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Plasma Metabolomics Analysis Identifies Abnormal Energy, Lipid, and Amino Acid Metabolism in Abdominal Aortic Aneurysms

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Background: Abdominal aortic aneurysm (AAA) is a complicated aortic dilatation disease. Metabolomics is an emerging system biology method. This aim of this study was to identify abnormal metabolites and metabolic pathways associated with AAA and to discover potential biomarkers that could affect the size of AAAs.





Material/Methods: An untargeted metabolomic method was used to analyze the plasma metabolic profiles of 39 patients with AAAs and 30 controls. Multivariate analysis methods were used to perform differential metabolite screening and metabolic pathway analysis. Cluster analysis and univariate analysis were performed to identify potential metabolites that could affect the size of an AAA.

Results: Forty-five different metabolites were identified with an orthogonal projection to latent squares-discriminant analysis model and the differences between them in the patients with AAAs and the control group were compared. A variable importance in the projection score >1 and P<0.05 were considered statistically significant. In patients with AAAs, the pathways involving metabolism of alanine, aspartate, glutamate, D-glutamine, D-glutamic acid, arginine, and proline; tricarboxylic acid cycling; and biosynthesis of arginine are abnormal. The progression of an AAA may be related to 13 metabolites: citric acid, 2-oxoglutarate, succinic acid, coenzyme Q1, pyruvic acid, sphingosine-1-phosphate, platelet-activating factor, LysoPC (16: 00), lysophosphatidylcholine (18: 2(9Z,12Z)/0: 0), arginine, D-aspartic acid, and L- and D-glutamine.

Conclusions: An untargeted metabolomic analysis using ultraperformance liquid chromatography-tandem mass spectrometry identified metabolites that indicate disordered metabolism of energy, lipids, and amino acids in AAAs.

MeSH Keywords: **Aortic Aneurysm, Abdominal • Chromatography, Liquid • Metabolism • Plasma • Energy Metabolism**

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Background

Abdominal aortic aneurysms (AAAs) are characterized by permanent localized dilation of the aorta [1]. An abdominal aorta diameter >3 cm typically is diagnostic of the condition [2]. Moreover, the rate of mortality associated with AAA rupture is extremely high at almost 70% [3]. Surgical treatment of AAAs has become increasingly sophisticated and less invasive, but it can only be used for aneurysms ≥ 5.5 cm [4]. There remains an urgent need to identify pathways and potential biomarkers that predispose patients to aneurysmal formation and that can be used to focus attention from surgery to medical approaches that can help limit the progression of small AAAs.

AAAs are characterized by progressive luminal dilation accompanied by aortic wall inflammation, decreased medial smooth muscle cells, and disruption of the extracellular matrix. Although studies have been conducted on a variety of biologically plausible markers for diagnosis and prediction of disease activity in AAA [5–7], there is still a need for a systematic understanding of biological disturbances associated with the condition. Metabolomics is a high-throughput omics technology that follows on the heels of genomics, transcriptomics, and proteomics, and which can be used to simultaneously measure and study a large number of small molecules (<1000 Da) [8]. The systematic identification and quantification of metabolites in biological systems with metabolomics has been extensively studied in clinical and preclinical studies as a way of identifying predictors of disease [9]. This study aimed to identify differences in plasma metabolites between patients with AAAs and a control group, and to identify potential biomarkers that could influence the size of AAAs.

Material and Methods

Chemicals and reagents

The 2-chloro-L-phenylalanine used in the present study was obtained from J&K Chemical (Beijing, China), the ketoprofen was purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.A.), and the acetonitrile and methanol (HPLC-grade) were supplied by Fisher Scientific (Pittsburgh, Pennsylvania, U.S.A.). The HPLC-grade formic acid was purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.A.).

Samples

We collected 69 plasma samples in this study, from 39 patients with AAAs that were identified on ultrasound at the First Affiliated Hospital of Zhengzhou University (AAA group) and from 30 volunteers who were recruited for the research (control group). The maximum diameter of the infrarenal

AAAs in all patients was measured by 2 experienced ultrasonographers. Because many risk factors are associated with AAA [10,11], baseline data from all of the participants were extracted from their medical records. Factors analyzed included age; sex; smoking status; history of chronic obstructive pulmonary disease (COPD), diabetes, or hypertension; serum lipid levels; and drug exposure.

All subjects fasted beginning at 10 p.m. the day before their plasma was collected. Patients were excluded if they had thoracic aortic aneurysms, aortic dissection, an AAA combined with inflammatory aortic disease, or a systemic connective tissue disorder. EDTA anticoagulant tubes were used to collect blood samples from all patients, which were immediately centrifuged at 3000 rpm for 15 min and then at 4000 rpm for 10 min. All of the samples were stored at -80°C before analysis.

UPLC-MS analysis

An untargeted metabolomics analysis was performed with an Ultimate 3000 Ultra Performance Liquid Chromatography system (Dionex, U.S.A.) coupled with a Thermo Q-Exactive Orbitrap mass spectrometer and autosampler. Extraction of metabolites was performed by sampling 100 μL of plasma into 300 μL of a methanol solution created following existing standards, which was then subjected to vortex mixing for 2 min and centrifugation at 13 000 rpm for 10 min to pellet the proteins.

To ensure the reliability of the experimental results, quality control (QC) samples were interpolated in the sequence to monitor acquisition of data. In the first run sequence, 6 QC samples were used to balance the instruments. A QC sample then was interspersed after every 7 samples from the participants. All samples were separated using an ACQUITY UPLC BEH C_{18} column (particle sizes 100 μm , 2.1 mm, and 1.7 μm) (Waters, Milford, Massachusetts, U.S.A.). The column temperature was maintained at 40°C and the flow rate at 0.3 mL/min (mobile phase A: water/0.1% formic acid; mobile phase B: acetonitrile). The gradient conditions were as follows: 0–0.5 min, 5% B; 0.5–1.0 min, 5% B; 1.0–9.0 min, 5–100% B; 9.0–12.0 min, 100% B; 12.0–15.0 min, 5% B. The sample injection volume was 5 μL .

Ion trapping was performed in both cation and anion modes, with a scanning range of 80 to 1200 m/z, a primary resolution of 70 000, and a secondary resolution of 17 500. The operating parameters in the positive mode were set as follows. The ion source temperature was 300°C , the ion transfer tube temperature was 320°C , the sheath gas flow rate was 40 arb, the auxiliary gas flow rate was 10 arb, and the spray voltage was 3.50 kV. In the negative mode, the conditions were an ion source temperature of 300°C , ion transfer tube temperature of 320°C , sheath gas flow rate of 38 arb, auxiliary gas flow rate of 10 arb, and spray voltage of 3.00 kV.

Table 1. Clinical and demographic characteristics of patients with abdominal aortic aneurysms and controls.

Characteristics	AAA (n=39)	Control (n=30)	P value
Age	72.26±6.773	71.40±5.31	0.425
Female gender, n (%)	5 (12.8)	9 (30.0)	0.073
Body mass index, kg/m ²	23.96±2.81	23.83± 3.31	0.441
Aortic diameter(mm)	48 (32.1–100.0)	18 (15.4–23.6)	<0.01
Hypertension, n (%)	15 (38.5)	11 (36.7)	0.54
Diabetes mellitus, n (%)	11 (28.2)	7 (23.3)	0.431
COPD, n (%)	4 (28.2)	3 (23.3)	0.647
Antihypertensive drugs	5 (12)	7 (23.3)	0.205
Hypoglycemic agents	5 (12)	6 (20.0)	0.315
Current smoker, n (%)	8 (26.7)	3 (10.0)	0.199
Ex-smokers, n (%)	4 (10.2)	5 (16.7)	0.612
Alcohol drinker, n (%)	5 (12.8)	4 (13.3)	0.612
Triacylglycerol, mmol/L	1.08±0.77	1.06±0.68	0.798
Total cholesterol, mmol/L	4.49±1.14	4.58±1.23	0.927
HDL-c, mmol/L	1.81±0.56	1.70±0.44	0.422
LDL-c, mmol/L	2.28±0.89	2.35±0.87	0.881
Statins, n (%)	6 (15.4)	4 (13.3)	0.546
Aspirin, n (%)	6 (15.4)	4 (13.3)	0.546
Fibrates, n (%)	2 (5.1)	1 (3.3)	0.599

HDL-c – high-density lipoprotein-cholesterol; LDL-c – low-density lipoprotein-cholesterol.

Data processing and statistical analysis

Univariate statistical analysis was performed using SPSS Statistics 21 software (IBM, U.S.A.) Clinical information about the group with AAAs and the control group was expressed as a mean±SD, median (minimum-maximum), or percentage. The *t*, chi square and Fisher exact tests were used to analyze differences in clinical characteristics between the 2 groups. Data were regarded as statistically significant at *P*<0.05.

The raw data collected through mass spectrometry analysis were processed with Compound Discoverer 3.0 software (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) to determine peak extraction, to compare peaks, and to perform peak analysis. SPSS Statistics 21 software was used to obtain *P* values for the processed dataset by doing an independent *t*-test. The principal component analysis (PCA) model is a multivariate, statistical, unsupervised method reflecting the distribution state of the real samples, whereas the orthogonal projection to latent squares-discriminant analysis (OPLS-DA) is a supervised, multivariate, statistical method that is able to determine the variable importance in the projection (VIP) score. In the present study, Pareto was the scaling method used for PCA and OPLC-DA. The obtained data were statistically analyzed. Finally, multivariate statistical analysis of the

normalized data was conducted by using R software (3.6.1). *P*<0.05 was considered statistically significant.

MetaboAnalyst (<http://www.metaboanalyst.ca>) was used for pathway prediction. Differential ion peaks were annotated using the Human Metabolome Database (HMDB) (<https://hmdb.ca/>) and the Kyoto Encyclopedia of Genes and Genomes database (<http://www.kegg.jp/kegg/pathway.html>). Cluster and univariate analyses of the obtained differential metabolites were performed based on aneurysm diameter to identify potential biomarkers that could affect the size of AAAs.

Results

Patient characteristics

Table 1 summarizes patient demographics, including age, gender, body mass index, history of hypertension, alcohol consumption, smoking status, history of COPD, medication history (statins, aspirin, fibrates), and levels of triglycerides, total cholesterol, high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c). The differences between these characteristics in the patients and the controls were not statistically significant. We conducted a PCA analysis for

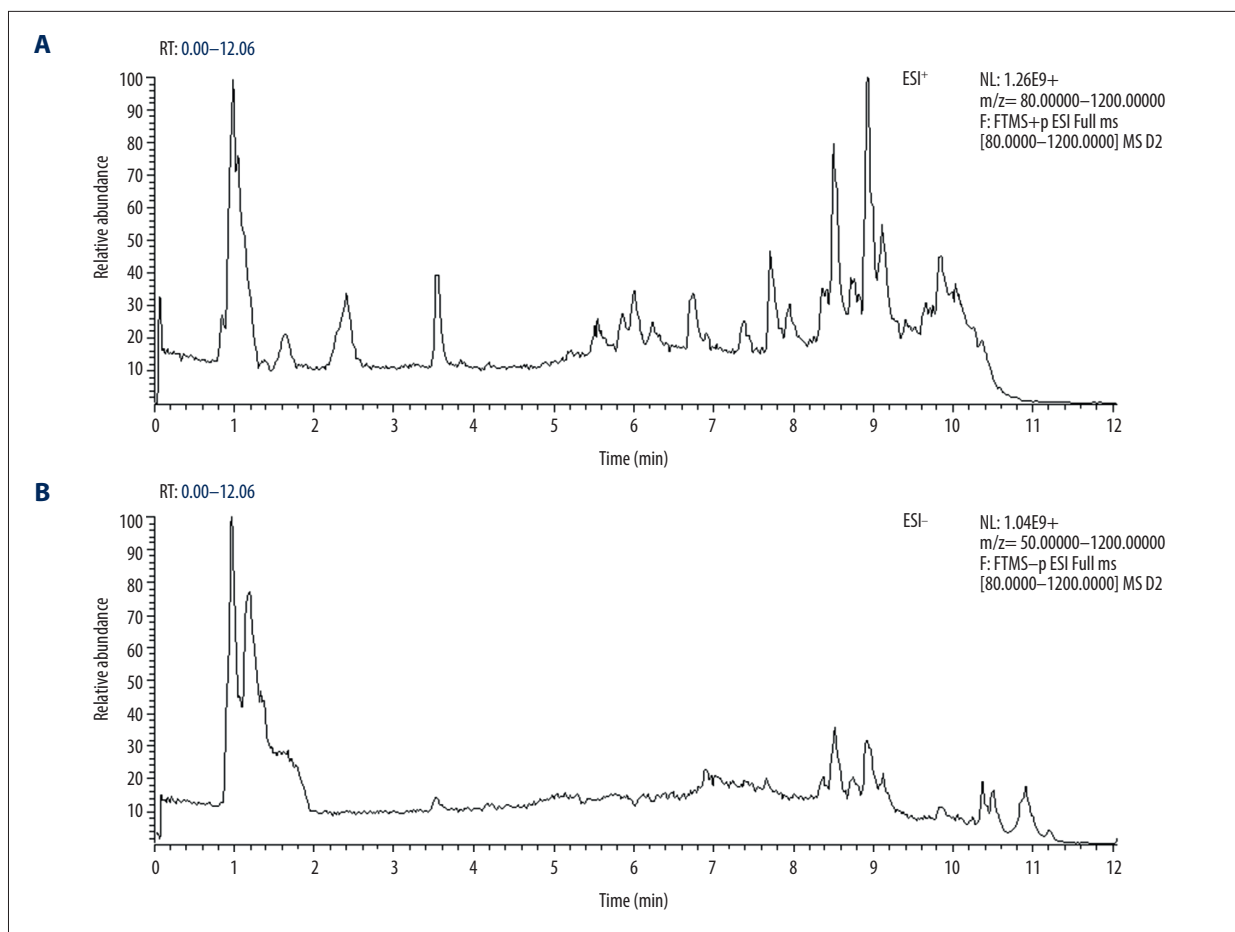


Figure 1. Total ion chromatography of plasma samples. (A) Electrospray ionization (ESI)⁺ mode. (B) ESI⁻ mode.

differential ions and the results showed that sex is not the main cause of metabolomic differences (Supplementary Figure 1).

Metabolic profiles of AAA

A total of 2605 ion peaks were extracted in the cation mode and 2344 ion peaks were extracted in the anion mode (Figure 1). In the score plot of the PCA model, the metabolic profile of AAA was distinctly different between controls, with the majority of samples presenting within a 95% Hotelling T2 ellipse (Figure 2). The first 2 principal components (PCs) accounted for 16.5% and 5.3% of the total variability in the positive ion mode and the first 2 PCs accounted for 16.5% and 7.6% of the total variability in the negative ion mode. The QC samples were well aggregated, reflecting good stability of and repeatability with the instruments and methods used in this study. The OPLS-DA model was performed between the 2 groups to further identify the metabolites that differed between the AAA and control groups (Figure 3). The reliability of the model was identified by 7-fold cross-validation with 200-time permutation tests. The R²_Y and Q²_Y values were 0.993 and 0.977 in the electrospray ionization (ESI)⁺ mode and the R²_Y and Q²_Y

values were 0.979 and 0.96 in the ESI⁻ mode, which demonstrated acceptable goodness of fit and high-quality predictability. Given the importance of projection (VIP) value >1 and P<0.05, a total of 121 ion peaks (65 in positive mode and 56 in negative mode) differed between the 2 groups (Figure 4). Using molecular weight and mass spectrometry information, a search was conducted of the HMDB to identify metabolites with significant differences. Forty-five compounds that differed most between groups, including amino acids, phospholipids, and fatty acids, were selected based on their clinical and biological significance (Table 2). With pathway analysis, 5 vital metabolic pathways were identified: metabolism of alanine, aspartate, and glutamate; metabolism of D-glutamine and D-glutamic acid; cycling of tricarboxylic acid (TCA); metabolism of arginine and proline; and biosynthesis of arginine (Figure 5). The cluster analysis of 45 metabolites showed a distinction between patients with large (>5.5 cm) and small (<5.5 cm) AAAs (Figure 6). With univariate analysis, another 13 metabolites were identified that may affect the size of AAAs (P<0.05) (Figure 7).

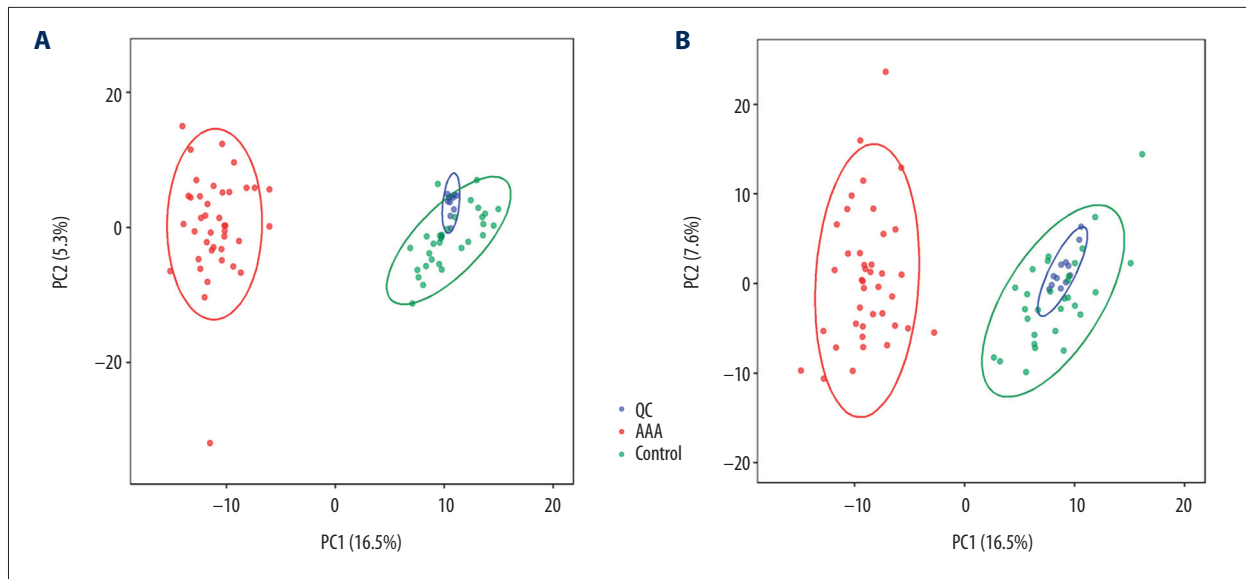


Figure 2. Principal component analysis score plot. (A) Electrospray ionization (ESI)⁺ mode. (B) ESI⁻ mode.

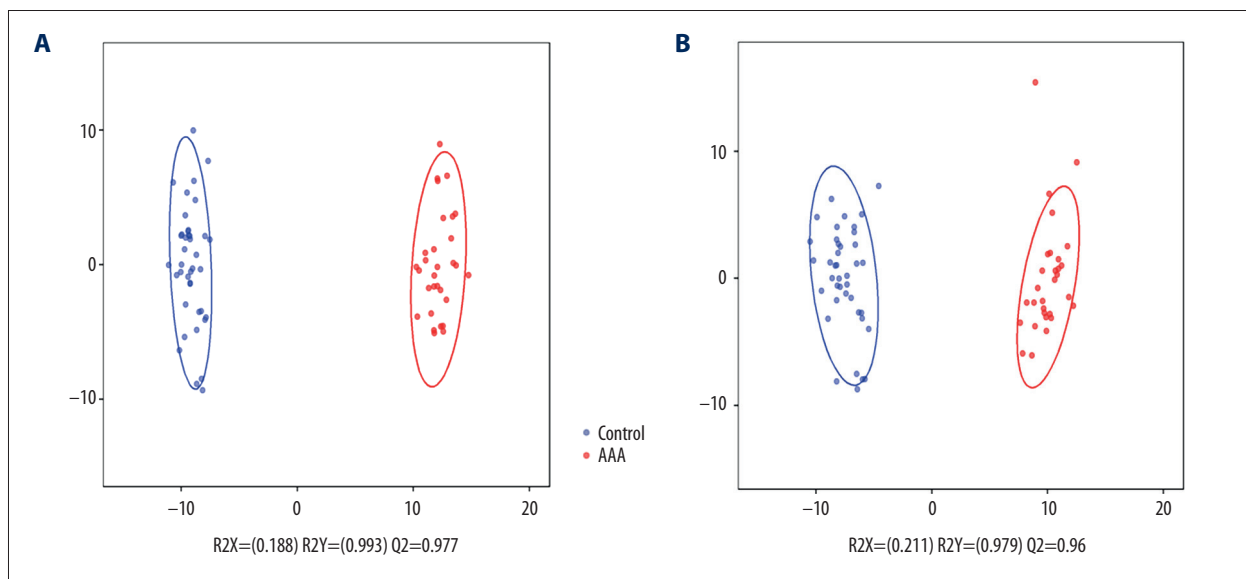


Figure 3. Orthogonal projection to latent squares-discriminant analysis model. (A) Electrospray ionization (ESI)⁺ mode. (B) ESI⁻ mode.

Discussion

AAA is an important, life-threatening disease, but its pathogenesis is very complicated. Metabolomics can reflect physical changes caused by various factors, such as disease, lifestyle, and environmental influences. In previous studies, using gas chromatography-mass spectrometry and hydrogen-1 nuclear magnetic resonance, Ruperez et al. that found aminomalonic acid and glycerol may be potential plasma biomarkers [12]. Ciborowski et al. distinguished large aneurysms and small aneurysms by analyzing the plasma metabolic profile in patients with AAAs [13]. With the development of metabolomics, more detailed data are needed to study the relationship between

metabolomics and AAAs. In our study, we used untargeted UPLC-MS to reveal the plasma metabolic profile of AAAs. There are aberrations in energy, lipid, and amino acid metabolism in AAAs. The groups with large and small AAAs, citric, 2-oxoglutarate, and succinic acids; CoQ1; and pyruvic acid differed in energy metabolism. Metabolites such as S1P, platelet-activating factor (PAF), LysoPC (16: 00), and LysoPC (18: 2(9Z,12Z)/0: 0) were significantly altered in lipid metabolism. The abnormalities in amino acid metabolism were mainly in arginine and L-glutamate (Figure 7).

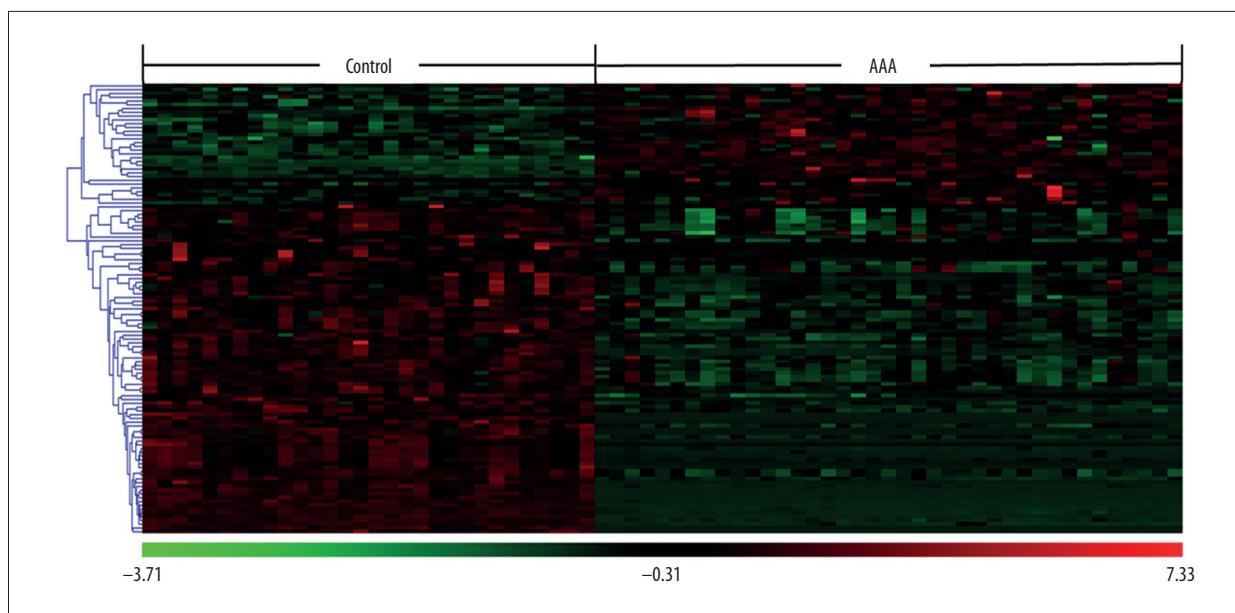


Figure 4. Clustering analysis of 121 differential ions. Rows represent differential ions and columns represent samples. Green denotes low intensity and red denotes high intensity.

Table 2. Results of identification of differential metabolites.

Metabolites	Mode	VIP	P	FC	Class	Pathway
2-Oxoglutaric acid	ESI-	1.58	<0.001	0.33	keto acids	TCA Cycle
Succinic acid	ESI-	1.15	<0.001	0.59	Dicarboxylic acids	TCA Cycle
Citric acid	ESI-	1.58	<0.001	0.31	Tricarboxylic acids	TCA Cycle
cis-Aconitic acid	ESI-	1.48	<0.001	0.01	Tricarboxylic acids	TCA cycle
S1P	ESI+	1.38	<0.001	0.52	Phosphosphingolipids	Sphingolipid metabolism
Phytosphingosine	ESI+	3.35	<0.001	0.64	Amines	Sphingolipid metabolism
D-Sphingosine	ESI+	1.81	<0.001	0.09	Amines	Sphingolipid metabolism
D-carnitine	ESI+	2.50	<0.001	1.40	Organonitrogen	Lipid metabolism
Hexanoylcarnitine	ESI+	1.07	<0.001	0.30	Fatty acid esters	Lipid metabolism
L-Octanoylcarnitine	ESI+	2.36	<0.001	0.35	Fatty acid esters	Lipid metabolism
9-Decenoylcarnitine	ESI+	1.87	<0.001	0.40	Fatty acid esters	Lipid metabolism
Decanoylcarnitine	ESI+	1.63	<0.001	0.41	Fatty acid esters	Lipid metabolism
LysoPC (16: 0/0: 0)	ESI+	2.10	<0.001	0.44	Glycerophosphocholines	Lipid metabolism
PAF	ESI+	1.34	<0.001	0.60	Glycerophosphoethanolamines	Lipid metabolism
LysoPC (18: 2(9Z,12Z)/0: 0)	ESI+	3.30	0.01	0.85	Glycerophosphocholines	Lipid metabolism
L-Glutamic acid	ESI-	1.3	<0.001	0.53	Amino acids, peptides, and analogues	D-Glutamine and D-glutamate metabolism
D-Glutamine	ESI-	1.18	<0.001	1.45	Amino acids, peptides, and analogues	D-Glutamine and D-glutamate metabolism
L-Glutamine	ESI+	2.51	<0.001	1.83	Amino acids, peptides, and analogues	D-Glutamine and D-glutamate metabolism
Creatine	ESI+	2.23	<0.001	1.55	Amino acids, peptides, and analogues	Arginine and proline metabolism

Table 2 continued. Results of identification of differential metabolites.

Metabolites	Mode	VIP	P	FC	Class	Pathway
L-Proline	ESI+	2.16	<0.001	1.33	Amino acids, peptides, and analogues	Arginine and proline metabolism
Arginine	ESI+	1.65	<0.001	3.15	Amino acids, peptides, and analogues	Arginine and proline metabolism
Creatinine	ESI+	2.53	<0.001	1.66	Amino acids, peptides, and analogues	Arginine and proline metabolism
Pyruvic acid	ESI-	1.08	<0.001	0.55	Alpha-keto acids and derivatives	Alanine, aspartate and glutamate metabolism
D-Aspartic acid	ESI+	1.45	<0.001	0.02	Amino acids, peptides, and analogues	Alanine, aspartate and glutamate metabolism
DL-Serine	ESI+	1.27	<0.001	1.33	Amino acids, peptides, and analogues	Glycine, serine and threonine metabolism
Taurine	ESI-	1.17	<0.001	0.62	Organosulfonic acids and derivatives	ABC transporters
Betaine	ESI+	3.35	<0.001	1.22	Amino acids, peptides, and analogues	ABC transporters
L-Phenylalanine	ESI+	5.68	<0.001	1.37	Amino acids, peptides, and analogues	ABC transporters
L-Isoleucine	ESI+	1.29	<0.001	1.32	Amino acids, peptides, and analogues	ABC transporters
Choline	ESI+	1.13	0.04	1.23	Organonitrogen compounds	ABC transporters
hypaphorine	ESI-	1.27	<0.001	0.39	Amino acids, peptides, and analogues	/
L-Threonic acid	ESI-	1.54	<0.001	0.04	Carbohydrates and carbohydrate conjugates	/
Glutaconic acid	ESI-	1.48	<0.001	0.05	Dicarboxylic acids and derivatives	/
DL-Tryptophan	ESI-	1.21	<0.001	2.07	Indolyl carboxylic acids and derivatives	/
[SP]Sphinganine-1-phosphate	ESI-	1.23	<0.001	0.53	Phosphosphingolipids	/
Monobutyl phthalate	ESI+	1.33	<0.001	0.31	Benzoic acids and derivatives	/
Piperine	ESI+	1.55	<0.001	0.10	/	/
N-Phenylacetylglutamine	ESI+	1.48	<0.001	4.00	Amino acids, peptides, and analogues	/
5-Aminopentanamide	ESI+	3.34	<0.001	0.18	Amino acids, peptides, and analogues	/
Glu-asp	ESI+	2.63	<0.001	0.01	Amino acids, peptides, and analogues	/
allylcysteine	ESI+	1.00	<0.001	0.40	Amino acids, peptides, and analogues	/
Oleamide	ESI+	1.98	<0.001	0.07	Fatty amides	/
Caffeine	ESI+	1.42	<0.001	0.14	Purines and purine derivatives	/
Dihydrothymine	ESI+	1.26	<0.001	2.61	Pyrimidines and pyrimidine derivatives	/
Ubiquinone-1(CoQ1)	ESI+	1.41	<0.001	0.40	Quinone and hydroquinone lipids	/

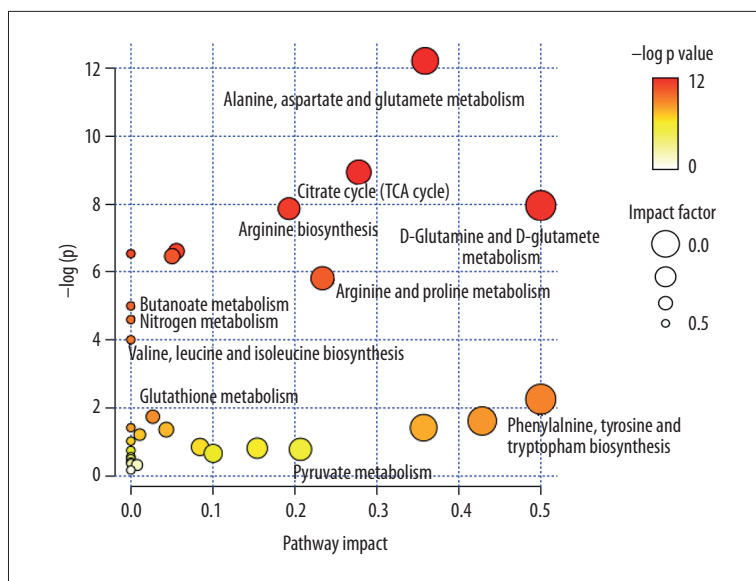


Figure 5. Pathway enrichment analysis of differential metabolites. The X axis represents the impact factor of the pathway topology analysis and the Y axis represents the P value of the pathway enrichment analysis ($-\log P$ value).

Energy metabolism

Three key metabolites of citric, 2-oxoglutaric, and succinic acids were downregulated in AAAs and their levels were lower in large AAAs, which indicates that the production of energy (adenosine triphosphate [ATP]) in the TCA cycle was disturbed [14]. Succinate is a substrate of succinate dehydrogenase that can promote the dehydrogenation of succinate to fumarate. Succinate dehydrogenase (also called complex II) couples 2 major pathways in mitochondria, the TCA cycle and the respiratory chain. Succinic acid exhibits 2-sidedness in blood vessels. A low concentration of succinic acid can induce vasodilation, whereas a high concentration can induce vasoconstriction [15]. Coenzyme Q (CoQ) plays a crucial role in the production of mitochondrial energy and reactive oxygen. CoQ is considered to be an antioxidant, and lack of it can lead to an increase in mitochondrial reactive oxygen species [16]. This could suggest that AAAs have mitochondrial dysfunction.

Lipid metabolism

Sphingosine 1-phosphate (S1P) is secreted by different blood cells and is taken up by HDL [17]. S1P interacts with various S1P receptors on the cell membrane. As a sensor to balance the inflammatory signal in the blood vessel wall, S1P stimulates S1P receptor-1 (S1PR1) in endothelial cells, which activates the endothelial chaperone downstream of Akt and promotes the production of NO [18,19]. On the other hand, S1PR1 activation inhibits the inflammatory activation of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, which reduces monocyte recruitment [20]. The anti-inflammatory effect of S1P may have contributed to atheroprotection. The downregulation of S1P receptors in aortic tissues also promotes the formation of AAAs [21]. PAF is an important

phospholipid mediator that can cause various platelet aggregation and inflammatory reactions and impair vascular endothelial function [22]. PAF is also related to the activity of metalloproteinases [23]. Platelet-activating factor-acetylhydrolase (PAF-AH) is a member of the phospholipase A2 enzyme superfamily and circulates in the blood together with plasma lipoprotein particles such as LDL and HDL, which can hydrolyze the acetyl group of PAF by converting PAF into hemolytic PAF [24]. We suspect that downregulation of PAF may be due to the higher level of PAF-AH activity in plasma of patients with AAAs than in healthy people [25]. In addition, the results of our study demonstrated that levels of LysoPC (16: 0/0: 0) and LysoPC (18: 2(9Z,12Z)/0: 0) decreased with the development of AAAs. LysoPC (16: 0) has been confirmed to be related to the inflammation and vulnerability of atherosclerotic plaque, which could affect the progression of AAAs [26,27].

Amino acid metabolism

Abnormal glutamate metabolism and dysfunctional metabolism of arginine metabolism also may play a role in the development of AAAs. Arginine is the source of nitric oxide (NO). Arginine deficiency can lead to the development of atherosclerosis, based on the fact that it leads to impairment of NO synthesis and consequently affects the function of vascular endothelial cells [28]. However, the formation of AAAs may be related to excessive NO produced by vascular smooth muscle cells, which promotes the degradation of elastin and the destruction of the extracellular matrix [29,30]. Glutamate and glutamine are precursors of L-arginine and a substrate for ATP and protein synthesis. Dysfunction of glutamine metabolism can promote the development of cardiovascular diseases by facilitating unrestricted growth and migration of vascular cells and deposition of the extracellular matrix [31]. As a derivative

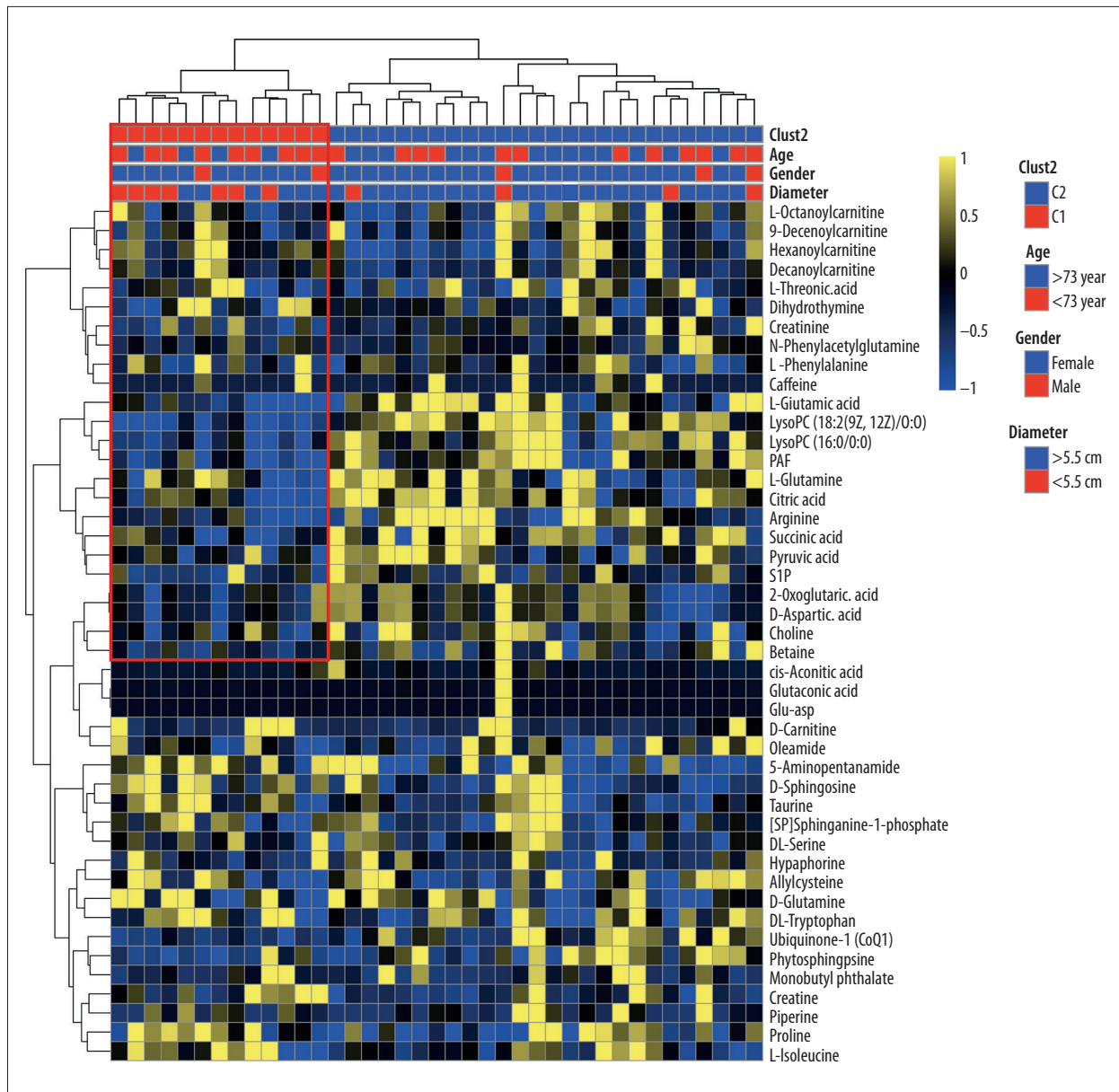


Figure 6. Cluster analysis of 45 differential metabolites in patients with abdominal aortic aneurysms. Rows represent differential metabolites, and columns represent samples.

of arginine, creatine levels increase, which is reflected in the reduced energy requirements of the tissue. High concentrations of phenylalanine may increase the risk of aortic disease because phenylalanine levels may be relevant to the activation of immunity and inflammation [32,33]. Proline is a component of collagen metabolites, and an increase in proline level may be related to the activation of collagen breakdown. The reduced amount of collagen makes the tissue more prone to rupture and dissection, which may accelerate AAA formation [33,34].

Study limitations

This article focused on identifying metabolites that differ between patients with AAAs and healthy individuals, based on untargeted plasma metabolomics, and discovering potential biomarkers that could affect the size of AAAs. Although we identified metabolites that differed in patients with large versus small AAAs, confirmation of our results in a small sample at a single center is required in an analysis of a large sample. Moreover, the precise relationship between these metabolites and AAAs is not yet clear. In future research, proteomics and

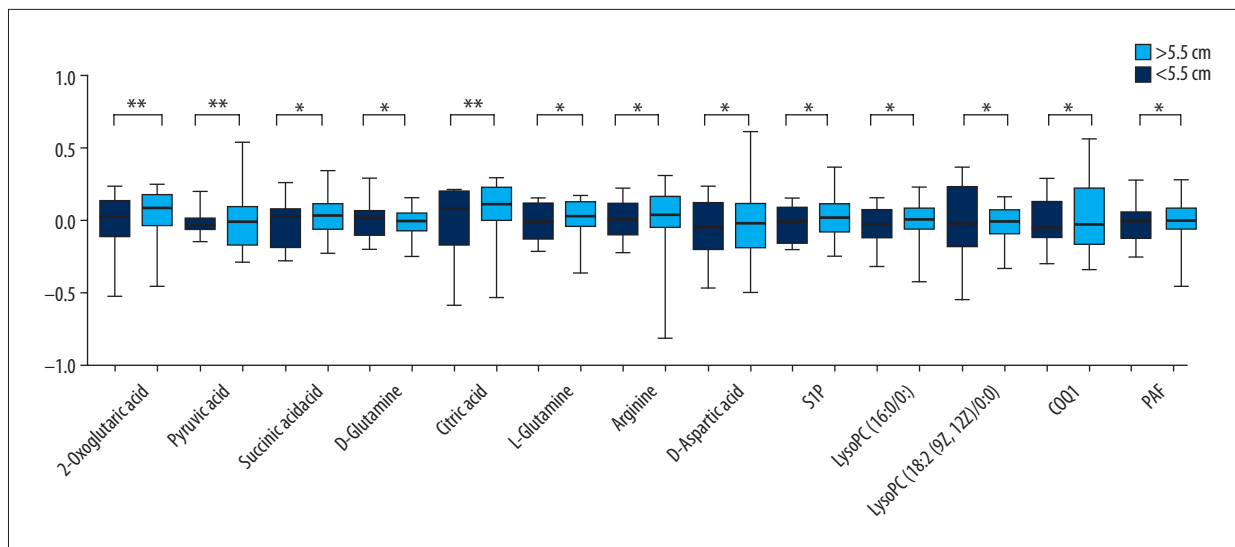


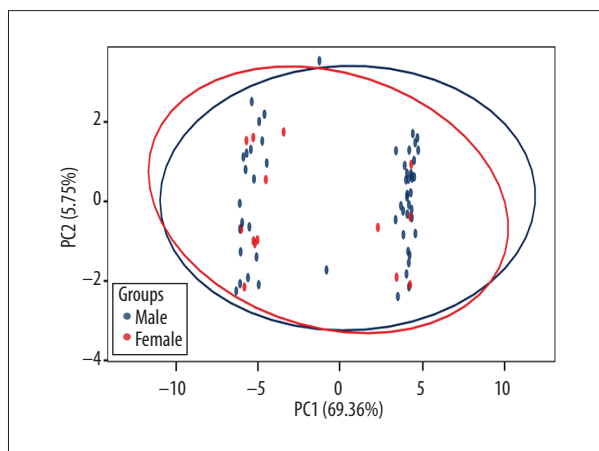
Figure 7. Difference in metabolites between large (>5.5 cm) and small (<5.5 cm) abdominal aortic aneurysms. * P<0.05 and ** P<0.01.

genomics should be used in combination to evaluate the specific significance of each of the metabolites.

Conclusions

We used untargeted metabolomics of UPLC-MS to identify different metabolites associated with AAAs and which are related to different metabolic aspects of the condition. The metabolites we found may indicate that metabolism of energy, lipids, and amino acids in AAAs is disordered.

Supplementary Data



Ethics statement

The research protocols for the present study were approved by the Ethics Committee at the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China).

Conflict of interest

None.

Supplementary Figure 1. Principal component analysis score plot of 121 ions that differ between men and women.

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