Supplementary materials

Stress hyperphenylalaninemia is associated with mortality in cardiac intensive care unit: clinical factors, genetic variants and pteridines

Chao-Hung Wang, MD, PhD [1,2]; Wei-Siang Chen, MD [1,3]; Min-Hui Liu, RN, NP, PhD [1,4]; Chi-Ying Lee, Msc[5]; Mei-Ying Wang, MD[1,3]; Chung-Yu Liang, MD[1,3]; Chien-Ming Chu, MD[6]; Huang-Ping Wu, MD[6]; Wen-Hsin Chen, MD[1,3]

[1] Heart Failure Research Center, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung;
[2] Chang Gung University College of Medicine, Taoyuan, Taiwan
[3] Intensive Care Unit, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung, Taiwan
[4] Department of Nursing, Chang Gung Memorial Hospital, Keelung, Taiwan
[5] Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, Taiwan
[6] Division of pulmonary, critical care and sleep medicine, Chang Gung Memorial Hospital, Keelung, Taiwan

Corresponding Author:
Chao-Hung Wang, MD, PhD,
Heart Failure Research Center,
Division of Cardiology, Department of Internal Medicine,
Chang Gung Memorial Hospital,
222 Mai Chin Road, Keelung, Taiwan
Tel. 886 2 24313131, ext. 2245
E-mail: bearty54@gmail.com; bearty@adm.cgmh.org.tw

Supplementary methods

Patient enrollment

From October 2017 to May 2021, patients with cardiovascular diseases were consecutively enrolled at the cardiac ICU based on the following inclusion criteria: They (1) had Acute Physiology And Chronic Health Evaluation (APACHE II) scores ≥15 or were intubated due to respiratory failure; (2) needed to stay in the ICU >48 hours; and (3) were older than 20 years old. The exclusion criteria were: (1) patients with comorbid disorders other than the main cause for admission that might compromise their survival within three months, such as terminal stage cancer; or (2) patients who died before baseline blood collection for measuring phenylalanine. All patients provided informed consent. The study was designed and carried out in accordance with the principles of the Declaration of Helsinki. Ethical approval was granted by the institutional Review Board of Chang Gung Memorial Hospital [(1) 201507968B0: approval date: August, 2017, study title: Metabolomics-Based Assessment of Short-Term Metabolic Disturbance after Acute Heart Failure, and in Response to Nutritional Supplements; (2) 201701750B0: approval date: August, 2018, study title: Clinical value of Metabolomics-based assessment of metabolism status tackling nutri-metabolic disturbance issues in intensively cared heart failure patients; (3) 201801514B0: approval date: January 2019, study title: Applications of metabolomics-based nutritional assessment and a simplified metabolic panel to patients in intensive care unit; and (4) 202000831B0: approval date: July 2020, study title: Establishment of key gene detection reagent kits and services for predicting short-term mentality in the intensive care unit).

Blood sampling and examination

Fasting blood samples were collected in EDTA-containing tubes in the early morning, the day after obtaining informed consent, and then every 3 to 4 days, twice a week. In the study for BH4 and pteridine measurement, additional blood was collected into 4-mL Vacutainer tubes containing 7.2 mg K_2-EDTA –Dickinson) and 0.1 mL 2% (w/v) dithiothreitol (Sigma Aldrich, Oakville, ON), yielding final concentration of 0.05% dithiothreitol. Plasma was separated in the dark, and frozen immediately at -80ºC until analysis. Measurement of phenylalanine and pteridines was described below. The Pmax was defined as the highest concentration of the phenylalanine throughout the whole period of stay in ICU. Measurement of other parameters, including albumin, estimated glomerular filtration rate (eGFR), C-reactive protein, hemoglobin, and alanine aminotransferase (ALT) was performed in the central laboratory.

Phenylalanine and tyrosine Measurements

Plasma concentrations of phenylalanine and tyrosine were quantified by ultra-performance liquid chromatography. Plasma samples (100µL) were precipitated with 10% sulfosalicylic acid. After protein precipitation and centrifugation, derivatization was initiated by AQC in acetonitrile. Amino acids were then analyzed using the ACQUITY UPLC System, consisting of a Binary Solvent Manager, a Sample Manager, and a Tunable UV detector. We used EmpowerTM 2 Software to control the system and collect data. Separations were performed on a 2.1×100mm ACQUITY BEH C18 column at a flow rate of 0.70mL/min. The average intra-assay coefficient of variation was 4.6% for phenylalanine and 4.3% for tyrosine. The total coefficient of variation was 3.7% for phenylalanine and 3.1% for tyrosine. The detection limit was 3.3 µM for phenylalanine and 2.0 µM for tyrosine. The linear range was 25–500 µM.

Analysis of Pteridines in plasma
Concentrations of biopterin, 7,8-dihydrobiopterin (BH2), and BH4 were quantified by liquid chromatography tandem mass spectrometry (ULPC-MS/MS). For biopterin, BH2 and BH4, methods of oxidation by acid iodine and alkaline iodine were modified from previous studies by Fukushima T et al and Fekkes D et al. The methods of UPLC-MS/MS were modified from previous study, and validated according to the guideline of bioanalysis from European Medicines Agency, European Union (EMEA, 2015). Briefly, analysis was performed on a Sciex API-4000 triple quadrupole mass spectrometer (Framingham, MA, USA) coupled with a Waters ACQUITY UPLC system (Milford, MA, USA). A calibration curve was prepared in water containing 0.05% DTT over different concentrations of pteridines. The precision expressed as coefficients of variation ranged from 4.05% to 10.09%. The accuracy ranged from 94.77% to 105.85%.

DNA extraction and quantification
After centrifugation, buffy coat was collected to extract gDNA by using Qiagen® DNA Mini kit based on the manufacturer's protocols (Qiagen, Valencia, CA, USA). DNA samples were quantified with a Qubit 3.0 fluorometer using a Qubit dsDNA HS Assay Kit according to the manufacturer's protocols (Thermo fisher, Waltham, MA, U.S.). DNA concentration should be ≥50 ng/μl.

Genotyping and quality control
A total of 270 samples were genotyped using standard Illumina protocols and commercially available genotyping arrays (Illumina, Inc., San Diego, CA): Infinium Asian Screening Array-24 v1.0 BeadChip. In total, 650,000 single nucleotide polymorphisms (SNPs) were genotyped in the arrays. The array aimed to capture coverage in Koreans, Mongolians, and Malaysians and was superior to existing reference populations, including populations of 2,000 Japanese, 1,600 Korean, hundreds of Taiwanese, 100 Malaysian, and 1000 Chinese (Mongolian and Singaporean) individuals. The clinical research content included variants associated with established cardiovascular diseases, relevant pharmacogenomics markers, and curated exonic content based on ClinVar, NHGRI, pharmacogenomic, HLA variant, ACMG, and ExAC databases. Samples were removed if any one of the following criteria was met: (1) per-individual call rate less than 97%; (2) wrongly assigned sex; (3) pihat greater than 0.2, by quality control performed using PLINK 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/). All samples were qualified. Variants were removed if any one of the following criteria was met: (1) genotyping call rate less than 97%; (2) p value in Hardy-Weinberg equilibrium test less than 0.00001 (Supplementary Figure 2).

In silico analysis of genetic variants
We sought to link risk variants to candidate genes of phenylalanine metabolic pathway by assessing their effect on gene expression levels. First, since blood phenylalanine concentration could predict the short-term mortality risk, we performed a pathway database analysis and built up the candidate gene list by KEGG. Second, since myopathies often arise from the cardiovascular tissue and cardiac muscle tissue, we identified eQTLs from related cardiac tissues in the Genotype-Tissue Expression– project (GTEx, released version 8.0). Finally, we mapped a gene expression and inferred the association between genetically predicted gene expression and risk of

interest. The targeted SNP selection criteria included (1) variants in phenylalanine-related pathways. According to KEGG, 4 pathways (hsa00360: phenylalanine metabolism, hsa00400: phenylalanine, tyrosine and tryptophan biosynthesis, hsa00350: tyrosine metabolism, and hsa00790: folate biosynthesis) were considered as phenylalanine-related pathways with 62 genes included in these pathways; (2) *p*-values <0.01 in at least one of the three genetic models: additive, dominant, and recessive models. Logistic regression model (for additive model) and Fisher exact test (for dominant and recessive models) were used to calculate p-values; (3) minor allele frequency <10%; (4) variants can regulate mapped or the nearest gene expression in GTEx; and (5) variants on linkage disequilibrium with $r^2 > 0.8$ were excluded (Supplementary Figure 2).

Direct genotyping

The direct genotyping of imputed SNPs (PCBD2, AKR1C3 and CBR1) was performed with TagMan® SNP Genotyping Assays (Catalogue number: 4351379) using the ABI PRISM 7700 system (Applied Biosystems, Foster City, CA). The reactions were run with non-template controls in each run and for each custom assay.

Statistical analyses

Results are expressed as the mean ± SD for variables with normal distribution, as the median [interquartile range (IQR)] for variables with skewed distribution, and as the number (percentage) for categorical variables. We compared data using the Mann-Whitney U test and Chi-square when appropriate. We estimated receiver operating characteristic (ROC) curve and used Youden's index to identify the cutoff value of variables. Area under the curve (AUC) of ROCs was presented. To calculate the genetic risk score (GRS), genetic variants were assigned values according to dominant or recessive inheritance model. Since the gene expression of PCBD2 and CBR1 was linear to the number of risk alleles, we used a dominant (additive) model for rs319598 (PCBD2) and rs20572 (CBR1). However, for rs17395698 (AKR1C3), the gene expression was similar in "0 risk allele" and "1 risk allele" and was only different in "2 risk alleles", so we used the recessive model. The 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. A univariate logistic regression model was used to determine the value of the variables for discriminating Pmax ≥11.2 μmol/dL from Pmax <8.5 μmol/dL. By logistic multivariable analysis, we adjusted for co-variates to better identify strong independent factors. Variables with a p value <0.05 in univariate analysis were included in the multivariable analysis.

We estimated the necessary sample size using the genetic association power calculator. 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. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. To compare time-dependent outcomes, we performed Kaplan-Meier analyses with a log-rank test. All statistical analyses were two-sided and performed using SPSS software (version 22.0, SPSS, Chicago, IL, USA). A p value of < 0.05 was considered significant.

Biopterin measurement

Experimental section

Chemicals and reagents

Potassium iodide, native biopterin, and biopterin-d_3 as the internal ssandard were purchased from Santa Cruz Biotechnlogy, Inc. (Dallas, Texas, USA). Butylated hydroxytoluene, formic acid, and hydrochloric acid were from Sigma-Aldrich (St. Louis, MO, USA). Iodine, ammounium hydroxide, ascorbic acid and dithioerythritol were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Milli-Q water was produced by a Millipore Direct-Q® 8 Ultrapure water system (Merck, Darmstadt, Germany). Stock solutions of biopterin and biopterin-d_3 were prepared according to the previous study (1), and stored at −80 °C. Working solutions were dilutd to the appropriated concentrations for UPLC-MS/MS analysis.

Pretreatment of plasma samples

Sample pretreatment process was based on the previous study with minor modification (1). Each sample was ready for biopterin preparation, acidic oxidation of BH_4 and BH_2 to biopterin, or basic oxidation of BH_2 to biopterin. Samples from acidic and basic oxidation were then pretreated by solid-phase extraction (SPE) to isolate the biopterin from the complex matrices. SPE was performed by utilizing the Oasis MCX cartridges (1 cc, 30 mg, Waters Corp. Milford, MA, USA). The samples were reconstructed and transferred for UPLC-MS/MS analysis.

UPLC-MS/MS

A Waters ACQUITY UPLC system (Waters Corp. Milford, MA, USA) equipped with a SCIEX API-4000 triple quadrupole mass spectrometer operated in positive electrospray ionization and scan mode of multiple reaction monitoring was applied to determine biopterin in this study. Separation of biopterin from the other interferences was achieved by a Hypersil Gold column (50 x 2.1 mm, 1.9 µm, Thermo Fisher Scientific Inc., Waltham, MA USA). Method validation of the UPLC-MS/MS method (in terms of linearity, limit of detection, limit of quantification), accuracy and precision were investigated to evaluate to feasibility for the analysis of Biopterin in plasma samples. Accuracy and precision were from 99.5% to 114.4% and from 0.8% to 11.7%, respectively. The results of method validation met the criteria of European Medicines Agency (EMA) (2).

Calculation of BH_2 and BH_4

According to the previous research, [BH_2] = [total biopterin after basic oxidation reaction] − [biopterin without oxidation reaction], and [BH_4] = [total biopterin after acidic oxidation reaction] − [total biopterin in basic oxidation reaction] (3). The UPLC-MS/MS chromatograms of biopterin in human plasma without and with oxidation reaction are shown in the Figure below.

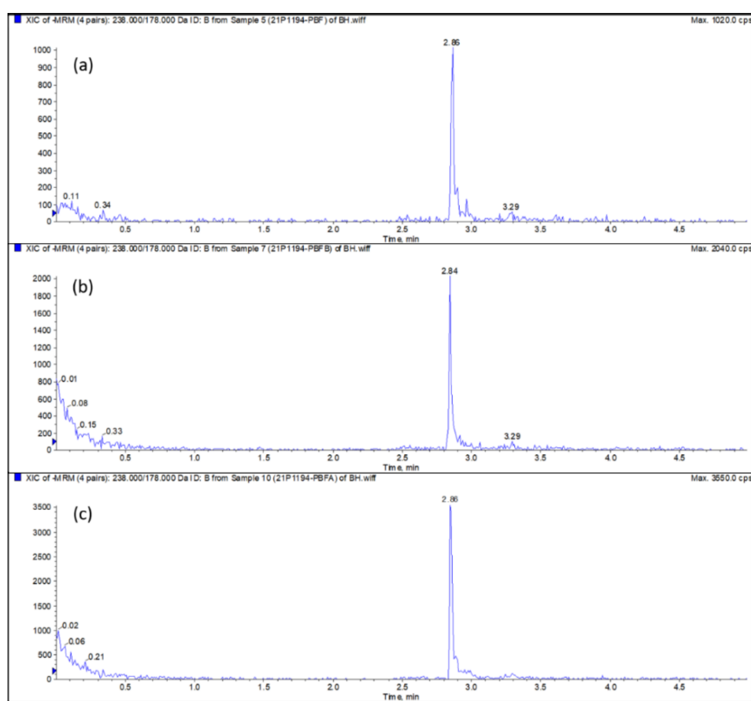
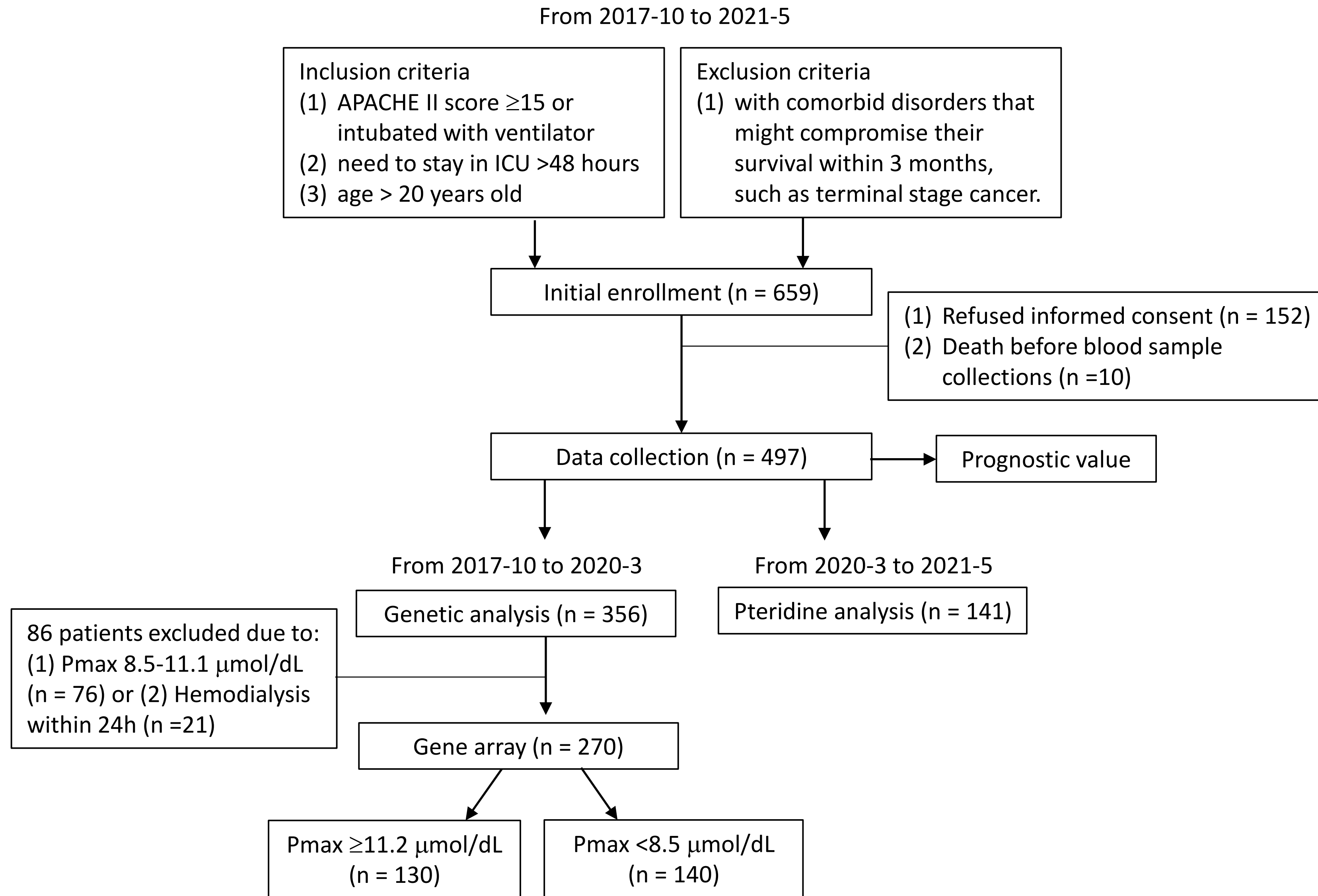


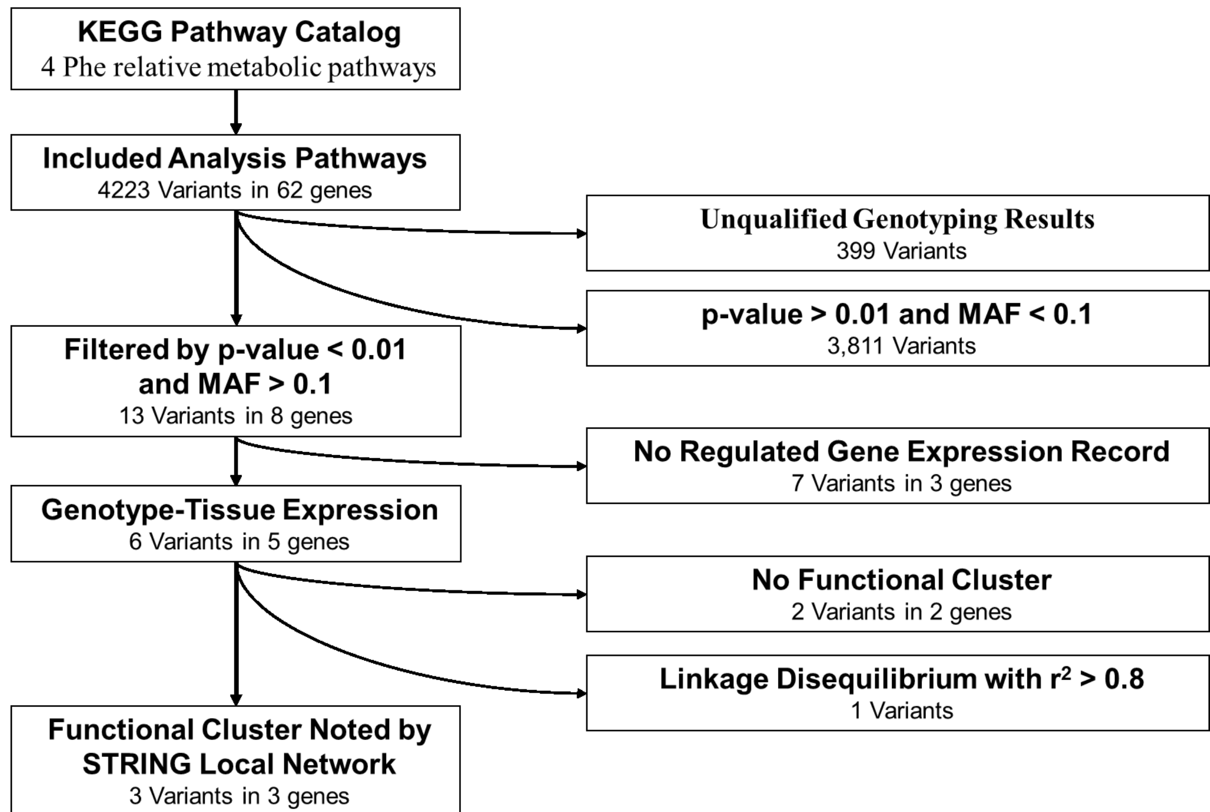
Figure. UPLC-MS/MS chromatograms of (a) biopterin in human plasma without oxidation reaction, (b) biopterin in human plasma with basic oxidation reaction, and (c) biopterin in human plasma with acidic oxidation reaction.

Reference

1. FISMEN, Lise, et al. Simultaneous quantification of tetrahydrobiopterin, dihydrobiopterin, and biopterin by liquid chromatography coupled electrospray tandem mass spectrometry. *Analytical biochemistry*, 2012, 430.2: 163-170.
2. EMEA (2015) Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev 1 Corr 2
3. VALDÉS, Cristian, et al. Simplified HPLC methodology for quantifying biological pterins by selective oxidation. *Journal of Chromatography B*, 2017, 1055: 113-118.

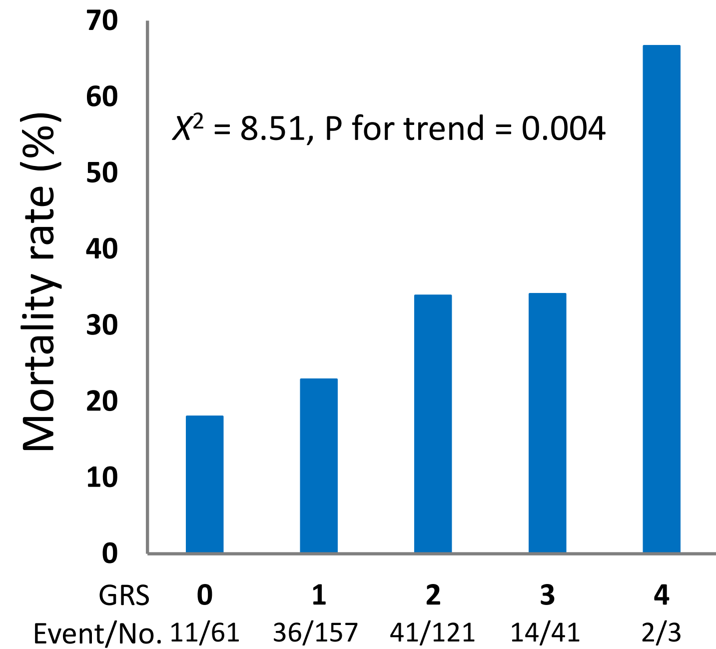


Supplementary Figure 1. Flow diagram of the study

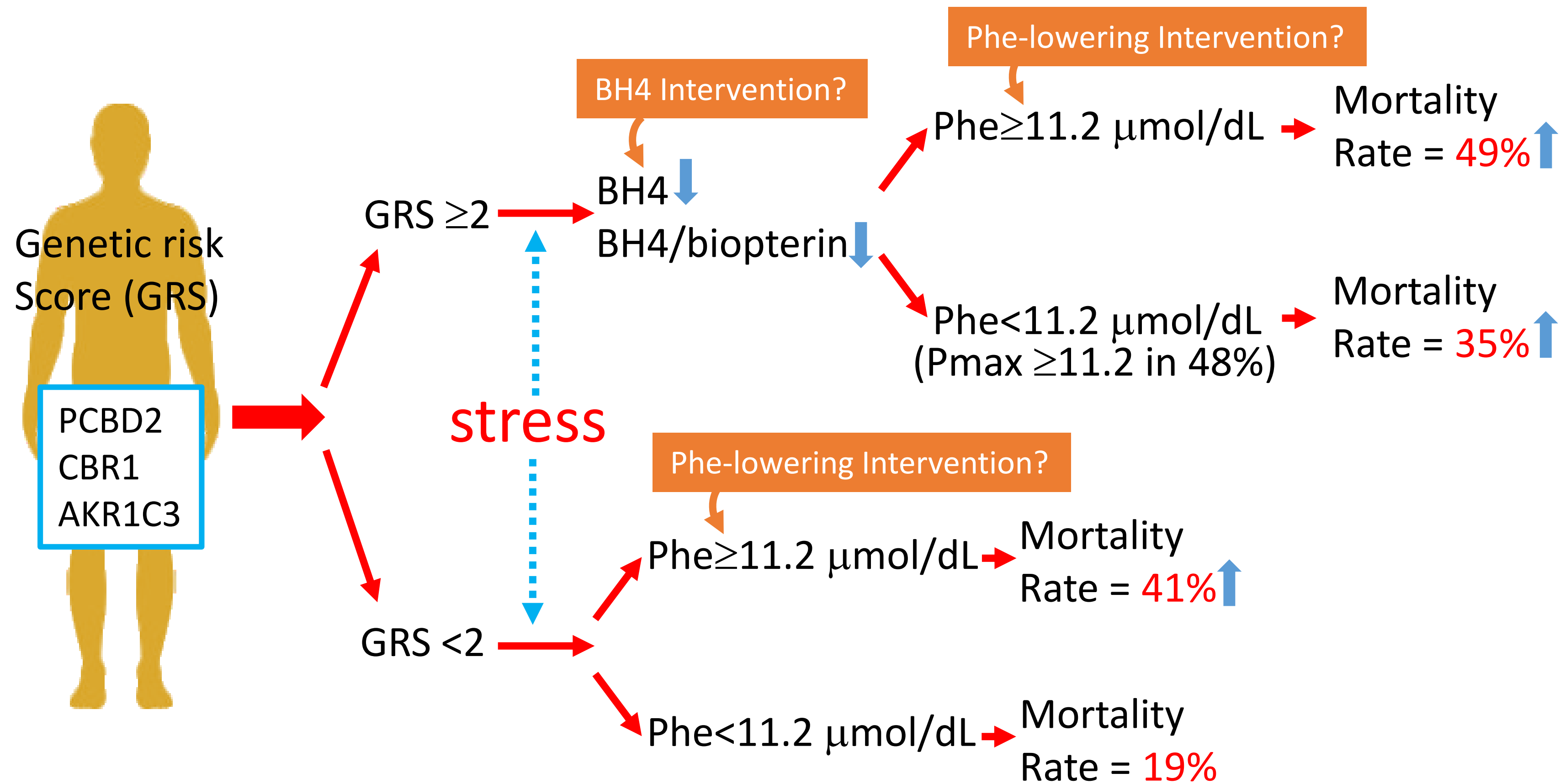


Supplementary Figure 2. The variant selection workflow and criteria.

According to KEGG, 4 pathways (hsa00360: phenylalanine metabolism, hsa00400: phenylalanine, tyrosine and tryptophan biosynthesis, hsa00350: tyrosine metabolism, and hsa00790: folate biosynthesis) were considered as phenylalanine-related pathways, and 62 genes were included in these pathways. The functional clusters noted by STRING were tetrahydrobiopterin biosynthetic process and ACT domain.



Supplementary Figure 3. The mortality rate in different genetic risk score (GRS) in patients with baseline phenylalanine level <11.2 μmol/dL



Supplementary Figure 4. Clinical implication of genetic screening by genetic risk score (GRS) and baseline phenylalanine (Phe) concentration. BH4, tetrahydrobiopterin; Pmax, maximal Phe concentration during stay in intensive care unit

Supplementary Table 1. COX univariate and multivariable analysis of phenylalanine level and clinical and laboratory variables for predicting mortality in 90 days (n = 497)

	Univariate		Multivariable (model 1)		Multivariable (model 2)	
variables	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Phenylalanine, baseline (μmol/dL)	1.087(1.068-1.106)	<0.001	1.069(1.046-1.092)	<0.001	1.076(1.053-1.101)	<0.001
Age (years)	1.016(1.003-1.029)	0.012	1.019(1.005-1.034)	0.008	1.009(0.995-1.024)	0.202
Non-cardiac reason*	1.565(1.134-2.165)	0.006	1.126(0.799-1.587)	0.497	1.148(0.815-1.616)	0.430
Diabetes mellitus	1.118(0.817-1.530)	0.487				
Hypertension	1.137(0.813-1.529)	0.453				
Atrial fibrillation	1.518(1.025-2.250)	0.037	1.274(0.833-1.947)	0.264	1.328(0.875-2.016)	0.183
C-Reactive Protein (log)	1.734(1.354-2.219)	<0.001	1.288(0.992-1.673)	0.058	1.399(1.085-1.805)	0.010
Cholesterol (mg/dL)	0.987(0.983-0.992)	<0.001	0.995(0.991-0.999)	0.008	0.994(0.990-0.998)	0.006
Albumin(log)	0.012(0.003-0.049)	<0.001	0.167(0.026-1.054)	0.057	0.158(0.024-1.031)	0.054
eGFR(log)	0.646(0.461-0.905)	0.011	1.115(0.748-1.660)	0.593	1.030(0.695-1.526)	0.882
SOFA score	1.212(1.160-1.265)	<0.001	1.158(1.095-1.224)	<0.001		
APACHE II score	1.122(1.092-1.153)	<0.001			1.087(1.053-1.122)	<0.001

HR, Hazard ratio; CI, confidence interval; eGFR, estimated glomerular filtration rate. Model 1, multivariable analysis adjusting for all significant clinical and laboratory variables in the univariate analysis and SOFA score; model 2, multivariable analysis adjusting for all significant clinical and laboratory variables in the univariate analysis and APACHE II score.*reasons for admission to intensive care unit

Supplementary Table 2. Demographic and laboratory data in patients with maximal phenylalanine level (Pmax) ≥11.2 μmol/dL versus <8.5 μmol/dL (n = 270)

Variables	All	Pmax <8.5 μmol/dL	Pmax ≥11.2 μmol/dL	
	n = 270	n = 140	n = 130	*p* value
Age (years)	70.1±13.2	70.4±13.19	69.9±13.32	0.739
Male (%)	175(64.8)	82(58.6)	93(71.5)	0.030
APACHE II score	17.8±5.57	17.6±5.2	18.1±6.0	0.445
SOFA score	6.01±2.99	5.65±2.75	6.40±3.19	0.039
Body mass index (kg/m^2)	24.3 (21.6-27.4)	24(21.3-27.3)	24(21.2-27.4)	0.690
Non-cardiac reason (%)*	196(72.6)	92(65.7)	104(80.0)	0.009
Co-morbidity				
Diabetes mellitus (%)	126(46.7)	67(47.9)	59(45.4)	0.715
Hypertension (%)	174(64.4)	87(62.1)	87(66.9)	0.447
Coronary disease (%)	123(45.6)	59(42.1)	63(48.5)	0.328
Atrial fibrillation (%)	47(17.4)	24(17.1)	23(17.7)	1.000
COPD (%)	28(10.4)	16(11.4)	12(9.2)	0.690
Ventilator use (%)	191(70.7)	101(72.1)	90(69.2)	0.688
Inotropic agent use (%)	68(25.2)	26(18.6)	42(32.3)	0.011
Days in ICU (day)	12.6±9.55	12±7.57	13.2±11.3	0.301
Laboratory data				
Hemoglobin (g/dL)	11.5±7.41	11.7±9.12	11.4±5	0.713
CRP (mg/L)	24.5(7.70-66.6)	24.8(7.54-57.2)	23.9(8.7-85.8)	0.190
Cholesterol (mg/dL)	141±60.0	146.4±45.89	135.3±72.07	0.142
Albumin (g/dl)	3.24(2.83-3.68)	3.3(2.9-3.7)	3.2(2.8-3.7)	0.255
eGFR (ml/min/1.73 m^2)	60.1±52.6	67.2±53.41	52.4±50.77	0.020
ALT (U/L)	32.0(18.5-66.0)	28.5(17.3-55.8)	38(19-84)	0.060
Bilirubin, total (mg/dL)	0.50(0.30-1.00)	0.5(0.3-0.7)	0.6(0.4-1.23)	<0.001
Creatine kinase (U/L)	48.0(15.8-138)	32.0(10.2-97.4)	67.4(21.2-199)	0.001
Phenylalanine (μmol/dL)	9.55±4.57	7.57±0.92	15.36±6.33	<0.001
Tyrosine (μmol/dL)	8.11±5.46	6.62±2.17	10.07±7.48	<0.001

Data are expressed as the mean ± SD for variables with normal distribution, median [interquartile range (IQR)] for variables with skewed distribution, and as number (percentage) for categorical variables. APACHE, acute physiology and chronic health evaluation; ALT, alanine aminotransferase; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); ICU, intensive care unit; SOFA, sequential organ failure assessment. *reasons for admission in intensive care unit.

Supplementary Table 3. Details of the 13 selected variants in the phenylalanine-related pathways to differentiate phenylalanine ≥11.2 μmol/dL from phenylalanine <8.5 μmol/dL in the genetic analysis (n = 270)

dbSNP	Mapped or Nearest Gene	Function	RAF	Chr.	Position	*p* value	KEGG pathway [†]	Gene expression trend*	BH4 biosynthesis	Proxy
rs35710857	CBR1	exonic	0.20	21	37444120	<0.001	hsa00790	upregulate	+	rs20572
rs76923303	TPO	intronic	0.86	2	1446942	0.009	hsa00350	NA		
rs10206020	TPO	intergenic	0.82	2	1557927	0.006	hsa00350	downregulate		
rs10879346	TPH2	intronic	0.45	12	72351835	0.004	hsa00790	no match		
rs1567919	TPO	intronic	0.86	2	1432014	0.006	hsa00350	no match		
rs17395698	AKR1C3	intronic	0.21	10	5104908	0.002	hsa00790	upregulate	+	
rs20572	CBR1	Exonic	0.20	21	37444973	<0.001	hsa00790	upregulate	+	rs35710857
rs319598	PCBD2	upstream	0.49	5	134240235	0.001	hsa00790	downregulate	+	
rs4570625	TPH2	upstream	0.45	12	72331923	0.002	hsa00790	NA		
rs4822454	MIF-AS1	intergenic	0.50	22	24255208	0.006	hsa00350	upregulate		
rs6582078	TPH2	intronic	0.44	12	72374891	0.003	hsa00790	no match		
rs7305115	TPH2	Exonic	0.45	12	72372862	0.005	hsa00790	no match		
rs9285933	PCBD2	intronic	0.20	5	134257519	0.004	hsa00790	no match	+	

BH4, tetrahydrobiopterin; Chr., chromosome; dbSNP, single nucleotide variations, microsatellites, and insertions /deletions database, version 150; RAF, risk allele frequency; Proxy: linkage disequilibrium $r^2 > 0.8$.

*Gene expression trend: gene expression with risk allele, based on the Genotype-Tissue Expression project to characterize the tissue-specific gene expression and regulation. NA, no significant eQTLs were found for SNP in all tissues; no match, no significant eQTLs matching itself.

[†]KEGG pathways included hsa00360 (phenylalanine metabolism), hsa00400 (phenylalanine, tyrosine and tryptophan biosynthesis), hsa00350 (tyrosine metabolism), and hsa00790 (folate biosynthesis).

Supplementary Table 4. Demographic and laboratory data in patients with different genetic risk scores (GRS) in the pteridine analysis (n = 141)

Variables	All	GRS=0	GRS=1	GRS=2	GRS=3	GRS=4	
	n=141	n=19	n=59	n=49	n=11	n=3	*P* for trend
Age (years)	70.8±13.8	68.6±14.8	72.0±12.8	70.7±14.5	70.3±15.3	63.0±12.3	0.472
Male (%)	100(70.9)	14(73.7)	35(59.3)	40(81.6)	8(72.7)	3(100)	0.110
APACHE II score	18.2±5.89	17.4±6.34	18.0±5.59	19.0±6.40	17.6±4.39	17.3±7.57	0.945
SOFA score	6.71±2.78	6.21±2.74	6.54±2.44	7.00±2.71	6.27±3.88	10.0±5.20	0.041
LVEF (%)	56.0±18.7	56.1±13.0	54.2±22.8	57.2±15.5	58.4±19.0	62.7±7.37	0.493
Body mass index (kg/m^2)	24.7±5.59	25.0±2.86	24.5±6.31	24.7±5.75	26.1±2.79	23.0±10.5	0.906
Co-morbidity							
Diabetes mellitus (%)	67(47.5)	8(42.1)	31(52.5)	21(42.9)	7(63.6)	0(0)	0.709
Hypertension (%)	90(63.8)	8(42.1)	42(71.2)	31(63.3)	7(63.6)	2(66.7)	0.427
Coronary disease (%)	59(41.8)	7(36.8)	26(44.1)	21(42.9)	5(45.5)	0(0)	0.772
Atrial fibrillation (%)	13(9.2)	2(10.5)	6(10.2)	4(8.2)	1(9.1)	0(0)	0.598
COPD (%)	12(8.5)	0(0)	3(5.1)	8(16.3)	1(9.1)	0(0)	0.106
Ventilator use (%)	88(62.4)	14(73.7)	36(61.0)	31(63.3)	5(45.5)	2(66.7)	0.326
Inotropic agent use (%)	38(27.0)	4(21.1)	20(33.9)	11(22.4)	1(9.1)	2(66.7)	0.761
Days in ICU (day)	9.0(4.0-15.5)	9.0(3.0-13.0)	10.0(4.0-17.0)	9.0(4.5-16.5)	8.0(7.0-12.0)	8.0(2.0-14.0)	0.281
Laboratory data							
Hemoglobin (g/dL)	11.9±5.99	11.7±3.26	12.8±8.49	11.5±3.13	10.9±2.84	8.50±0.53	0.291
CRP (mg/L)	36.9(10.2-106)	19.4(3.84-166)	31.4(11.1-77.2)	26.0(10.2-86.4)	87.7(25.0-178)	236(158-366)	0.001
Cholesterol (mg/dL)	142±72.0	149±54.4	149±96.2	138±46.5	119±40.0	95.0±41.6	0.141
Albumin (g/dl)	3.2(2.85-3.64)	3.15(2.68-3.85)	3.22(2.90-3.60)	3.15(2.93-3.55)	3.52(2.78-3.70)	2.71(2.60-3.49)	0.767
eGFR	37.4(15.0-64.7)	45.6(23.5-74.6)	37.4(14.6-62.8)	34.9(15.6-65.5)	25.2(10.8-61.2)	78.8(3.68-276)	0.018
ALT (U/L)	27(14-61.5)	22.0(10.0-113)	30.0(14.0-51.0)	25.0(20.0-60.5)	27.0(12.0-74.0)	31.0(27.0-81.0)	0.957
Bilirubin, total (mg/dL)	0.7(0.4-1.0)	0.60(0.40-0.70)	0.70(0.40-1.00)	0.60(0.35-1.10)	0.80(0.60-1.20)	0.40(0.20-0.70)	0.953
Creatine kinase (U/L)	162(53-454)	383(38.0-838)	128(47.0-518)	162(62.0-381)	231(68.0-368)	26.0(20.0-880)	0.551
BH2 (ng/mL)	3.45±1.19	3.32±1.41	3.56±1.13	3.33±1.19	3.40±1.34	4.43±0.31	0.183

BH4 (ng/mL)	5.64±2.50	6.44±2.69	5.88±2.58	5.46±2.32	4.15±2.15	4.13±1.46	0.047
BH4/BH2 ratio	1.84±1.08	2.35±1.49	1.78±0.943	1.84±0.965	1.51±1.29	0.949±0.404	0.026
BH4/(total biopterin)	0.60±0.13	0.65±0.13	0.61±0.11	0.60±0.13	0.52±0.18	0.47±0.09	0.009

Data are expressed as the mean ± SD for variables with normal distribution, median [interquartile range (IQR)] for variables with skewed distribution, and as number (percentage) for categorical variables. APACHE, acute physiology and chronic health evaluation; ALT, aspartate and alanine aminotransferase; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); LVEF, left ventricular ejection fraction; SOFA, sequential organ failure assessment.

Supplementary Table 5. Baseline demographic and laboratory data in patients with different baseline phenylalanine levels (n = 497)

Variables	Baseline phenylalanine ＜11.2 μmol/dL	Baseline phenylalanine ≥ 11.2 μmol/dL	
	n = 383	n = 114	*P*-value
Age (years)	72.3±13.1	68.2±13.1	0.004
Male (%)	228(59.5)	85(74.6)	0.004
APACHE II score	18.2±5.50	18.5±7.13	0.622
SOFA score	6.33±3.03	7.11±3.87	0.025
LVEF (%)	57.4±24.3	51.5±34.0	0.057
Body mass index (kg/m^2)	24.0(21.3-27.3)	24.0(21.2-27.4)	0.960
Non-cardiac reason (%)*	198(51.7)	62(54.4)	0.697
Co-morbidity			
Diabetes mellitus (%)	182(47.5)	52(45.6)	0.720
Hypertension (%)	248(64.8)	76(66.7)	0.706
Coronary disease (%)	162(42.3)	55(48.2)	0.261
Atrial fibrillation (%)	57(14.9)	16(14.0)	0.822
COPD (%)	34(8.9)	7(6.1)	0.351
Ventilator use (%)	271(70.8)	70(61.4)	0.059
Inotropic agent use(%)	113(29.5)	46(40.4)	0.029
Days in ICU (day)	10(5-18)	8(2-18)	0.002
Laboratory data			
Hemoglobin (g/dL)	11.3±6.3	10.8±3.3	0.467
CRP (mg/L)	32.8(8.7-89.0)	23.9(7.6-64.4)	0.530
Cholesterol (mg/dL)	140.2±55.8	125.8±47.7	0.016
Albumin (g/dl)	3.4±2.1	3.3±1.2	0.732
eGFR	40.0(14.6-77.0)	28.5(11.0-62.3)	0.034
Bilirubin, total (mg/dL)	0.5(0.3-0.8)	0.7(0.5-1.7)	<0.001
Creatine kinase (U/L)	65.0(21.1-199)	102(44.3-344)	0.001
Tyrosine (μM)	69.1±26.3	138.1±96.9	<0.001

Data are expressed as the mean ± SD for variables with normal distribution, median [interquartile range (IQR)] for variables with skewed distribution, and as number (percentage) for categorical variables. APACHE, acute physiology and chronic health evaluation; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (ml/min/1.73 m^2); LVEF, left ventricular ejection fraction; SOFA, sequential organ failure assessment. *reasons for admission in intensive care unit.