5.35	0.446	19.6 ± 7.34	17.4 ± 6.80	0.101
Sequential Organ Failure Assessment score	6.30 ± 3.20	6.36 ± 2.81	0.846	7.68 ± 3.84	6.53 ± 3.84	0.110
Left ventricular ejection fraction (%)	55.1 ± 19.4	60.3 ± 29.2	0.063	56.3 ± 43.1	46.5 ± 20.1	0.153
Body mass index (kg/m^2)	23.8 (21.0–27.0)	24.4 (21.5–27.6)	0.347	24.3 (21.6–26.5)	23.4 (21.2–27.6)	0.738
Noncardiac, *n* (%)[a]	111 (50.9)	87 (52.7)	0.726	30 (52.6)	32 (56.1)	0.707
Comorbidity, *n* (%)						
Diabetes mellitus	104 (47.7)	78 (47.3)	1.000	27 (47.4)	25 (43.9)	0.851
Hypertension	138 (63.3)	110 (66.7)	0.518	37 (64.9)	39 (68.4)	0.843
Coronary disease	100 (45.9)	62 (37.6)	0.117	27 (47.4)	28 (49.1)	1.000
Atrial fibrillation	30 (13.8)	27 (16.4)	0.562	9 (15.8)	7 (12.3)	0.788
Chronic obstructive pulmonary disease	20 (9.2)	14 (8.5)	0.858	2 (3.5)	5 (8.8)	0.438
Ventilator use, *n* (%)	155 (71.1)	116 (70.3)	0.910	39 (68.4)	31 (54.4)	0.178
Inotropic agent use, *n* (%)	65 (29.8)	48 (29.1)	0.910	29 (50.9)	17 (29.8)	0.035
Days in ICU (d)	10 (5–17)	10 (5–18)	0.595	9 (2–19)	6 (1–13)	0.087
Laboratory data						
Hemoglobin (g/dL)	11.0 ± 4.1	11.6 ± 8.4	0.378	11.1 ± 3.5	10.5 ± 3.2	0.300
C-reactive protein (mg/L)	27.5 (7.1–78.2)	40.4 (10.7–100)	0.082	29.9 (11.7–75.5)	21.3 (7.0–63.6)	0.209
Cholesterol (mg/dL)	143.9 ± 63.8	135.2 ± 42.8	0.114	123.7 ± 50.9	127.8 ± 44.7	0.655
Albumin (g/dL)	3.4 ± 2.7	3.2 ± 0.61	0.377	3.0 ± 0.8	3.5 ± 1.5	0.034
Estimated glomerular filtration rate (mL/min/1.73 m^2)	40.0 (15.3–72.8)	41.1 (12.8–80.6)	0.640	25.6 (11.4–49.5)	31.0 (9.99–80.3)	0.453
Bilirubin, total (mg/dL)	0.5 (0.3–0.8)	0.5 (0.3–0.7)	0.508	0.6 (0.5–1.5)	1 (0.5–1.9)	0.395
Creatine kinase (U/L)	72.0 (25.0–183)	62.3 (19.6–211)	0.604	119 (45.6–614)	91.3 (36.6–256)	0.306
Phenylalanine (μmol/dL)	7.70 ± 1.53	7.92 ± 1.69	0.190	17.25 ± 8.12	15.15 ± 6.41	0.127
Tyrosine (μmol/dL)	6.91 ± 2.69	6.91 ± 2.55	0.994	14.32 ± 9.76	13.25 ± 9.69	0.610

GRS = Genetic Risk Score.

[a]Reasons for admission in ICU.

Data are expressed as the mean ± SD for variables with normal distribution, median (interquartile rangeR) for variables with skewed distribution and as number (percentage) for categorical variables.

is relative, an observation supported by the increased level of tyrosine.

Genetic Variants in SHP

Phenylalanine overload substantially expedites the oxidation of BH4 to quinonoid BH2, and then the recycling pathway is mandatory to reduce quinonoid BH2 back to BH4 (17). BH4 recycling involves three critical enzymes (Fig. 1*B*) (22). BH4 was initially oxidized into 4a-Carbinolamine-BH4, followed by reduction to quinonoid BH2 and BH4 by pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase, respectively. Our data revealed that elevated phenylalanine was associated with the polymorphism in PCD gene but not with the polymorphism in the other two enzymes. The de novo production of BH4 from guanosine triphosphate (GTP) also involves three main enzymes, including GTP cyclohydrolase I, pyruvoyl tetrahydropterin synthase, and sepiapterin reductase (SR) (Fig. 1*B*). SR is the main enzyme in the process of producing BH4 from pyruvoyl tetrahydropterin. Based on the findings in patients with congenital defects in SR, alternate pathways consisting of carbonyl reductase and aldose reductases can functionally mask this defect (23). Genetic polymorphisms at the alternate SR pathways are potentially of interest (24). It is noteworthy that increased GRSs derived from SHP-related SNPs were associated with higher mortality risk. Nevertheless, further studies are needed to investigate how these genetic variants are functionally regulated in response to critical stress.

Clinical Implications

In our study cohort, GRS greater than or equal to 2 was present in 44.7% of patients in the ICU. SHP presented in 49.1% of the patients with GRS greater than or equal to 2, with 25.7% presenting with SHP in the beginning and 23.4% later. Each increase of phenylalanine by 1 μmol/dL was associated with an 8.7% relative increase in mortality risk. The clinical application of phenylalanine concentration and genetic polymorphism was compensatory (**Supplementary Fig. 4**, http://links.lww.com/CCM/H178). In patients without SHP in the beginning, GRS greater than or equal to 2 early predicted the development of deficient bioavailability of BH4 in response to stress, SHP, and subsequent mortality and also indicated close monitoring of phenylalanine levels. Prior studies have noted that inadequate BH4 bioavailability gives rise to immune dysfunction (25), nitric oxidase uncoupling, and dysregulated microvascular perfusion (17), all of which are factors associated with poor outcomes. The question left is whether and when adequate supplementation of BH4 improves the outcome of patients with GRS greater than or equal to 2. Although the measurement of phenylalanine is not currently available for most ICUs, it could be achieved based on the existing platform of daily screening for phenylketonuria among newborns or enzyme-mediated assays. Genetic screening to identify patients with GRS greater than or equal to 2 could be achievable within 3 hours after hospitalization in this era of infectious diseases and precision medicine.

The extremely elevated phenylalanine (> 60 μmol/dL) in phenylketonuria is associated with severely impaired brain development and early mortality in newborns (6). Intriguingly, a strong association has been observed between mild-to-moderate phenylalanine elevation and mortality in our patients (11.2–53.4 μmol/dL) and those with sepsis or critical illness (7, 9). In patients with GRS less than 2, SHP could be attributed to increased tissue breakdown along with multiple organ dysfunction, which is related to mortality. Although phenylalanine elevation might be a byproduct of metabolism at critical status, recent studies have suggested that dysregulated phenylalanine catabolism plays a key role in cardiac aging (26), and hyperphenylalaninemia has a direct toxic effect on organs with active inflammation (27). In addition, the elevation of phenylalanine indicates the decompensation of phenylalanine catabolism by overloaded alternative pathways that produce toxic metabolites such as phenylpyruvate, phenyllactate, and phenylacetate, known to promote oxidative stress (28–30). Nevertheless, whether SHP or phenylalanine-derived toxic metabolites play a direct role in poor outcomes needs further investigation.

Study Limitations

There are a few limitations to this study. First, the sample size was small due to difficulties enrolling patients with critical status. A larger sample size could better explore the network of potential genetic variants and the benefits of early genetic screening. Second, the function of the identified genetic polymorphisms

needs further investigation. Third, the relationship between phenylalanine levels and BH4 bioavailability would be better interpreted by serial measurements of pteridines in blood or urine. Finally, patients were at a single center and heterogeneous. However, the multivariable analysis demonstrated our findings were significant independent of reasons for admission, supporting the generalizability to polyvalent ICUs. On the other hand, multicenter studies are warranted for future clinical application.

CONCLUSIONS

SHP in patients admitted to the ICU was associated with a worse prognosis. The mechanisms involved in phenylalanine elevation include genetic polymorphisms located at de novo synthesis and recycling pathways of BH4. In patients without SHP, genetic polymorphisms associated with SHP measured using a GRS of greater than or equal to 2 was associated with the subsequent SHP and higher mortality risk. Integration of genetic screening, pteridine measurement, and monitoring of phenylalanine levels offers innovative ways to assess patients in critical condition and provides crucial information for precision medicine to improve outcomes in critical care.

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