Serum Metabolome Analysis by Liquid Chromatography–mass Spectrometry Reveals Differences between Asymptomatic Hyperuricemia and Gout

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Abstract

Background: Hyperuricemia (HUA) and gout are highly prevalent metabolic diseases caused by a high level of urate. This study aimed to investigate changes in the serum metabolic profile alterations and identify potential biomarkers of gout in patients with HUA by Liquid chromatography–mass spectrometry (LC-MS)-based metabolomics approach.

Methods: To this end, we conducted a study in two parts. Firstly, serum samples were collected from 50 subjects, including 20 patients with asymptomatic hyperuricemia (AH), 20 patients with gout and 10 healthy controls (HCs). Secondly, 10 newly diagnosed HUA subjects were recruited as a febuxostat treatment group. They were treated with 40 mg/d febuxostat, and the plasma samples were required in the 0, 6, 12, 18 and 24 weeks during the treatment. LC–MS combined with partial least squares–discriminant analysis was used to distinguish between samples from patients and HCs. Clinical measurements and pathway analysis were performed to help elucidate metabolic changes.

Results: Through serum metabolic profiling, 60 metabolites, including lipids and amino acids, were found to be significantly altered in patients with AH or gout. The biomarker and clinical data showed differences between patients with HUA or gout and healthy individuals. According to pathway analysis, the metabolism of taurine and hypotaurine; alanine, aspartate, and glutamate; and D-arginine and D-ornithine were significantly perturbed in patients with HUA. Moreover, 27 differential metabolites were identified between the AH and gout subgroups. Additionally, we found changes in the expression level of some metabolites during febuxostat treatment through a 24-week follow-up study.

Clonclutions: Taken together, we identified the biomarker signature for HUA (AH and gout). Metabolomics signatures could be used as new strategies for the prevention and early diagnosis of gout.

Background

Gout is a form of inflammatory arthritis associated with excruciating pain triggered by the deposition of monosodium urate (MSU) in the joints, tendons, and surrounding tissues [1]. Deposition of MSU crystals, and an imbalance between urate production and urate excretion, are the main causes of chronic hyperuricemia (HUA). However, while in many individuals with HUA, MSU crystals form and acute attacks of gouty arthritis occur, this is not always the case. Despite the fact that at least two-thirds of HUA individuals are expected to remain asymptomatic, 22% of subjects with urate levels higher than 9 mg/dL develop gout within 5 years[2]. Clinical diagnoses of gout based on serum urate (SUA) may be inaccurate. The rate of progression from asymptomatic hyperuricemia (AH) to clinically evident gout varies and mainly depends on SUA levels [3]. Potential biomarkers for gout are currently being studied, because early diagnosis and treatment could prevent, or at least decrease, MSU-associated tissue damage [4].

Metabolomics is the study of (ideally) all metabolites in a biological system. Currently, the most popular methods used for metabolomics research are nuclear magnetic resonance spectroscopy, gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry (LCMS), and ultra-

performance LCMS. LCMS is the most widely used platform for metabolomics studies due to its high power and sensitivity, simple sample pretreatment process, and wide coverage of metabolites[5]. LCMS is especially useful in the analysis and identification of biomarkers.

Metabolites are the end products of cellular regulatory processes and may provide more information on the biological pathways involved. The detection and functional characterization of such pathways is crucial to improve the management and treatment of patients with HUA and gout. In the present study, we employed a LCMS-based metabolomics approach to monitor metabolic alterations in subgroups of AH and gout patients, and to obtain the serum metabolic signatures of a febuxostat treatment group. The effects of febuxostat on metabolic profiles were further evaluated in the context of urate-lowering therapy. Our aim was to investigate changes in the serum metabolic profile of urate and identify potential biomarkers of gout in patients with HUA.

Methods

Study population.

In the study 1, 40 HUA patients were involved, including 20 newly diagnosed AH subjects and 20 newly diagnosed gout subjects. In addition, 10 healthy individuals were recruited as a control group. In the study 2, 10 newly diagnosed HUA subjects were recruited as a febuxostat treatment group. They were treated with 40 mg/d febuxostat, and the plasma samples were required in the 0, 6, 12, 18 and 24 weeks during the treatment. The diagnosis of HUA and gout according to the latest clinical criteria for the classification of gout proposed by the ACR and EULAR in 2015[6]. Following subjects were excluded from the study: (i) female; (ii) bellow 18 years old; (iii) clinical data was incomplete; (iv) previous therapy with urate-lowering medication; (v) patients with cancer, blood disease, polycystic kidney disease, solitary kidney, urinary tract stenosis, or kidney diseases except diabetic kidney disease. The study was performed according to the principles of the Declaration of Helsinki and was approved by the ethics committee of Shanghai Jiao Tong University Afliated Sixth People's Hospital. All study participants provided written informed consent prior to enrolment.

Clinical and laboratory assessments.

The clinical data of the subjects were recorded, including height, weight, waist circumference, hip circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP). The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. waist-to-hip ratio (WHR) was the ratio of waist circumference to hip circumference. All subjects were assessed after fasting overnight for at least 10 h. All the biochemical indices were measured on a Hitachi 7600 analyzer (Hitachi, Tokyo, Japan). Venous blood samples were collected after an overnight fast for measurements of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Alanine aminotransferase (ALT), aspartate aminotransferase (AST), GGT and

Hemoglobin A1c (HbA1c). The fractional of urate excretion (FEUA) (%) = [serum creatinine (μmol/L) × 24 -hour urine urate (μmol)] / [(serum urate (μmol/L) × 24 -hour urine creatinine (μmol)) × 100%. All subjects underwent standard clinical and laboratory evaluations.

Serum sample preparation.

The dried plasma extracts were reconstituted with 120 μL MeOH/water (50:50, v:v) solution, containing 0.3 μM D9-carnitine, 0.2 μM 13C6-phenylalanine, 0.03 μM D3-Palmitoyl carnitine detection internal standard to monitor the response stability of the used mass spectrometer during profiling analysis. (Notice: in order to suppress solvent effect for both RP and HILIC analyses, 50% MeOH-H2O was employed for reconstitution.)

LC-MS conditions.

The metabolic profiles were acquired by using Q Exactive quadruple-Orbitrap high resolution mass spectrometer coupled with Ultimate 3000 UHPLC system (Thermo Scientific, San Jose, USA). The reverse phase (RP) chromatographic separation of metabolites was conducted on a Hypersil GOLD C8 column (2.1 × 100 mm, 1.9 μm, Thermo Scientific), and hydrophilic interaction phase (HILIC) separation on a ACQUITY UPLC BEH Amide column (2.1 × 100 mm, 1.7 μm, Waters). 5 μL and 2 μL aliquots of the reconstituted metabolite extracts were injected for RP and HILIC analyses, respectively. Mobile phases containing 0.1% formic acid-water and 0.1% formic acid-acetonitrile were utilized for RP separation with 15 min fast gradient. On the other hand, for HILIC analysis, a binary mobile phase comprising of 15% water in acetonitrile containing 10 mM ammonium acetate with pH adjusted to 10 with ammonium hydroxide, and 50% water in acetonitrile containing the same buffer salt was employed. Every 10 randomized samples a pooled QC sample was injected to monitor the analytical performance, there were 7 QCs in the batch totally.

Mass spectrometer was operated under electrospray positive and negative ionization mode for RP and HILIC separation, respectively. The ion source parameters were set as below: sheath gas 45 arb, aux gas 10 arb, heater temperature 350℃ (300℃ for HILIC), ion transfer capillary temperature 300℃, S-lens voltage 50%. The 70 ~ 1000 m/z ionized metabolic features were profiled under 70,000 FWHM resolution with 250 ms maximum ion injection time and 1e6 AGC target. Full scan-ddMS2 data of the pooled QC sample was repeated injected to acquire high resolution accurate mass MS2 spectra for metabolite structural confirmation. The key settings of ddMS2 mode include: 17500 FWHM resolution, 1e5 AGC target, 1.0 Da precursor isolation window, loop count 3, stepped NCE 15%, 30% and 45%, dynamic 5 ~ 8 s. And the top 100 background ions in the solvent blank were put into exclusion list to improve the ddMS2 quality.

Untargeted metabolic profiling data process.

The raw spectral data were processed by using Compound Discoverer 2.1 (Thermo Scientific, San Jose) for chromatographic alignment, metabolic component detection and deconvolution, gap re-filling, artifacts exclusion, elemental prediction and structural elucidation. The metabolites were firstly annotated by searching a local endogenous metabolite and lipid database within 5 ppm mass tolerance, and further confirmed through searching against an in-house built MS2 spectral library and mzCloud library (www.mzcloud.org). A proprietary Highchem HighRes algorithm was utilized to calculate the spectral similarity between reference and the retrieved spectra with the similarity score threshold 50.

Statistical analysis.

The data was imported into SIMCA-P 14.1 (Umetrics AB, Umea, Sweden) to perform partial leastsquares – discriminant analysis (PLS-DA). Permutation test was exploited to verify the fitting degree of PLS-DA model. R2 represents the explanation capacity of the model, while Q2 stands for the predictive capacity of the model. Student's t test was analyzed on Excel (Microsoft, USA) software, and $p < 0.05$ was considered to be a significant difference. The differential metabolites were imported to Multi Experiment Viewer (MEV) software and generated a heat map to visualize the relative levels of metabolites in different groups. Metabolic pathway analysis was conducted on MetaboAnalyst Web site (http://www.metaboanalyst.ca). GraphPad Prism 6 was used for statistical analysis. Results were presented as mean (S.D.) for continuous variables and as a percentage for categorical variables. Statistical significance was set at $P < 0.05$ unless annotated otherwise.

Results

Patient demographics and clinical outcomes.

The anthropometric and metabolic characteristics of the study subjects are shown in Table 1. There were no statistically significant differences in age or body mass index between the control and HUA patients. The HUA group had a significantly higher waist-to-hip ratio, diastolic blood pressure ($p < 0.05$), triglyceride level, and SUA level ($p < 0.01$) than those without HUA. Compared to healthy controls (HCs), high-density lipoprotein cholesterol and fractional excretion of urate were significantly lower in HUA patients ($p < 0.01$).

Table 1
Baseline characteristics for the HUA(AH and Gout subgroups) and Control groups.

	HC(n = 10)	HUA(n = 40)	AH(n = 20)	Gout(n = 20)
Age(year)	41.80 ± 13.53	40.23 ± 9.27	37.30 ± 11.44	43.15 ± 5.23#
BMI(Kg/m^2)	23.12 ± 3.01	24.83 ± 2.63	25.84 ± 1.57	24.41 ± 2.89
WHR	0.89 ± 0.07	0.96 ± 0.08*	0.97 ± 0.14	0.95 ± 0.07
SBP(mmHg)	116.50 ± 8.78	126.53 ± 14.95	123.55 ± 10.55	130.25 ± 18.82
DBP(mmHg)	74.50 ± 9.13	83.47 ± 10.58*	80.05 ± 7.47	87.75 ± 12.46#
ALT(U/L)	21.20 ± 14.05	32.95 ± 27.87	36.35 ± 30.58	29.55 ± 25.20#
AST(U/L)	22.30 ± 5.85	25.88 ± 13.48	28.20 ± 15.43	23.55 ± 11.11
TC(mmol/L)	4.95 ± 0.98	4.92 ± 1.09	4.89 ± 1.08	4.94 ± 1.13
TG(mmol/L)	1.25 ± 0.66	2.85 ± 1.82 **	2.80 ± 1.78	2.90 ± 1.91
HDL-C(mmol/L)	1.33 ± 0.26	0.95 ± 0.23 **	0.90 ± 0.24	1.00 ± 0.22
LDL-c(mmol/L)	3.04 ± 0.89	2.67 ± 0.91	2.57 ± 0.92	2.77 ± 0.91
SCr(umol/L)	80.00 ± 12.21	79.55 ± 15.73	76.35 ± 13.35	82.75 ± 17.56
SUA(umol/L)	316.20 ± 60.88	476.4 ± 104.45**	470.48 ± 36.12	482.35 ± 144.97
HbA1c(%)	5.51 ± 0.20	5.80 ± 2.02	5.57 ± 0.46	5.85 ± 2.22
FEUA(%)	6.89 ± 1.72	4.66 ± 1.43**	4.09 ± 0.80	4.76 ± 1.51
Body mass index, BMI; Waist-to-Hip Ratio, WHR; Systolic blood pressure, SBP; Diastole blood pressure, DBP; Alanine aminotransferase, ALT；Aspartate aminotransferase, AST；Total cholesterol, TC； Triglyceride, TG；High-density lipoprotein cholesterol, HDL-C；Low-density lipoprotein cholesterol, LDL-C；Screatinine, SCr；Serum urate, SUA；Glycosylated hemoglobin, HbA1c；The fractional of urate excretion, FEUA;				
*p < 0.05, **p < 0.01, vs Control; #p < 0.05, ##p < 0.01, vs AH group, adjusted for age.				

Analysis of serum metabolite profiles by an untargeted method.

All serum samples of the HC and HUA patients were analyzed with an untargeted LCMS method. In total, 1,207 and 757 variables were analyzed in the electrospray ionization + and − modes, respectively. The 373 variables derived by combining these data were included in the subsequent univariate and multivariate

analyses. The coefficients of variation of internal standard D_9-carnitine, $^{13}C_6$-phenylalanine, and D_3-palmitoyl carnitine peak areas in seven quality controls evenly inserted into the analytical batch were 5.4, 5.9, and 13.4%, respectively. According to a stability analysis demonstrated, that the analytical method performed well.

We conducted a partial least squares–discriminant analysis (PLS-DA). As shown in Fig. 1A, a PLS-DA model was designed to identify metabolites that can distinguish between HC and HUA (including AH and gout). The R^2Y and Q^2 values were 0.787 and 0.466, respectively. A PLS-DA score plot was generated to show the metabolic profiles of the HCs and HUA patients. We validated the model using a permutation test, and the intercepts of R^2 and Q^2 were 0.648 and − 0.205, respectively (Fig. 1B); there was no overfitting. However, the PLS-DA model could not distinguish between AH and gout, as shown in Fig. 1C.

Metabolic differences between the healthy control and hyperuricemia groups.

Figure 2A shows the metabolic alterations in the HC and HUA groups. Compared to the HC group, some metabolites were significantly changed in the HUA subjects, such as urate and most of the free fatty acids (FFAs), lysophosphatidylcholines, acylcarnitines, and amino acids. As shown in the Venn diagram in Fig. 2B, the levels of 86 and 98 metabolites were altered in the AH and gout subgroups, respectively, compared to the HC group; 60 of these were common to both subgroups. Furthermore, alterations of some metabolites, including glutamate (Glu), 2oxoglutarate, taurine, choline, and non-essential amino acids, were significantly associated with the formation of HUA (Fig. 2C).

The metabolites in the HC and HUA groups were subjected to a metabolic pathway analysis. Pathways with an impact value > 0.1 ($p < 0.05$, HC vs. HUA) were considered significantly perturbed and are shown in Fig. 2D; these included taurine and hypotaurine; alanine (Ala), aspartate and Glu; and Darginine and Dornithine.

Potential metabolic biomarkers of gout.

In total, 27 differential metabolites were identified between the AH and gout subgroups (Fig. 3A). Some metabolites, such as phenylalanine (Phe), kynurenine, and aspartic acid, showed a significant increase in the gout group compared to the AH group, while tetracosahexaenoic acid showed a significant decrease (Fig. 3B – E).

Metabolic changes induced by febuxostat.

HUA treatment is based on dietary and lifestyle management, as well as pharmacological intervention. Drug treatment is aimed at the inhibition of urate production, such as via allopurinol or febuxostat, and

promoting the excretion of urate. The National Institute for Health and Clinical Excellence concluded that febuxostat is more effective than standard doses of allopurinol[7].

We evaluated the effects of febuxostat on metabolic profiles in the context of urate-lowering therapy. In total, 10 HUA patients were treated with 40 mg/d of febuxostat, and their SUA levels after 0, 6, 12, 18, and 24 weeks are shown in Fig. 4. Under this therapeutic regimen, febuxostat treatment resulted in a significant ($p < 0.05$) decrease in SUA levels after 24 weeks, followed by a return to normal levels. We created a PLS-DA loading plot to display the metabolites differing in expression before versus after febuxostat treatment (Fig. 5A – B). The metabolites on the right-hand side of Fig. 5C showed higher levels after treatment than before, especially FFAs like oleic, linolenic, and palmitate acids. By contrast, Lcarnitine and urate levels were lower than before.

Discussion

The metabolomics approach has been successfully applied in recent years to identify early signals or biomarkers of abnormalities[8], and to characterize biological pathways [9] and diagnose disease [8, 10]. LC-MS is a powerful approach for the study of metabolomics due to its simple sample pretreatment process, wide coverage of metabolites, and high sensitivity [11]. In the present study, we used LC-MS-based metabolomics to analyze metabolites in the serum of patients with AH and gout, to better understand predisposing factors for gout. We investigated alterations in the metabolome in AH and gout patients: significant alterations in FFAs, acylcarnitines, amino acids, bile acids, and lipids species were observed and could be useful to differentiate between HCs and HUA patients. Specifically, Glu, 2-oxoglutarate, taurine, choline, and some non-essential amino acids might play important roles in the formation of urate. The process of urate formation also appeared to involve the metabolism of taurine and hypotaurine; Ala, aspartate and Glu; and D-arginine and D-ornithine. Although the metabolite changes were not significantly different between the AH and gout groups according to the PLS-DA score plot, the metabolic alterations observed might explain the occurrence of gout to some extent.

Certain amino acids participate in the biosynthesis of purine and subsequent formation of urates. For example, amino acids such as glutamine, glycine (Gly), and serine are implicated in the formation of urate in gout[12]. Therefore, it is reasonable to postulate that amino acids play important roles in the pathogenesis of gout. The serum levels of Ala, aspartic acid, oxoglutaric acid, glutamine, and L-glutamic acid were higher in the HUA group. Ala, aspartate, and Glu are important in energy metabolism. In addition, glutamine and aspartate are important for the biosynthesis of endogenous purine nucleotide through the *de novo* synthesis pathway, which requires a significant amount of energy[13]. Glutamine and hypoxanthine are intermediary products in the formation of urate in birds, and it has been suggested that the mechanism may be the same in HUA patients. Therefore, glutamine may be an intermediate in the formation of both urea and urate[14]. Ts'ai-fan Yu reported an abnormally high plasma glutamic acid

level in AH patients, and the elevated glutamate level was attributed to a deficiency of glutamic dehydrogenase[15]. In the presence of intracellular accumulation of Glu in cases of glutamic dehydrogenase deficiency, renal production of ammonium may be reduced due to its inhibitory effect on glutaminase. As a result of a renal blockade preventing ammonia formation, surplus glutamine may be instead be used for urate synthesis.

Oxoglutaric acid (2-oxoglutarate), which can be produced from Glu by oxidative deamination (via glutamate dehydrogenase), is a key molecule in the tricarboxylic acid cycle and plays a fundamental role in determining the overall rate of this important metabolic process. Several other amino acids have also been shown to be differential metabolites, including arginine (Arg), leucine (Leu), isoleucine (Ile), and serine. Our findings are consistent with those of Kaplan et al.[16], who found significantly elevated levels of serum Ala, Ile, Leu, valine (Val), tyrosine (Tyr), Phe, and lysine (Lys) in patients with gout. Yű et al. [12] suggested that amino acids like glutamine, Gly, and serine are involved in the formation of urate in gout, as also observed in our study. An imbalance of amino acid homeostasis is closely associated with the formation of HUA.

Furthermore, many dipeptides were significantly changed in our HUA group, such as Arg-asparagine (Asn), Gly-Val, Leu-Gly, and Glu-Tyr. It has been reported that tryptophan-containing dipeptides inhibit xanthine oxidase (XO). Current understanding of dipeptides is incomplete, and other dipeptides may therefore be interesting targets for novel treatments and strategies for preventing HUA and related diseases.

Changes in taurine and hypotaurine metabolism were found in the HUA group. Compared to the HC group, the taurine level was significantly lower in the HUA group. Taurine is a sulfur amino acid, like methionine, cystine, cysteine, and homocysteine. It can be synthesized by the body from cysteine when vitamin B6 is present. Taurine efficiently decreased elevated XO activities and reduced urate formation in hyperuricemic rats. Moreover, it prevented any decrease in the mRNA and protein expression levels of urate transporters, and regulated renal urate excretion[17]. Therefore, taurine might be a promising agent for the treatment of HUA.

Previous studies found that HUA patients experienced changes in lipid metabolism similar to those seen during the course of cardiovascular disease[18]. The results of the present study suggested that abnormal fatty acid metabolism may be one of the metabolic pathways involved in the pathogenesis of HUA. Palmitic acid and oleic acid are involved in the synthesis and metabolism of fatty acids, elongation of fatty acids in mitochondria, synthesis of unsaturated fatty acids, and other biochemical reaction processes. The elevated plasma levels of these fatty acids in our patients with HUA suggested that disorders of fatty acid metabolism may occur in HUA. A marked alteration in HUA was related to the metabolism (β-oxidation and transition) of FFAs. Compared to the HC group, most FFAs were significantly increased in the HUA group. FFAs are a key energy source, and β-oxidation of fatty acids is reflected in the acylcarnitine profile[19].

Carnitine is essential for the transport of long-chain fatty acids from the cytosol to the intramitochondrial space in mammalian cells, and thus plays a major role in fatty acid oxidation [20]. Short-chain acyl derivatives of L-carnitine also prevent the lipid peroxidation induced in various cardiovascular tissues by an excess of oxygen free radicals. In the presence of acetyl-L-carnitine, a significant reduction of XO activity has been detected[21]. According to the results of the present study, L-carnitine levels in the HUA patients were reduced after the febuxostat treatment, while oleic, linolenic, and palmitate acids increased. Febuxostat is an XO inhibitor, and the increased oxidation of FFAs seems to be due to alteration of XO activity via effects on L-carnitine.

Long-term elevation of urate leads to various complications, among which gout is the most common. In individuals with HUA, gout is caused by an inflammatory reaction that arises in response to the deposition of urate, in the form of MSU crystals, in articular joints and bursal tissues. In our study, compared to the gout group, the levels of Phe, aspartic acid, and kynurenine were significantly higher in the AH group.

Phe is an essential amino acid and the precursor of Tyr. Like Tyr, Phe is also a precursor for catecholamines including tyramine, dopamine, epinephrine, and norepinephrine. Phe is highly concentrated in a number of high-protein foods, including meat, cottage cheese, and wheat germ. An additional dietary source of Phe is artificial sweeteners containing aspartame. Aspartame, a low-calorie sweetener, was shown to have antipyretic, analgesic, and anti-inflammatory actions, and to delay osteoarthritis in animal models[22]. Disruption of rheumatoid factor activity by aspartame has been proposed to alleviate the pain and immobility associated with chronic inflammation of joints [23]. In 1981, a chemotactic factor having a molecular mass of 8,400 was identified in gouty synovial fluid. Amino acid analysis demonstrated that this chemotactic factor was relatively rich in aspartic acid, glycine, serine, glutamic acid, and Ala[24].

Kynurenine, a metabolite of tryptophan, was also significantly changed in our gout group. Tryptophan and its catabolites have been found to suppress T cell-driven local inflammatory responses through indoleamine 2,3-dioxygenase[25]. Significantly elevated levels of one or more tryptophan metabolites were measured in the urine of active rheumatoid arthritis patients with comorbidities, especially hydroxykynurenine and kynurenine [26]. In summary, these results indicate that kynurenine may play an important role in the onset of gout-related inflammation. Moreover, aspartic acid may be involved in the chemotaxis of inflammation, and Phe is involved in the inflammation of joints.

The present study had some limitations. First, it included a small sample, and there was a bias about which patients had aspiration performed. Second, we could not investigate the relationship between plasma urate and serum metabolite levels due to a limited amount of data. Last, we did not examine the mechanisms underlying the metabolic alterations caused by HUA and gout.

Conclusions

Taken together, our results showed that novel serum metabolites may be important in the pathogenesis of HUA, particularly alterations in the metabolism of taurine and hypotaurine; Ala, aspartate, and Glu; and D-arginine and D-ornithine. Further exploration of the metabolic differences between HCs and HUA patients found significant increases in Ala, aspartate, Glu, and oxoglutaric acid, which are closely involved in energy consumption. Arginine, Leu, Ile, and serine were elevated in the HUA group, indicating a disturbance in amino acid homeostasis. We hypothesized that febuxostat inhibited the activity of XO, decreased urate, and consumed L-carnitine, leading to changes in lipid metabolism. Taurine might reduce urate formation, as well as promote renal urate excretion, so the low level thereof in our HUA group merits attention. Furthermore, Phe, aspartic acid and kynurenine may be critical in the occurrence of gout. However, further study is needed to validate our findings and clarify the mechanism underlying the progression from AH to gout.

Abbreviations

HUA: Hyperuricemia; LC-MS: Liquid chromatography–mass spectrometry; AH: Asymptomatic hyperuricemia; MSU: Monosodium urate; BMI: Body mass index; WHR: Waist-to-Hip Ratio; SBP: Systolic blood pressure; DBP: Diastole blood pressure; ALT: Alanine aminotransferase；AST: Aspartate aminotransferase；TC: Total cholesterol；TG: Triglyceride；HDL-C: High-density lipoprotein cholesterol；LDL-C: Low-density lipoprotein cholesterol；SCr: Screatinine；SUA: Serum urate；HbA1c: Glycosylated hemoglobin；FEUA: The fractional of urate excretion.

Declarations

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None declared.

Author Contributions

YWM, KFG: Analysed the data and wrote the manuscript. MW, XTC: Analysed and acquisition of data. SQ: Critical revision of the manuscript. HBC: Study conception and design, critical revision of the manuscript, and approval of the final version of the manuscript. All authors approved the submitted version.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Informed consent was obtained from all the individuals who participated in the study. The study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Reginato AM, Mount DB, Yang I, Choi HKJNRR: **The genetics of hyperuricaemia and gout**. 2012, **8**(10):610-621.
2. Campion EW, Glynn RJ, DeLabry LO: **Asymptomatic hyperuricemia. Risks and consequences in the Normative Aging Study**. *The American journal of medicine* 1987, **82**(3):421-426.
3. Priyanka C, Edward R, Lorna C, Jane R, Hider SL, Mallen CDJR: **Health-related quality of life in gout: a systematic review**. 2013(11):11.
4. Marilisa B, Giuseppe CAF, Maddalena V, Claudio BJVH, Management R: **An evidence-based review on urate-lowering treatments: implications for optimal treatment of chronic hyperuricemia**. 2017, **Volume 13**:23-28.
5. Dettmer K, Aronov PA, Hammock BD: **Mass spectrometry-based metabolomics**. *Mass spectrometry reviews* 2007, **26**(1):51-78.
6. Neogi T, Jansen TLTA, Dalbeth N, Fransen J, Schumacher HR, Berendsen D, Brown M, Choi H, Edwards NL, Janssens HJEMJAotRD: **2015 Gout classification criteria**. 2015, **74**(10):1789-1798.
7. Wang X, Zhao M, Su G: **Febuxostat for the management of hyperuricaemia in people with gout**.

8. Li Y, Ju L, Hou Z, Deng H, Zhang Z, Wang L, Yang Z, Yin J, Zhang Y: **Screening, verification, and optimization of biomarkers for early prediction of cardiotoxicity based on metabolomics**. *Journal of proteome research* 2015, **14**(6):2437-2445.
9. Ratnasekhar C, Sonane M, Satish A, Mudiam MK: **Metabolomics reveals the perturbations in the metabolome of Caenorhabditis elegans exposed to titanium dioxide nanoparticles**. *Nanotoxicology* 2015, **9**(8):994-1004.
10. Chan GH, Ho EN, Leung DK, Wong KS, Wan TS: **Targeted Metabolomics Approach To Detect the Misuse of Steroidal Aromatase Inhibitors in Equine Sports by Biomarker Profiling**. *Analytical chemistry* 2016, **88**(1):764-772.
11. Dettmer K, Aronov PA, Hammock BD: **Mass spectrometry-based metabolomics**. 2007, **26**(1):51-78.
12. Yü TF, Adler M, Bobrow E, Gutman AB: **Plasma and urinary amino acids in primary gout, with special reference to glutamine**. *The Journal of clinical investigation* 1969, **48**(5):885-894.
13. Boza JJ, Moënnoz D, Bournot CE, Blum S, Zbinden I, Finot PA, Ballèvre O: **Role of glutamine on the de novo purine nucleotide synthesis in Caco-2 cells**. *European journal of nutrition* 2000, **39**(1):38-46.
14. ÖRSTRÖM Å, Örström MJAMS: **The Rôle of Glutamine in Metabolism, with Special Regard to the Formation of Uric Acid in Gout**. 1950, **138**.
15. Yű Ta-f, Medicine CKJAiE, Biology: **The Natural History of Hyperuricemia Among Asymptomatic Relatives of Patients with Gout**. 1980, **122A**:1.
16. KAPLAN DJAoIM: **Serum and Urinary Amino Acids in Normouricemic and Hyperuricemic Subjects**. 1965, **62**(4).
17. Feng Y, Sun F, Gao Y, Yang J, Wu G, Lin S, Hu J: **Taurine decreased uric acid levels in hyperuricemic rats and alleviated kidney injury**. *Biochemical and biophysical research communications* 2017, **489**(3):312-318.
18. Peng TC, Wang CC, Kao TW, Chan JY, Yang YH, Chang YW, Chen WL: **Relationship between hyperuricemia and lipid profiles in US adults**. *BioMed research international* 2015, **2015**:127596.
19. Adams SH, Hoppel CL, Lok KH, Zhao L, Wong SW, Minkler PE, Hwang DH, Newman JW, Garvey WT: **Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women**. *The Journal of nutrition* 2009, **139**(6):1073-1081.
20. Borum PR: **Carnitine**. *Annual review of nutrition* 1983, **3**:233-259.
21. Di Giacomo C, Latteri F, Fichera C, Sorrenti V, Campisi A, Castorina C, Russo A, Pinturo R, Vanella A: **Effect of acetyl-L-carnitine on lipid peroxidation and xanthine oxidase activity in rat skeletal muscle**. *Neurochemical research* 1993, **18**(11):1157-1162.
22. LaBuda CJ, Fuchs PN: **A comparison of chronic aspartame exposure to aspirin on inflammation, hyperalgesia and open field activity following carrageenan-induced monoarthritis**. *Life sciences* 2001, **69**(4):443-454.

23. Ramsland PA, Movafagh BF, Reichlin M, Edmundson AB: **Interference of rheumatoid factor activity by aspartame, a dipeptide methyl ester**. *Journal of molecular recognition : JMR* 1999, **12**(5):249-257.
24. Phelps P, Andrews R, Rosenbloom J: **Demonstration of chemotactic factor in human gout: further characterization of occurrence and structure**. *The Journal of rheumatology* 1981, **8**(6):889-894.
25. Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH: **Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy**. *Nature immunology* 2001, **2**(1):64-68.
26. Flinn JH, Price JM, Yess N, Brown RR: **EXCRETION OF TRYPTOPHAN METABOLITES BY PATIENTS WITH RHEUMATOID ARTHRITIS**. *Arthritis and rheumatism* 1964, **7**:201-210.

Figures

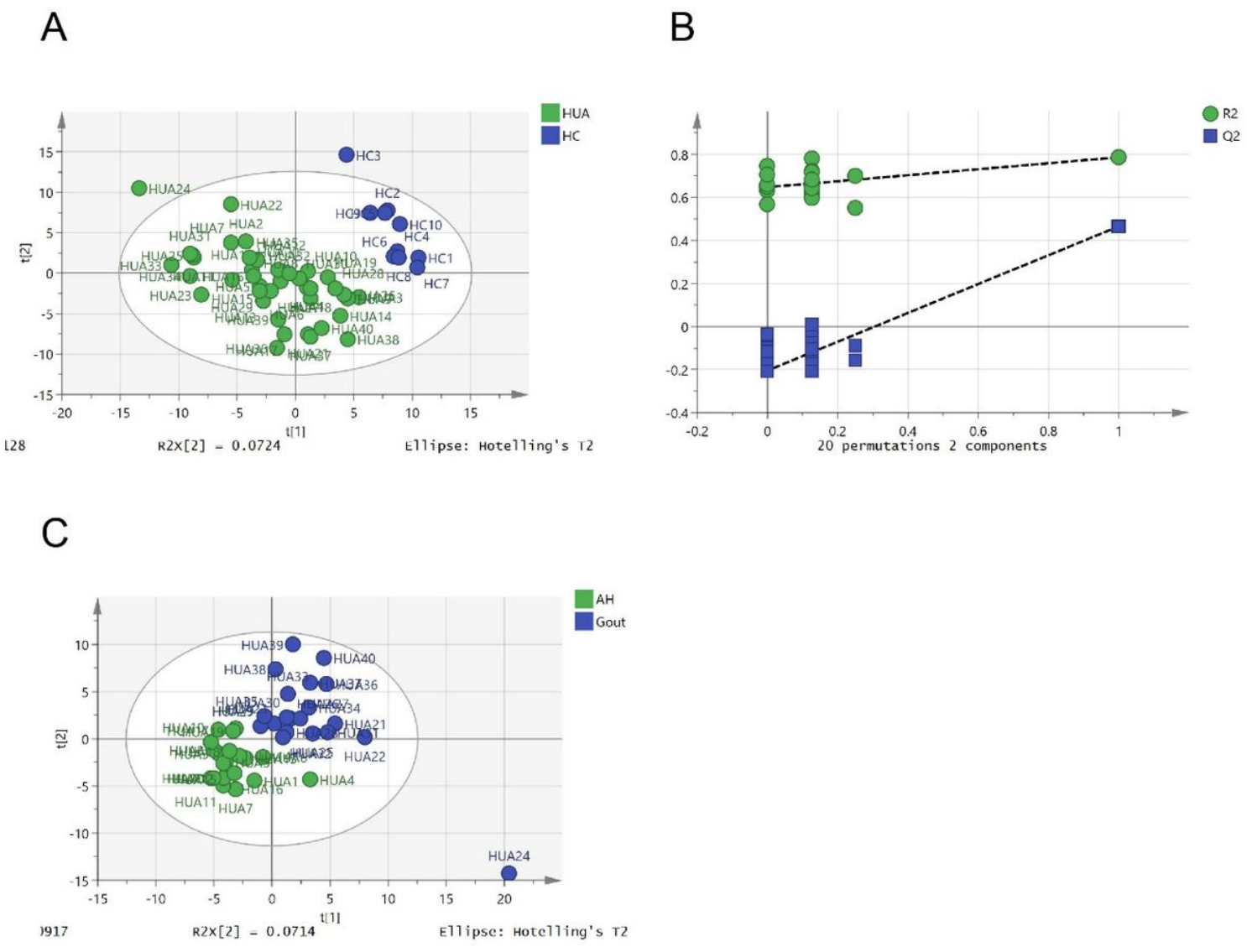

Figure 1

Partial least squares–discriminant analysis (PLS-DA) score plots. (A) PLS-DA score plot of healthy control and high urate (hyperuricemia [HUA; including asymptomatic HUA] and gout) groups. Data obtained in electrospray ionization + and − modes were combined. The R2Y, Q2 and A* values were 0.787, 0.466, and 2, respectively. (B) Validation of the PLS-DA score plot. In the permutation test the R2 and Q2 intercept values were 0.648 and -0.205, respectively. (C) PLS-DA score plot of asymptomatic HUA and gout groups. Data obtained in electrospray ionization + and − modes were combined. The R2Y, Q2 and A* values were 0.783, 0.102 and 2, respectively.

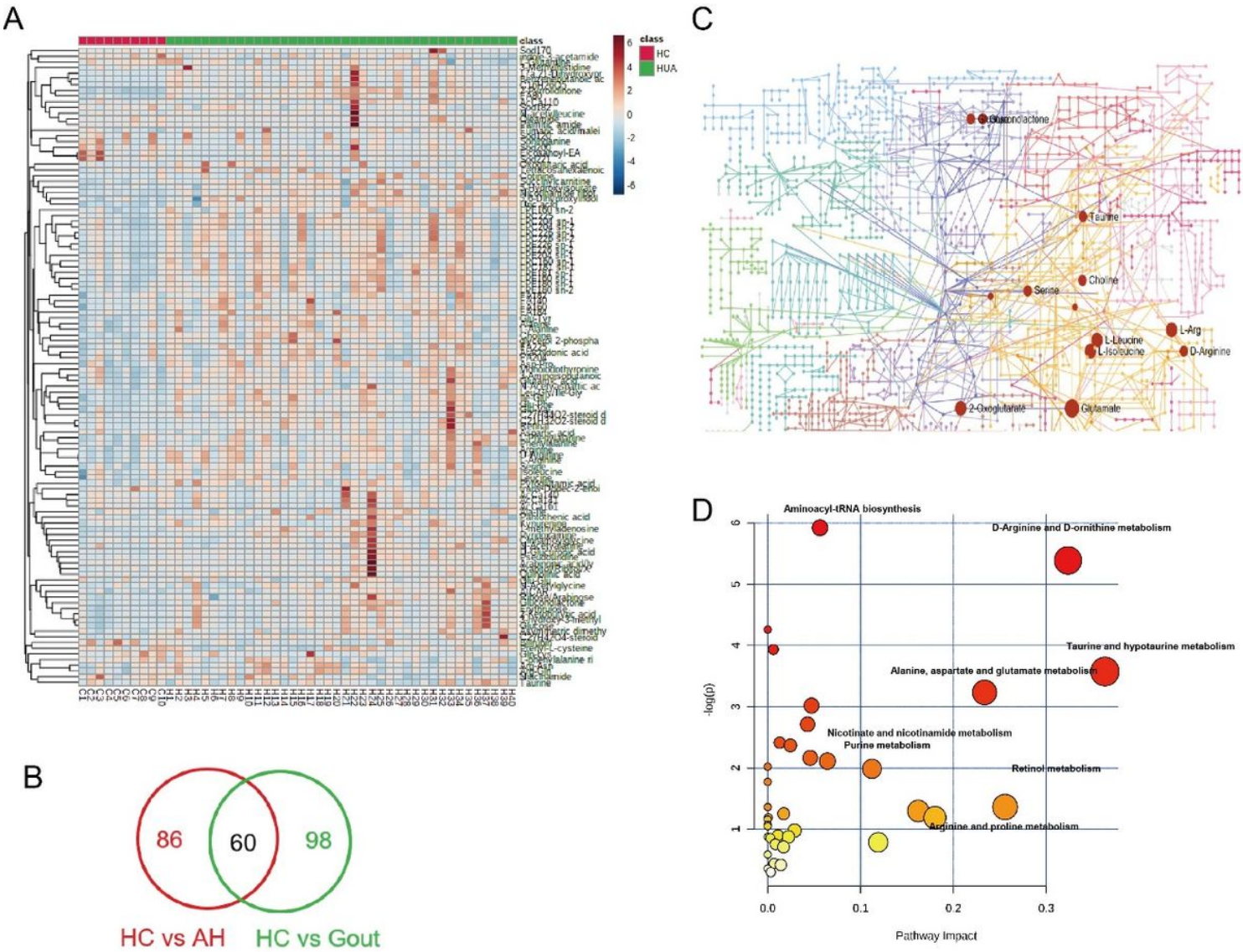

Figure 2

(A) Heat map of differential metabolites among the gout, asymptomatic hyperuricemia (HUA), and healthy control (HC) groups; red and blue indicate high and low levels of metabolites, respectively, while white indicates an equal level between groups. (B) Venn diagram of differential metabolites between the HCs and HUA patients. (C) Significantly different metabolites s. (D) Disrupted pathways in the HUA (including asymptomatic HUA and gout) groups; a small p value and large pathway impact value indicate that the pathway is significantly perturbed.

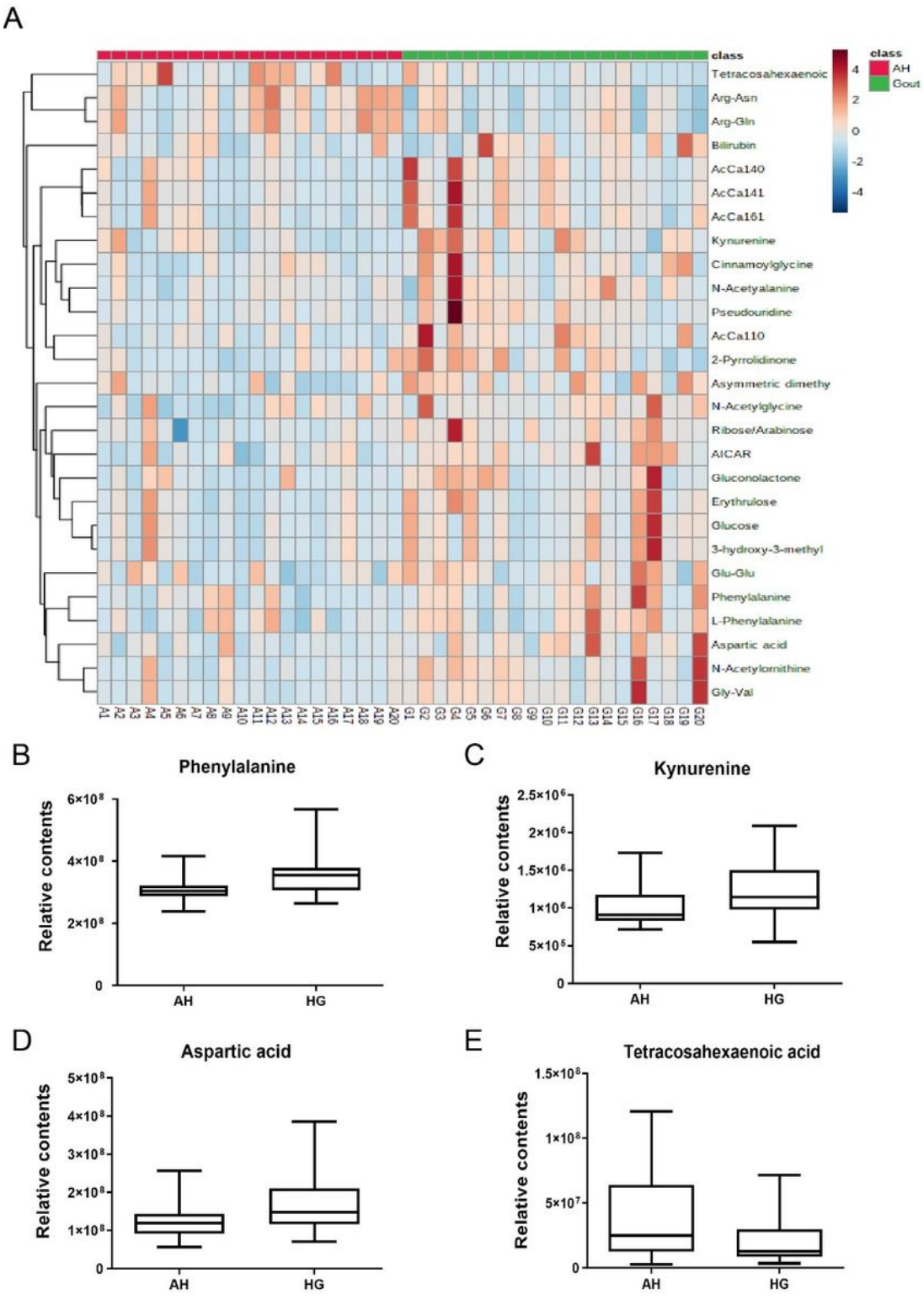

Figure 3

(A) Heat map of differential metabolites between the asymptomatic HUA and gout groups; yellow and blue indicate high and low levels of metabolites, respectively, while black indicates an equal level between groups. (B–E) Contents of four significantly changed metabolites: (B) phenylalanine, (C) kynurenine, (D) aspartic acid, and (E) tetracosahexaenoic acid.

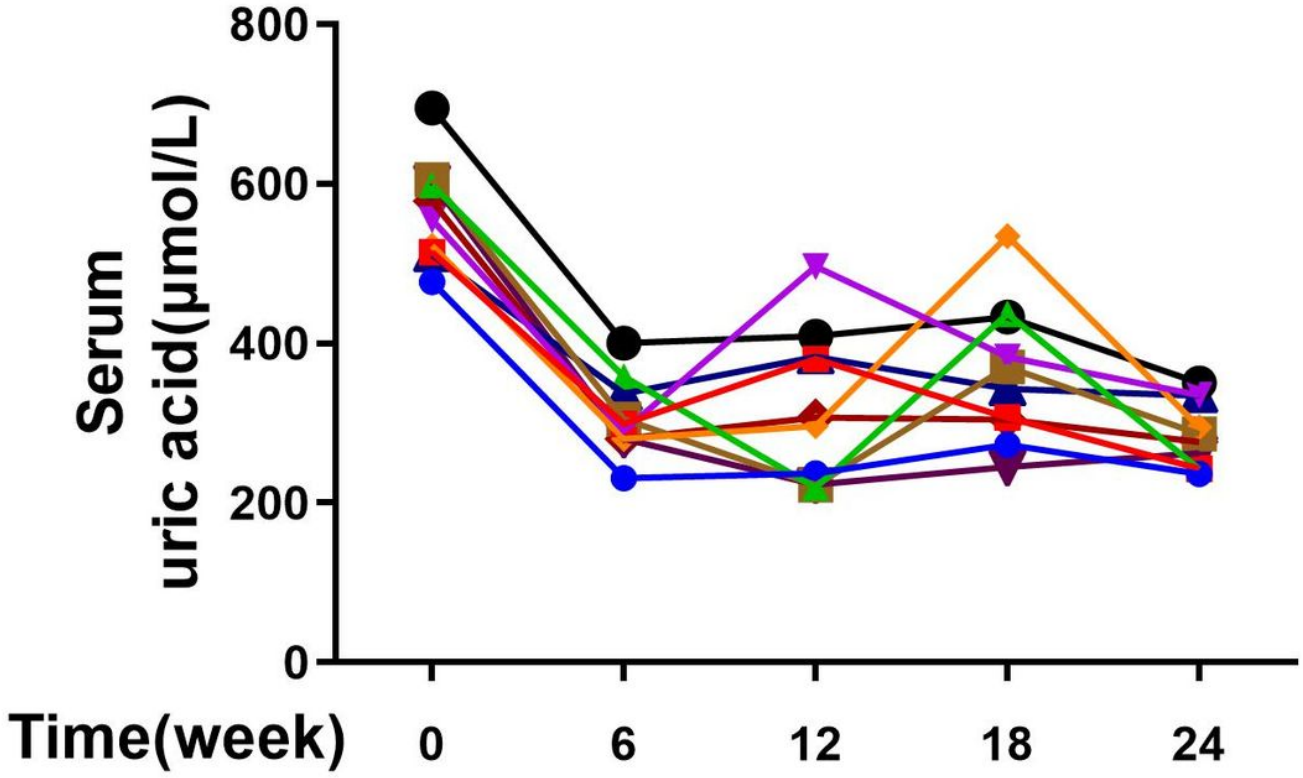

Figure 4

Serum urate levels of patients treated by febuxostat after 0, 6, 12, 18, and 24 weeks.

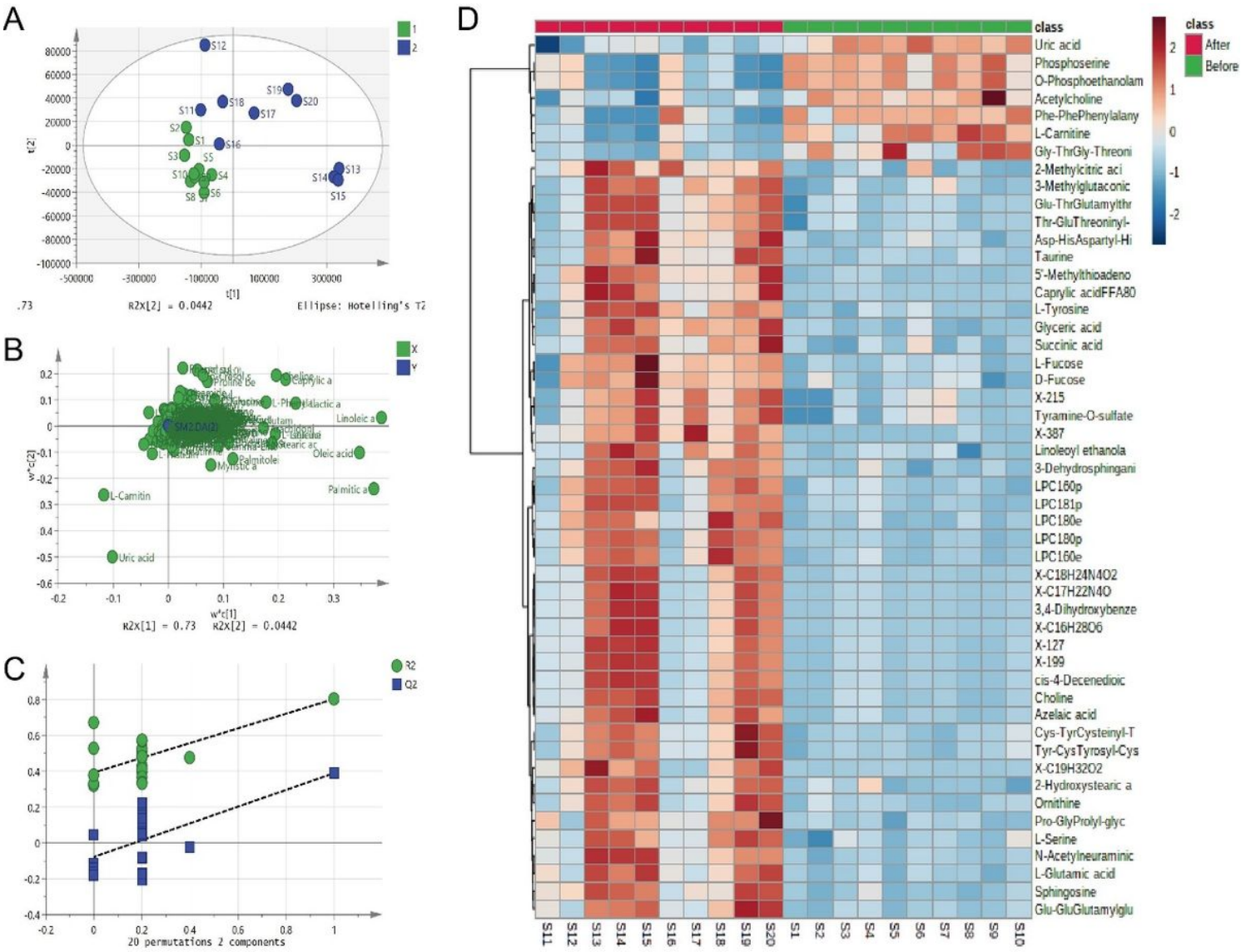

Figure 5

(A) Partial least squares–discriminant analysis (PLS-DA) score plot showing metabolite expression before and after febuxostat treatment. Data obtained in electrospray ionization + and − modes were combined. The R2Y, Q2 and A* values were 0.774, 0.39 and 2, respectively. (B) Validation of the PLS-DA score plot. In the permutation test, the R2 and Q2 intercept values were 0.432 and -0.0221, respectively. (C) PLS-DA loading plot showing metabolite expression before and after febuxostat treatment. (D) Heat map of the top 50 differential metabolites between before and after febuxostat treatment; red and blue indicate high and low levels of metabolites, respectively, while white indicates an equal level between groups.