**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 greater 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

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*See also p. 1670.*

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.
(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 hyperphenylalanemia is a prognostic biomarker for poor outcomes, rather than just an essential amino acid in patients with cardiovascular diseases.

Hyperphenylalanemia 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 hyperphenylalanemia 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 hyperphenylalanemia 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.

**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 included 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).

**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.
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 PCBD2 genes, respectively (Fig. 1B) (described in the Statistical Analyses section and in the Supplementary Methods, http://links.lww.com/CCM/H178).

### TABLE 1. Demographic and Laboratory Data

| Variables                              | Whole Cohort | Death | SURVIVOR | p   |
|----------------------------------------|--------------|-------|----------|-----|
|                                        | N = 497      | N = 156 | N = 341  |     |
| 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/m²)                | 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 m²) | 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 |

*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.
Genetic Polymorphisms and Clinical Factors Associated With SHP

In univariate analysis, factors with ability of discriminating patients with Pmax 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 to 11.2 μmol/dL versus Pmax less than 11.2 μmol/dL (right panel). 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. 2A–D) (Supplementary Table 4, Fig. 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 to 11.2 μmol/dL versus Pmax less than 11.2 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 = dihydrobioperin, CBR = carbonyl reductase, DHFR = dihydrofolate reductase, DHPR = dihydropyridine reductase, GTP = guanosine triphosphate, GTPCH = GTP cyclohydrolase, PAH = phenylalanine hydroxylase, PCD = pterin-4a-carbinolamine dehydratase, PTPS = 6-pyruvoyl tetrahydropterin synthase, qBH2 = quinonoid BH2, SR = sepiapterin reductase. 1′-OXPH4 = 6-(1′-oxo-2′-hydroxypropyl)-tetrahydropterin, 2′-OXPH4 = 6-(1′-hydroxy-2′-oxopropyl)-tetrahydropterin.
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

**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)

| Variables                        | Univariate | Multivariable (Model 1) | Multivariable (Model 2) |
|----------------------------------|------------|-------------------------|-------------------------|
|                                  | 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 reasona              | 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 Scoreb              | 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.
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. 1A, left panel), GRS greater than or equal to 2 did not significantly provide additional prognostic value (Fig. 1C, 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.
**TABLE 3.** Baseline Demographic and Laboratory Data in Patients With Different Baseline Phenylalanine Levels and Genetic Risk Scores (*N* = 497)

| Variables                                      | Baseline Phenylalanine < 11.2 μmol/dL | Baseline Phenylalanine ≥ 11.2 μmol/dL | p   | Baseline Phenylalanine < 11.2 μmol/dL | Baseline Phenylalanine ≥ 11.2 μmol/dL | p   |
|-----------------------------------------------|---------------------------------------|---------------------------------------|-----|---------------------------------------|---------------------------------------|-----|
|                                              | GRS < 2  | GRS ≥ 2  | p      | GRS < 2  | GRS ≥ 2  | p      |
|                                              | N = 218 | N = 165 |       | N = 57  | N = 57  |       |
| 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/m²)                       | 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                             |                                     |                                      |     |                                      |                                      |     |
| Hypertension                                  |                                     |                                      |     |                                      |                                      |     |
| Coronary disease                              |                                     |                                      |     |                                      |                                      |     |
| Atrial fibrillation                           |                                     |                                      |     |                                      |                                      |     |
| Chronic obstructive pulmonary disease         |                                     |                                      |     |                                      |                                      |     |
| 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 m²) | 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.

*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 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. 1B) (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. 1B). 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 oxide 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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