**Supplementary Materials**

**Supplementary Table 1: Absolute quantification of plasma AAs.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Metabolites (µmol/L)** | **NC (*n*= 57)** | **HCM (*n* = 109)** | ***Z* values** | ***P* values** | **HNCM (*n* = 52)** | **HOCM (*n* = 57)** | ***Z* values** | ***P* values** |
| Glutamate | 79.49 (51.19, 106.50) | 86.06 (64.34, 117.37) | −1.546 | 0.122 | 87.33 (64.25, 117.46) | 83.54 (64.92, 115.73) | −0.206 | 0.837 |
| Glycine | 450.19 (325.36, 654.68) | 413.62 (288.56, 574.23) | −2.104 | 0.035 | 470.93 (319.09, 601.28) | 389.39 (282.96, 478.21) | −1.935 | 0.053 |
| Lysine | 165.09 (134.98, 182.75) | 160.02 (143.38, 187.49) | −0.269 | 0.788 | 159.72 (143.08, 187.61) | 161.07 (143.19, 188.43) | −0.400 | 0.689 |
| Aspartate | 24.19 (6.28, 51.24) | 26.36 (8.16, 48.44) | −0.575 | 0.565 | 34.23 (8.06, 52.64) | 24.23 (7.97, 36.13) | −0.928 | 0.353 |
| Arginine | 56.51 (46.20, 75.75) | 63.00 (50.78, 77.24) | −1.425 | 0.154 | 57.26 (39.92, 70.35) | 70.06 (56.04, 85.21) | −3.258 | 0.001 |
| Serine | 116.29 (100.87, 137.15) | 104.59 (90.40, 120.84) | −2.797 | 0.005 | 105.56 (92.15, 119.99)  | 103.54 (89.91, 121.30) | −0.382 | 0.702 |
| Methionine | 24.40 (20.53, 28.82) | 25.62 (21.85, 28.85) | −0.837 | 0.403 | 25.20 (22.10, 28.79) | 25.86 (21.69, 29.28) | −0.067 | 0.947 |
| Phenylalanine | 63.29 (57.54, 70.15) | 64.68 (56.96, 73.45) | −0.612 | 0.541 | 68.83 (60.55, 78.48) | 60.28 (53.70, 68.56) | −3.422 | 0.001 |
| Tyrosine | 65.10 (56.16, 72.32) | 60.77 (53.81, 72.12) | −0.901 | 0.368 | 64.33 (57.07, 77.62) | 57.83 (52.36, 68.62) | −2.366 | 0.018 |
| Leucine | 62.32 (46.62, 113.42) | 58.71 (49.85, 97.20) | −0.487 | 0.626 | 63.00 (49.70, 102.81) | 57.34 (50.09, 91.77) | −0.874 | 0.382 |
| Isoleucine | 94.90 (57.78, 129.78) | 104.49 (63.03, 132.76) | −0.820 | 0.412 | 107.56 (64.57, 135.23) | 99.07 (59.64, 132.35) | −0.212 | 0.832 |
| Histidine | 124.62 (76.41, 164.86) | 134.38 (80.71, 168.11) | −0.891 | 0.373 | 133.02 (81.93, 167.23) | 141.90 (78.06, 169.54) | −0.346 | 0.729 |
| Proline | 345.59 (226.75, 480.80) | 237.55 (167.32, 399.20) | −2.787 | 0.005 | 300.35 (195.70, 478.27) | 186.62 (149.34, 364.97) | −2.973 | 0.003 |
| Valine | 265.17 (231.47, 313.63) | 287.80 (242.01, 321.17) | −1.210 | 0.226 | 283.21 (243.61, 323.62) | 287.88 (233.14, 313.84) | −0.752 | 0.452 |
| Threonine | 122.14 (92.78, 150.28) | 114.93 (95.01, 129.99) | −1.630 | 0.103 | 118.79 (95.40, 131.56) | 112.73 (95.01, 128.48) | −0.959 | 0.338 |
| Alanine | 223.21 (147.33, 509.47) | 195.51 (158.34, 444.56) | −0.545 | 0.586 | 226.50 (175.75, 521.83) | 179.65 (144.23, 408.16) | −2.451 | 0.014 |
| Asparagine | 57.14 (48.63, 71.46) | 58.47 (47.68, 68.73) | −0.212 | 0.832 | 63.92 (50.30, 72.56) | 55.39 (47.09, 63.95) | −2.111 | 0.035 |
| Creatine | 29.62 (21.09, 40.25) | 29.72 (22.42, 41.66) | −0.185 | 0.853 | 26.00 (19.31, 36.34) | 33.28 (24.24, 44.77) | −2.396 | 0.017 |
| Citrulline | 30.18 (24.18, 38.57) | 38.22 (31.26, 46.10) | −4.148 | <0.001 | 38.82 (30.29, 46.12) | 36.24 (31.80, 46.10) | −0.309 | 0.757 |
| Glutamine | 568.14 (194.08, 795.86) | 750.06 (235.08, 855.58) | −2.928 | 0.003 | 746.28 (225.64, 878.45) | 779.15 (238.15, 829.21) | −0.388 | 0.698 |
| Cysteine | 1.81 (0.39, 2.74) | 2.48 (0.62, 3.33) | −2.968 | 0.003 | 2.39 (0.49, 3.67) | 2.50 (0.67, 3.22) | −0.504 | 0.615 |
| Creatinine | 27.75 (23.20, 36.25) | 36.36 (26.38, 41.59) | −3.230 | 0.001 | 36.97 (26.65, 42.87) | 34.88 (25.23, 40.81) | −1.122 | 0.262 |
| Tryptophan | 58.88 (49.71, 69.69) | 58.89 (47.89, 72.35) | −0.178 | 0.859 | 65.91 (52.36, 77.48) | 52.61 (45.20, 66.62) | −3.325 | 0.001 |
| Hydroxyproline | 95.02 (11.59, 240.70) | 171.62 (13.97, 292.19) | −1.617 | 0.106 | 201.06 (13.70, 306.31) | 168.31 (14.59, 292.19) | −0.249 | 0.804 |
| Ornithine | 91.22 (65.70, 109.93) | 80.71 (58.47, 108.99) | −1.284 | 0.199 | 96.55 (85.05, 120.79) | 62.81 (50.45, 81.92) | −5.351 | <0.001 |
| Taurine | 17.38 (14.20, 21.80) | 16.65 (10.27, 24.20) | −0.783 | 0.434 | 17.59 (13.67, 24.30) | 14.92 (6.47, 24.25) | −1.377 | 0.168 |
| Cystine | 145.40 (115.22, 191.75) | 177.90 (132.72, 240.23) | −2.817 | 0.005 | 181.97 (132.56, 256.11) | 174.33 (134.22, 231.77) | −0.516 | 0.606 |
| Choline | 461.43 (410.26, 520.69) | 546.39 (467.05, 643.79) | −4.457 | <0.001 | 569.67 (489.85, 718.88) | 519.63 (437.50, 624.40) | −2.160 | 0.031 |
| Aminoadipic acid | 1.37 (0.85, 1.64) | 1.54 (1.16, 1.95) | −2.716 | 0.007 | 1.54 (1.18, 1.94) | 1.54 (1.13, 1.97)  | −0.176 | 0.860 |

Data are presented as median (Q1, Q3). AAs: Amino acids; HCM: Hypertrophic cardiomyopathy; HNCM: Non-obstructive HCM; HOCM: Obstructive HCM; NC: Normal control.

**Supplementary Table 2: Comparison of differential AA expression between HCM patients without HP and NC**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Metabolites (µmol/L)** | **NC (*n* = 57)** | **HCM (without HP) (*n* = 69)** | ***Z* values** | ***P* values** |
| Serine | 116.29 (100.87, 137.15) | 102.54 (89.63, 119.08) | -2.801 | 0.005 |
| Glycine | 450.19 (325.36, 654.68) | 409.87 (305.78, 574.20) | -2.228 | 0.031 |
| Proline | 345.59 (226.75, 480.80) | 215.41 (159.99, 374.46) | -2.953 | 0.003 |
| Citrulline | 30.18 (24.18, 38.57) | 38.59 (32.71, 46.98) | -4.291 | <0.001 |
| Glutamine | 568.14 (194.08, 795.86) | 750.06 (225.94, 862.08) | -2.713 | 0.007 |
| Cystine | 145.40 (115.22, 191.75) | 172.39 (132.60, 220.37) | -2.394 | 0.017 |
| Creatinine | 27.75 (23.20, 36.25) | 35.79 (26.38, 40.92) | -3.125 | 0.002 |
| Cysteine | 1.81 (0.39, 2.74) | 2.82 (0.68, 3.63) | -3.340 | 0.001 |
| Choline | 461.43 (410.26, 520.69) | 553.24 (476.01, 658.35) | -4.311 | <0.001 |
| Aminoadipic acid | 1.37 (0.85, 1.64) | 1.51 (1.14, 1.88) | -2.316 | 0.021 |

Data are presented as median (Q1, Q3). AA: Amino acid; HCM: Hypertrophic cardiomyopathy; HP: Hypertension; NC: Normal control.

**Supplementary Table 3: Comparison of differential AA expression between HCM with HP and without HP.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Metabolites (****µmol/L)** | **HCM (with HP) (*n* = 40)** | **HCM (without HP) (*n* = 69)** | ***Z* values** | ***P* value** |
| Serine | 111.01 (94.53, 121.73) | 102.54 (89.63, 119.08) | -1.578 | 0.115 |
| Glycine | 422.00 (246.53, 582.59) | 409.87 (305.78, 574.20) | -0.572 | 0.567 |
| Proline | 294.92 (171.86, 452.00) | 215.41 (159.99, 374.46) | -1.088 | 0.277 |
| Citrulline | 36.07 (29.70, 44.13) | 38.59 (32.71, 46.98) | -1.389 | 0.165 |
| Glutamine | 754.88 (251.07, 853.14) | 750.06 (225.94, 862.08) | -0.258 | 0.797 |
| Cystine | 184.08 (136.74, 286.62) | 172.39 (132.60, 220.37) | -1.151 | 0.250 |
| Creatinine | 37.17 (25.89, 42.72) | 35.79 (26.38, 40.92) | -0.138 | 0.890 |
| Cysteine | 2.36 (0.46, 2.82) | 2.82 (0.68, 3.63) | -1.701 | 0.089 |
| Choline | 544.67 (455.91, 629.25) | 553.24 (476.01, 658.35) | -0.503 | 0.615 |
| Aminoadipic acid | 1.62 (1.16, 2.11) | 1.51 (1.14, 1.88) | -0.836 | 0.403 |

Data are presented as median (interquartile range). AA: Amino acid; HCM: Hypertrophic cardiomyopathy; HP: Hypertension.

**Supplementary Table 4: DeLong test of the three ROCs.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Model** | **AUC (95% CI)** | **Delong test (*P*\*)** | **Delong test (*P*†)** |
| AUC (overall) | 0.82 (0.75–0.89) | – | 0.739 |
| AUC (HCM without HP) | 0.84 (0.76–0.91) | 0.739 | – |
| AUC (HCM with HP) | 0.79 (0.69–0.89) | 0.636 | 0.463 |

*P*\*: Compared with overall AUC. *P*†: Compared with AUC of HCM without HP. AUC: Area under the ROC curve; CI: Confidential interval; HCM: Hypertrophic cardiomyopathy; HP: Hypertension; ROCs: Receiver operating characteristics.



**Supplementary Figure 1:** The ion chromatogram of AAs and derivatives. (A) Total ion chromatogram of AAs and derivatives in all samples. (B) Total ion chromatogram of AAs and derivatives in QC samples. AAs: Amino acids; QC: Quality control.



**Supplementary Figure 2:** Assessment of the two screening models with RF, LR, and SVM. (A) Importance ranking of biomarkers by RF for HCM *vs.* NC (Panel A). (B) Based on 5-fold cross-validation, ROC, sensitivity, and specificity were calculated by RF, LR, and SVM for HCM *vs.* NC (Panel A). (C) Importance of biomarkers were calculated by RF for HOCM *vs.* HNCM (Panel B). (D) Based on 5-fold cross-validation, ROC, sensitivity, and specificity of Panel B were calculated by RF, LR, and SVM for HOCM *vs.* HNCM. HCM: Hypertrophic cardiomyopathy; HNCM: Non-obstructive HCM; HOCM: Obstructive HCM; LR: Logistic regression; NC: Normal control; RF: Random Forest; ROC: Receiver operating characteristic; SVM: Support vector machine.



**Supplementary Figure 3:** Correlogram of selected AAs and derivatives in each panel. (A) Correlation of the four AAs and derivatives (choline, proline, cysteine, and glycine) in Panel A. (B) Correlation of the three AAs (ornithine, proline, and arginine) in Panel B. AAs: Amino acids; R: Coefficient of correlation.

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**Supplementary Figure 4:** Forest plot demonstrating AUC, sensitivity, specificity, and odds ratio for sex, age and BMI subgroups. AUC: Area under the receiver operating characteristic (ROC) curve; BMI: Body mass index.



**Supplementary Figure 5:** Receiver operating characteristic (ROC) curves of Panel A in in HCM patients with or without HP. The blue line showed the overall AUC of HCM *vs.* NC. The red line showed the AUC of HCM with a previous history of HP *vs.* NC. The yellow line showed the AUC of HCM without HP history *vs.* NC. AUC: Area under the ROC curve; HCM: Hypertrophic cardiomyopathy; HP: Hypertension; NC: Normal control; ROC: Receiver operating characteristic.