Novel biomarkers identifying hypertrophic cardiomyopathy and its obstructive variant based on targeted amino acid metabolomics

Lanyan Guo1, Bo Wang2, Fuyang Zhang1, Chao Gao1, Guanyu Hu1, Mengyao Zhou2, Rutaow Wang1, Hang Zhao1, Wenjun Yan1, Ling Zhang1, Zhiling Ma1, Weiping Yang1, Xiong Guo1, Chong Huang1, Zhe Cui1, Fangfang Sun1, Dandan Song1, Liwen Liu2, Ling Tao1

1Department of Cardiology, Xijing Hospital, The Fourth Military Medical University, Xi’an, Shaanxi 710032, China; 2Department of Ultrasound, Xijing Hospital, The Fourth Military Medical University, Xi’an, Shaanxi 710032, China.

Abstract

Background: Hypertrophic cardiomyopathy (HCM) is an underdiagnosed genetic heart disease worldwide. The management and prognosis of obstructive HCM (HOCM) and non-obstructive HCM (HNCM) are quite different, but it also remains challenging to discriminate these two subtypes. HCM is characterized by dysmetabolism, and myocardial amino acid (AA) metabolism is robustly changed. The present study aimed to delineate plasma AA and derivatives profiles, and identify potential biomarkers for HCM.

Methods: Plasma samples from 166 participants, including 57 cases of HOCM, 52 cases of HNCM, and 57 normal controls (NCs), who first visited the International Cooperation Center for HCM, Xijing Hospital between December 2019 and September 2020, were collected and analyzed by high-performance liquid chromatography–mass spectrometry based on targeted AA metabolomics. Three separate classification algorithms, including random forest, support vector machine, and logistic regression, were applied for the identification of specific AA and derivatives compositions for HCM and the development of screening models to discriminate HCM from NC as well as HOCM from HNCM.

Results: The univariate analysis showed that the serine, glycine, proline, citrulline, glutamine, cystine, creatinine, cysteine, choline, and aminoacidic acid levels in the HCM group were significantly different from those in the NC group. Four AAs and derivatives (Panel A; proline, glycine, cysteine, and choline) were screened out by multiple feature selection algorithms for discriminating HCM patients from NCs. The receiver operating characteristic (ROC) analysis in Panel A yielded an area under the ROC curve (AUC) of 0.83 (0.75–0.91) in the training set and 0.79 (0.65–0.94) in the validation set. Moreover, among 10 AAs and derivatives (arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline) with statistical significance between HOCM and HNCM, 3 AAs (Panel B; arginine, proline, and ornithine) were selected to differentiate the two subgroups. The AUC values in the training and validation sets for Panel B were 0.83 (0.74–0.93) and 0.82 (0.66–0.98), respectively.

Conclusions: The plasma AA and derivatives profiles were distinct between the HCM and NC groups. Based on the differential profiles, the two established screening models have potential value in assisting HCM screening and identifying whether it is obstructive.

Keywords: Hypertrophic cardiomyopathy; Amino acids; Targeted metabolomics; Biomarkers; Algorithm

Introduction

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease worldwide with an unexpectedly high prevalence of 1:500 to 1:200.[1,2] It is estimated that HCM affects nearly 20 million people worldwide. However, only 10% of patients with HCM are clinically identified by a non-invasive imaging approach.[3,4] According to the left ventricular outflow tract gradient (LVOTG), obstructive HCM (HOCM) is defined as a maximum LVOTG (LVOTGmax) ≥30 mmHg; otherwise, it is classified as non-obstructive HCM (HNCM). HOCM is a common but catastrophic cause of sudden cardiac death (SCD) in young athletes and results in tremendous emotional, social, financial, and medical burdens for families across the country.[4] However, LVOTG is dynamic and is greatly influenced by myocardial contractility and preload, and enhanced
myocardial contractility or decreased preload increases LVOTG.\[5\]

To date, the screening and diagnosis of HCM and even HOCM is still largely dependent on imaging modalities, including echocardiography (Echo), cardiac magnetic resonance and computed tomography, and genetic testing.\[6-11\] These imaging modalities have a series of limitations such as high interobserver variability, high intraobserver variability, high false-negative rate, high medical cost, high dependence on professional guidance, low popularity, increased radiation exposure, and intolerance among certain patients. These weaknesses profoundly limit the universal screening, especially among those living in undeveloped regions or countries. Therefore, it is necessary to explore an objective, convenient, and easily available approach to apply in clinical practice for HCM initial screening.

Circulatory biomarkers have been widely used in the early diagnosis or large-scale screening of diseases. However, biomarkers for HCM screening remain clinically lacking. Non-targeted and targeted metabolomics have emerged as powerful tools for mapping circulating metabolite changes and screening candidate biomarkers across a spectrum of cardiovascular diseases.\[15\] To date, the potential of metabolomics in the discovery and establishment of HCM biomarkers has not been studied. We have provided evidence that the dysregulation of amino acid (AA) metabolism is tightly associated with pathological cardiac remodeling in response to ischemic insult.\[16,17\] Recent clinical observations have also indicated that alterations in circulating AAs correlate with adverse cardiovascular events.\[18-21\] A multi-omics analysis has demonstrated that myocardial AA metabolism is robustly changed in HCM.\[22\] Thus, we explored whether circulating AA and derivatives concentrations are changed in patients with HCM and if they could serve as promising biomarkers for HCM screening.

In the present study, we recruited 166 participants, including 57 normal controls (NCs), 57 cases of HOCM, and 52 cases of HNCM, to depict the AA and derivatives profiles, and identify possible screening biomarkers utilizing high-performance liquid chromatography–mass spectrometry (HPLC–MS)-based targeted AA metabolomics. Three separate classification algorithms, including random forest (RF), support vector machine (SVM), and logistic regression (LR), were applied for the identification of specific AA and derivatives compositions for HCM and the development of screening models to discriminate HCM from NC as well as HOCM from HNCM.

### Methods

#### Ethical approval

This prospective, single-center observational study was approved by the Ethics Committee of Xijing Hospital, Fourth Military Medical University (No. KY20150120-1), and it complied with the Declaration of Helsinki. Written informed consent was obtained from all participants.

### Study population

A total of 166 fasting participants who first visited the International Cooperation Center for HCM, Xijing Hospital between December 2019 and September 2020 were consecutively recruited for the present study. The study flowchart is shown in Figure 1. For sample size calculation, at least 10 events per variable are needed.

HCM was diagnosed according to the 2014 European Society of Cardiology guidelines for the diagnosis and management of HCM.\[6\] LVOTG was measured with color-guided continuous-wave Doppler Echo (Philips Medical Systems, Bothell, Washington, USA) at rest and provocation, such as Valsalva maneuver or exercise stress with a supine bicycle exercise (semirecumbent and tilting bicycle ergometer; Lode BV, Groningen, Netherlands).\[23,24\] The presence of a peak LVOTG $\geq$ 30 mmHg at rest and with exercise stress is considered indicative of HOCM, while HNCM is defined by LVOTG $<$ 30 mmHg at rest and with exercise stress.\[16,23\] NCs were individuals who were suspected of having HCM and visited the International Cooperation Center for HCM in Xijing Hospital, but were eventually precluded from having HCM due to normal ventricular wall thickness. All participants underwent transthoracic echocardiography independently conducted by two experienced ultrasound technicians. All
procedures were in accordance with the guidelines of the American Society of Echocardiography.[16]

**Inclusion and exclusion criteria**

The inclusion criteria were as follows: (1) age between 18 and 80 years; (2) clinical diagnosis of HCM[19] as indicated by a maximal end-diastolic wall thickness \( \geq 15 \text{ mm} \) anywhere in the left ventricle that is not explained solely by loading conditions. More limited hypertrophy (13–14 mm) can be diagnostic when present in family members of a patient with HCM; and (3) informed consent for the present study. The exclusion criteria were as follows: (1) severe or acute inflammation within 1 month; (2) autoimmune diseases; (3) uncontrolled hypertension (HP); (4) moderate-severe aortic stenosis; (5) end-stage liver or renal failure; (6) other health behaviors that may affect the study, such as intemperance; (7) needle phobia and recent history (<1 month) of blood transfusion or hemodialysis; (8) severe non-cardiac disease with an expected survival of <1 year; and (9) unwillingness to participate.

**Targeted AA metabolomics in plasma**

To exclude the dietary impact on plasma AA and derivatives levels, venous blood samples were collected from participants enrolled in the study after overnight fasting. Blood samples were collected in ethylenediaminetetraacetic acid vacutainer tubes and centrifuged at 644 \( \times g \) for 10 min, and aliquoted samples were stored at \(-80^\circ \text{C} \) until processing. Plasma samples (100 \( \mu \text{L} \)) were added to 400 \( \mu \text{L} \) of precooled methanol acetonitrile solution (1:1, v/v), vortexed for 60 s, and placed at \(-20^\circ \text{C} \) for 1 h to precipitate protein. The samples were then centrifuged at 14,000 \( \times g \) at 4\( ^\circ \text{C} \) for 20 min, and the supernatant was separated and tested by MS using an Agilent 1290 Infinity ultrahigh-performance liquid chromatography system and 5500 QTRAP mass spectrometer (AB SCIEX, Framingham, MA, USA). A quality control (QC) sample was set up, among each interval of a certain number of experimental samples, to detect and evaluate the stability and repeatability of the detection system, and peaks with a relative standard deviation (RSD) >30% in QC samples were removed from the peak table. The standard mixture of AA metabolites was used for the correction of chromatographic retention time and the identification of metabolites. Multireaction monitoring mode was used to detect the ion pair. MultiQuant 3.0.3 (AB SCIEX, Framingham, MA, USA) was used to extract the chromatographic peak area and retention time.

**Statistical analysis**

Continuous variables were presented as the mean ± standard deviation or median (Q1, Q3) when appropriate, and categorical variables were presented as counts (percentages). The independent \( t \)-test was used for the comparison of two continuous variables with normal distribution, and non-normally distributed data were compared using a two-sided nonparametric Mann–Whitney \( U \)-test. \( \chi^2 \) or Fisher’s exact test was used for the comparison of unordered categorical variables. Spearman’s rank correlation analysis was performed to analyze the correlations between AAs and other parameters in HCM.

Variables with \( P < 0.05 \) were considered significant AA metabolites and were used as alternatives for further selection analysis. To identify potential biomarkers for HCM, we used three integrated methods, including RF, SVM, and LR, based on multiple feature selection algorithms. We applied a voting procedure to select potential biomarkers, which rewarded features appearing in the optimal subsets by the ensemble methods. The selected variables were ranked in decreasing order by their contribution to the classification model. Several highly ranked metabolites that led to the highest and most stable area under the receiver operating characteristic (ROC) curve (AUC) value of the model were considered potential biomarkers for developing screening HCM panels. Thereafter, the screening models were developed and assessed according to LR, SVM, and RF. We performed a 5-fold cross-validation for each of the three classifiers. Finally, the screening model was presented as LR. Youden’s index was used to define the optimal discriminative cutoff value. The ROC curve was applied to evaluate the performance of the statistical model. In addition, 70% of the samples were randomly selected as the training set, and the remaining 30% of the samples were selected as the validation set in each group. All statistical analyses were performed using SPSS statistics 26.0 (IBM, Armonk, NY, USA) and R software (version 4.0.2) (https://www.r-project.org/).

**Results**

**Baseline characteristics**

A total of 166 participants were enrolled in this study, including 57 NCs and 109 cases of HCM (52 cases of HNCM and 57 cases of HOCM). The demographic and clinical characteristics were shown in Table 1. The mean age was 47.4 ± 14.3 years in the HCM group and 34.0 ± 11.8 years in the NC group \( (t = -6.078, P < 0.001) \). Males accounted for 72.5% (\( n = 79 \)) and 43.9% (\( n = 25 \)) in the HCM and NC groups \((\chi^2 = 14.046, P < 0.001)\), respectively. Body mass index (BMI, 25.14 ± 3.93 kg/m\(^2\)) vs. 23.38 ± 3.87 kg/m\(^2\), \( t = -2.536, P = 0.012 \) and systolic blood pressure (125.61 ± 18.73 mmHg vs. 120.00 ± 13.23 mmHg, \( t = -2.222, P = 0.028 \)) were higher in HCM than NC. 36.7% of HCM patients had a previous history of HP, which was significantly higher than that of NC \((36.7\% \text{ vs. } 0, \chi^2 = 27.558, P < 0.001)\), and all received regular antihypertensive drugs and the blood pressure was under control.

There was no significant difference in end-diastolic volume, end-systolic volume, or left ventricular ejection fraction between the HCM and NC groups. The ratio of early diastolic mitral valve velocity to atrial contraction mitral valve velocity (E/A) was significantly lower \((0.85 [0.68, 1.35] \text{ vs. } 1.48 [1.21, 1.66], Z = -4.893, P < 0.001)\), while the ratio of early diastolic mitral valve velocity to early diastolic mitral annular velocity (E/e') was significantly higher in HCM as compared with NC \((13.86 [10.45, 17.27] \text{ vs. } 8.14 [6.91, 9.34], Z = -7.054, P < 0.001)\),
which reflected left ventricular diastolic dysfunction. The diameters of the left atrium (39.06 ± 6.07 mm vs. 30.29 ± 3.90 mm, t = -3.770, P < 0.001), the maximum wall thickness (MWT; 22.57 ± 5.51 mm vs. 8.12 ± 1.43 mm, t = -25.783, P < 0.001), LVOTGrest (21.00 [4.00, 77.00] mmHg vs. 3.20 [2.75, 4.00] mmHg, Z = -6.928, P < 0.001), and LVOTGmax (53.00 [16.75, 124.00] mmHg vs. 9.00 [8.00, 14.00] mmHg, Z = -7.465, P < 0.001) in the HCM group were significantly higher than those in the NC group. The MWT (23.84 ± 5.22 mm vs. 21.17 ± 5.32 mm, t = -2.594, P = 0.011), LVOTGrest (76.00 [43.00, 103.00] mmHg vs. 4.00 [3.00, 6.00] mmHg, Z = -8.974, P < 0.001), and LVOTGmax (122.00 [92.00, 149.50] mmHg vs. 16.25 [8.95, 22.00] mmHg, Z = -8.992, P < 0.001) were also significantly different between the HOCM and HNCM groups.

### Plasma AA and derivatives levels and their correlations with baseline characteristics

Targeted AA metabolomics was performed, and 31 AA metabolites were measured in the plasma samples. The total ion chromatogram indicated the reliability of the metabolomics analysis [Supplementary Figure 1, http://links.lww.com/CM9/B159]. The results revealed that, except for spermidine and putrescine, 29 metabolites were absolutely quantified in the samples. There were significant differences in plasma serine, glycine, proline, citrulline, glutamine, cystine, creatinine, cysteine, choline, and aminoacidic acid levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HOCM and HNCM groups [Supplementary Table 1, http://links.lww.com/CM9/B159]. Correlations between 29 metabolites levels and baseline characteristics were analyzed. There were significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups. In addition, there were also significant differences in plasma arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline levels between the HCM and NC groups.
the training set, and the remaining 30% of the samples were selected as the validation set in each group. The ROC curve was applied to evaluate the performance of the classifiers. Independent panels with distinct combinations of AA metabolites were developed for the discrimination of HCM from NC and HOCM from HNCM.

First, to differentiate the HCM and NC groups, four metabolites (Panel A: proline, glycine, cysteine, and choline) were filtered out because their combination showed the optimum discrimination efficacy and practical performance [Figure 3A, B and Supplementary Figure 2A, http://links.lww.com/CM9/B159]. The correlation of selected metabolites was poor in Panel A (all $R < 0.70$; Supplementary Figure 3A, http://links.lww.com/CM9/B159). The ROC analyses, utilizing RF, SVM, and LR, showed similar efficacy with an approximate AUC of 0.80 in distinguishing the HCM and NC groups [Figure 3C and Supplementary Figure 2B, http://links.lww.com/CM9/B159]. The following screening formula was deduced using LR to differentiate the HCM and NC groups:

$$Y = \frac{1}{1 + e^{-Z}};$$

where

$$Z = 0.63 - 0.68 \times \text{proline} - 1.24 \times \text{glycine} + 1.01 \times \text{cysteine} + 1.05 \times \text{choline}.$$

The expression level of the AAs and derivatives after log10 conversion was brought into the probability $P$ value.
calculated by the above panel formula. The best cutoff value for classification was derived from Youden’s index. Figures 3D and 3E show the relationships among the accuracy, sensitivity, specificity, and cutoff value. If the Y-value was >0.54, it indicated HCM; otherwise, it indicated NC. The corresponding accuracy, sensitivity, and specificity were 0.78, 0.80, and 0.75. Finally, 70% of the samples were randomly selected as the training set, and the remainder was selected as the validation set. The AUC values of the training and validation sets were 0.83 (0.75–0.91) and 0.79 (0.65–0.94), respectively [Figure 3F]. These results demonstrated that this screening model has good efficacy in the discrimination of HCM patients from NCs.

Statistical differences in age, sex, BMI, and previous history between the NC and HCM groups were observed in the baseline characteristics [Table 1]. To test whether the mismatch of clinical characteristics influenced the model establishment and discriminating efficacy, we performed a sensitivity analysis stratified by age, sex, and BMI. The analysis exhibited a consistent AUC value of approximately 0.80 across all the subgroups [Supplementary Figure 4, http://links.lww.com/CM9/B159]. These results indicated that the establishment and discriminating efficacy of the model was not influenced by age, sex, or BMI. Furthermore, to test the effect of a previous history of HP on discriminative ability, we performed an additional exploratory analysis by removing 40 cases of HCM combined with HP history. There was still a significant difference between the HCM without HP and NC groups in terms of ten differential AAs and derivatives [Supplementary Table 2, http://links.lww.com/CM9/B284]. In addition, there was no evident difference between HCM patients with HP and without HP in terms of the ten AAs and derivatives [Supplementary Table 3, http://links.lww.com/CM9/B284]. The subgroup analysis showed that in HCM patients with or without HP, the AUC values were 0.79 (0.69–0.89) and 0.84 (0.76–0.91), respectively [Supplementary Figure 5, http://links.lww.com/CM9/B159]. The DeLong test showed that there was no statistical significance between the overall AUC and the AUC of HCM patients with or without HP (P = 0.636 and 0.739, respectively), or between the AUC of HCM patients with HP and without HP (P = 0.463) [Supplementary Table 4, http://links.lww.com/CM9/B159]. These results suggested that the history of HP had limited influence on the discriminative ability of the screening model.

**Screening model to discriminate HOCM from HNCM**

Another major clinical challenge is to distinguish HOCM from HNCM. Therefore, we aimed to construct a predication panel to further differentiate HOCM from HNCM. Only arginine, proline, and ornithine were left in the model (Panel B) [Figure 4A, B and Supplementary Figure 2C, http://links.lww.com/CM9/B159]. The poor correlation of the selected metabolites is presented in
Supplementary Figure 3B, http://links.lww.com/CM9/B159, and the following screening formula was deduced:

\[ Y = \frac{1}{1 + e^{-Z}} \]

where

\[ Z = 0.11 + 0.73 \times \text{arginine} - 0.39 \times \text{proline} - 1.11 \times \text{ornithine}. \]

The AUC values and discriminative efficacy analyzed by LR and SVM were better than those analyzed by RF [Figure 4C and Supplementary Figure 2D, http://links.lww.com/CM9/B159]. If the Y-value was >0.53, it indicated HOCM; otherwise, it indicated HNCM. The corresponding accuracy, sensitivity, and specificity were 0.80, 0.81, and 0.79, respectively [Figures 4D and E]. The AUC values of the training and validation sets were 0.83 (0.74–0.93) and 0.82 (0.66–0.98), respectively [Figure 4F]. These results highlighted that this screening model has promising potential to differentiate HOCM from HNCM.

**Discussion**

HCM is an underdiagnosed monogenetic cardiovascular disease accompanied by metabolic disorders. Growing evidence indicates that aberrations in myocardial energy metabolism contribute to the development of hypertrophy and heart failure.\(^{27,28}\) In the present study, we profiled plasma AAs and derivatives, and identified AA metabolite panels with high discriminative accuracy to distinguish HCM individuals. Furthermore, multiple AAs and derivatives were significantly correlated with clinical parameters, such as the MWT and LVOTG\(_{\text{rest}}\). These findings provide an opportunity for developing panels of plasma biomarkers to help physicians initially identify HCM.

HCM may be defined clinically, genetically, and histologically with high clinical heterogeneity. Some individuals remain asymptomatic throughout their lifetime with little need for treatment, while some may exhibit a progressive course, such as severe heart failure, stroke, chest pain, or even SCD.\(^{29}\) It remains challenging to diagnose HCM promptly in clinical practice. Thus, a tremendous clinical challenge exists to differentiate or predict which individuals experience HCM. Even though HCM is diagnosed, it is still difficult to discern HOCM and HNCM, owing to the vulnerable LVOTG. Developing novel non-invasive, objective, and available screening biomarkers may help identify HCM in the population and improve care quality, especially in medically underserved patients. Metabolites are promising tools to understand the pathophysiological changes involved in disease onset and progression, and they can be used as biomarkers for disease prediction, diagnosis, and prognosis.\(^{30,31}\) Metabolomic technologies, such as ultra-performance liquid chromatography-mass spectrometry, (semi)quantitatively measure hundreds of unique metabolites, identifying a broad range of metabolic pathways, and only small volumes of biofluids.

![Figure 4: Screening model to discriminate HOCM from HNCM. (A) Weight ranking of candidate AAs. (B) Cumulative AUC of AAs in the discrimination of HOCM and HNCM. (C) ROC curves analyzed by LR, SVM, and RF. (D) Curve of the accuracy and the corresponding cutoff values. (E) Curve of specificity, sensitivity, and the corresponding cutoff values. (F) ROC curves of the training and validation sets. AAs: Amino acids; AUC: Area under the ROC curve; CI: Confidence interval; HCM: Hypertrophic cardiomyopathy; HNCM: Non-obstructive HCM; HOCM: Obstructive HCM; LR: Logistic regression; NCs: Normal controls; RF: Random forest; ROC: Receiver operating characteristic; SVM: Support vector machine.](http://links.lww.com/CM9/B159)
are needed. Moreover, sophisticated machine learning algorithms are utilized to analyze heterogeneous datasets to discover useful patterns that would be difficult for even well-trained professionals to identify, and they have been generally recognized as an effective approach in clinical diagnostics, precision treatments, and health monitoring.

Specifically, aberrations in cardiac AA metabolism have been shown to be associated with cardiac hypertrophy and heart failure progression. Emerging evidence has demonstrated that metabolic aberrations may participate in the initiation and development of HCM, even in preclinical HCM. By utilizing targeted AA metabolomics, the present study investigated the circulating AA and derivatives profile in HCM patients. Interestingly, plasma arginine, phenylalanine, tyrosine, ornithine, proline, alanine, asparagine, creatine, tryptophan, and choline levels correlated with LVOTG, and ornithine levels correlated with MWT, indicating that the above metabolites are associated with the severity of HCM. These results suggested that systemic AA and derivatives dysmetabolism may be involved in the pathogenesis of HCM and that changes in the plasma AA and derivatives profile may have the potential to detect or diagnose HCM.

Univariate analysis showed that 10 AAs and derivatives (serine, glycine, proline, citrulline, glutamine, cystine, creatinine, cysteine, choline, and aminoacidic acid) in the HCM groups were significantly different from those in the NC group. After systematically profiling AA metabolic alterations in HCM, four metabolites (proline, glycine, cysteine, and choline) were selected as a potential AA and derivatives panel for discerning HCM from the NC, considering discriminative efficacy and clinical utility. In the training and validation sets, the AUC values of the ROC analysis were 0.83 and 0.79, respectively, demonstrating the good value of detecting HCM.

To minimize the confounding factors that may affect the metabolomic differences, we included a “pure” NC group accompanied by no common comorbidities. The mean age of the “pure” NC group was 34 years, which may limit generalizability to the general population. Among the different factors that may influence systemic metabolism, age and sex were not matched between HCM patients and their NC counterparts. In the past 10 years, the average age of patients diagnosed with HCM has increased by 10 years to 51 years, and male patients account for 60% of the cases. In the present study, the average age of HCM patients was nearly 47 years, and males accounted for 72% of the cases. Although the baseline characteristics were mismatched in the NC and HCM groups, the sensitivity analysis stratified by age, sex, and BMI showed a consistent AUC value of approximately 0.80 across all the subgroups. Similarly, the previous history of HP had a limited effect on the discriminative performance. These results indicated that age, sex, BMI, and previous history of HP exerted a limited influence on the establishment and discriminating efficacy of the model.

Several AAs and derivatives have been reported to be involved in the regulation of cardiovascular pathology. Plasma homocysteine levels are associated with the incidence of cardiovascular diseases. Homocysteine reduction improves cardiac function and ameliorates pathological structural remodeling. Choline ameliorates cardiac dysfunction by regulating the expression of genes involved in the ketone body and fatty acid metabolism. This process may be involved in the activation of the sirtuin 3/adenosine monophosphate (AMP)-activated protein kinase pathway. Accumulating evidence demonstrates that choline supplementation prevents myocardial hypertrophy, fibrosis, and the inflammatory response induced by pressure overload. The elevation in plasma choline levels is associated with the incidence of heart failure. Glycine promotes the synthesis of the antioxidant metabolite, glutathione, and it reduces the production of mitochondrial oxidized protein and increases adenosine triphosphate, thereby improving cardiac function and ventricular remodeling. The decrease in plasma glycine levels observed in HCM patients indicates that HCM patients are in a state of oxidative stress. Proline improves myocardial remodeling and reduces infarct size and cardiomyocyte death after myocardial infarction. Proline plays a protective role in postischemic heart failure by promoting oxidative phosphorylation and improving the redox state. The above studies suggest that cysteine, choline, glycine, and proline may play an important role in the pathogenesis of HCM, and more research is needed to confirm these findings. The panel comprised of the four AAs and derivatives has the potential to be used for broad population-based screening of HCM.

Left ventricular outflow tract obstruction (LVOTO) has malignant effects on patients. A previous study has reported that HNCM patients have higher morbidity and arrhythmic risk than HOCM patients, while HOCM patients, who are generally much older, have a higher BMI, New York Heart Association class, and overall risk. Additionally, therapy choice and prognosis are quite different between HOCM and HNCM patients, and septal incision therapy may be recommended for medical refractory patients with LVOTG ≥ 50 mmHg. Therefore, provocative maneuvers, despite their relatively high cost and limited availability, should be considered in patients with low peak resting gradients (ie, <30 mmHg) to induce the presence of LVOTO, particularly for those with symptoms, even if provocative tests have some lethal risks.

In the present study, among the 10 AAs and derivatives (arginine, phenylalanine, tyrosine, proline, alanine, asparagine, creatine, tryptophan, ornithine, and choline) with statistical significance between the HOCM and HNCM groups, three metabolites (arginine, proline, and ornithine) were validated as potential biomarkers to further differentiate HOCM from HNCM with similar AUC values of 0.83 and 0.82 in the training and validation sets, respectively. Arginine levels have been linked with heart failure in a rat model. HCM patients show specific features of endothelial dysfunction detectable in peripheral blood that are more severe in HOCM than HNCM. In the present study, the level of plasma arginine increased in HCM, particularly in HOCM, and the levels of proline and ornithine, as the main products of
arginine metabolism, decreased, indicating that arginine metabolism disorders occur in HCM, particularly for those with HOCM. Therefore, this panel can be used as a supplement to distinguish whether there is an obstruction for HCM patients, especially those with inconclusive Echo results or contraindications to stress Echo. Recognizing the existence of obstruction is helpful to guide clinical treatment strategies.

The present study developed two AA and derivatives panels to discriminate HCM patients from NCs as well as HOCM from HNCM patients with high sensitivity and specificity. It should be noted that the AAs and derivatives selected in these panels are different, which may indicate that distinct pathological mechanisms exist in AA metabolism during HNCM and HOCM progression.

Limitations
The present study had several limitations. First, the study was performed in a single center, even though the center is the largest HCM center in northwestern China. Second, a total of 166 participants were enrolled in the study, resulting in a relatively small sample size. Third, because plasma metabolites reflect systemic inflammation and metabolic changes, the mechanistic understanding of such differential AA metabolites involved in HCM pathophysiology and whether the changes in plasma AA and derivatives profiles in HCM contribute to disease progression is still undetermined. Fourth, the present study used targeted AA metabolomics to discriminate HCM patients from NCs. The adaptation of non-targeted metabolomics may adequately map the metabolic changes in these patients. Fifth, genotypes were not tested for all the patients, even though the clinical HCM phenotypes may be independent of the genotypes. Finally, validation of these results in a large prospective cohort is required to address the value for future clinical application in HCM initial screening. Therefore, large-scale, multiple-center, and non-targeted metabolomics studies combined with genomics are needed to validate the application of metabolic changes detection in HCM screening and detection.

Conclusions
The present study delineated the distinct AA and derivatives profiles in HCM patients compared to NCs. Utilizing multiple algorithms, two separate AA and derivatives panels were established to discriminate HCM patients from NCs as well as HOCM from HNCM. These easily available screening models may have potential value as alternative non-invasive and objective biomarkers for HCM screening and diagnosis. Further proper identification of HOCM from HNCM by health professionals may assist in prompt diagnosis and precise clinical care.

Funding
This research was funded by the National Key Research & Development Program of China (No. 2018YFA0107400) and Program for Chang-Jiang Scholars and Innovative Research Team in University (No. PCSIRT-14R08).

Conflicts of interest
None.

References
1. Maron BJ. Clinical course and management of hypertrophic cardiomyopathy. N Engl J Med 2018;379:653–668. doi: 10.1056/NEJMra1710575.
2. Sensenari C, Ingle J, Maron MS, Maron BJ. New perspectives on the prevalence of hypertrophic cardiomyopathy. J Am Coll Cardiol 2015;65:1249–1254. doi: 10.1016/j.jacc.2015.01.019.
3. Maron MS, Hellawell JL, Lucove JC, Farkaneh-Far R, Olivetto L. Occurrence of clinically diagnosed hypertrophic cardiomyopathy in the United States. Am J Cardiol 2016;117:1651–1654. doi: 10.1016/j.amjcard.2016.02.044.
4. Burns J, Jean-Pierre P. Disparities in the diagnosis of hypertrophic obstructive cardiomyopathy: a narrative review of current literature. Cardiol Res Pract 2018;2018:3750879. doi: 10.1155/2018/3750879.
5. Gekse JB, Soraja P, Ommen SR, Nishimura RA. Variability of left ventricular outflow tract gradient during cardiac catheterization in patients with hypertrophic cardiomyopathy. JACC Cardiovasc Interv 2011;4:704–709. doi: 10.1016/j.jcin.2011.02.014.
6. Authors/Task Force Members, Elliott PM, Anastasakis A, Borger MA, Borggreve M, Cecchi F, et al. 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy: The task force for the diagnosis and management of hypertrophic cardiomyopathy of the European Society of Cardiology. Eur Heart J 2014;35:2733–2779. doi: 10.1093/eurheartj/eht284.
7. Turner MC, Kerut EK, McKinnie J, Davis M, Hinton C. Cardiac CT angiography in the emergency room: apical hypertrophic cardiomyopathy presenting as acute coronary syndrome. Echocardiography 2017;34:773–775. doi: 10.1111/echo.13499.
8. Qin L, Chen C, Gu S, Zhou M, Xu Z, Ge Y, et al. A radiomic approach to predict myocardial fibrosis on coronary CT angiography in hypertrophic cardiac hypertrophy. J Cardiol 2021;337:113–118. doi: 10.1016/j.jcid.2021.04.060.
9. Puntmann VO, Gekker R, Duckett S, Mirels J, Schnackenburg B, Graefe M, et al. Left ventricular chamber dimensions and wall thickness by cardiovascular magnetic resonance: comparison with transthoracic echocardiography. Eur Heart J Cardiovasc Imaging 2013;14:240–246. doi: 10.1093/ehjci/jes145.
10. Augusto JB, Davies RH, Bhuma AN, Knott KD, Seraphim A, Allarh M, et al. Diagnosis and risk stratification in hypertrophic cardiomyopathy using machine learning wall thickness measurement: a comparison with human test-retest performance. Lancet Digit Health 2021;3:e20–e28. doi: 10.1016/S2589-7500(20)30267-3.
11. Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, et al. 2020 AHA/ACC guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: a report of the American College of Cardiology/American Heart Association Joint Committee on clinical practice guidelines. Circulation 2020;142; e358–e631. doi: 10.1161/CIR.0000000000000937.
12. Captur G, Manisty CH, Raman B, Marchi A, Wong TC, Ariga R, et al. Maximal wall thickness measurement in hypertrophic cardiomyopathy: biomarker variability and its impact on clinical care. JACC Cardiovasc Imaging 2021;14:2123–2134. doi: 10.1016/j.jcmg.2021.03.032.
13. Manrai AK, Funke BH, Rehm HL, Olesen MS, Maron BA, Scolovits P, et al. Genetic misdiagnoses and the potential for health disparities. N Engl J Med 2016;375:655–665. doi: 10.1056/NEJMsa1507092.
14. Teramoto R, Fujino N, Konno T, Nomura A, Nagata Y, Tsuda T, et al. Late gadolinium enhancement for prediction of mutation-positive hypertrophic cardiomyopathy on the basis of panel-wide sequencing. Circ J 2018;82:1139–1148. doi: 10.1253/circj.CJ-17-1012.
15. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. Circulation 2012;126:1110–1120. doi: 10.1161/CIRCULATIONAHA.111.060368.
16. Wang W, Zhang F, Xia Y, Zhao S, Yan W, Wang F, et al. Defective branched chain amino acid catabolism contributes to cardiac dysfunction and remodeling following myocardial infarction. Am J
