European Review for Medical and Pharmacological Sciences 2019; 23: 3940-3950

Identification of psoriasis vulgaris biomarkers in human plasma by non-targeted metabolomics based on UPLC-Q-TOF/MS

S.-S. LI[1], Y. LIU[2], H. LI[3], L.-P. WANG[4], L.-F. XUE[1], G.-S. YIN[5], X.-S. WU[1]

[1]Department of Pharmacy, the First Affiliated Hospital of Jinan University, Guangzhou, China
[2]Department of Pharmacy, Shunde Longjiang Hospital, FoShan, China
[3]Chinese Internal Medicine, Guangzhou Red Cross Hospital, Guangzhou, China
[4]Food Safe Laboratory, China National Analytical Center, Guangzhou Guangdong Institute of Analysis, Guangzhou, China
[5]Department of Laboratory Medicine, the First Affiliated Hospital of Jinan University, Guangzhou, China

Shasha Li and Ying Liu contributed equally to this work

Abstract. – OBJECTIVE: The aim of the study was to investigate the endogenous metabolites of patients with psoriasis vulgaris which will be helpful for the diagnosis of the disease and to provide the evidence of pathogenesis and the formulation for the individualized dosage regimen.

PATIENTS AND METHODS: This study investigated the plasma metabolomic profiling between the psoriasis vulgaris patients (N=12) and the healthy volunteers (N=12) using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) metabolomic techniques. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to identify and visualize the metabolic data clusters.

RESULTS: A total of 22 differential metabolites contributing to the clusters were identified, among which the levels of threonine (p<0.001), leucine (p<0.001), phenylalanine (p<0.001), tryptophan (p=0.018), palmitamide (p<0.001), Linoleic amide (p<0.001), oleamide (p<0.001), stearamide (p<0.001), cis-11- eicosenamide (p< 0.001), trans-13-Docosenamide (p<0.001), uric acid (p=0.034), LysoPC (16:0) (p<0.001), LysoPC (18:3) (p<0.001), LysoPC (18:2) (p=0.024), LysoPC (18:1) (P=0.012) and LysoPC (18:0) (p=0.002) were significantly higher in the plasma of psoriasis vulgaris patients compared with the healthy controls, whereas oleic acid (p<0.001), arachidonic acid (p<0.001) and N-linoleoyl taurine (p<0.001) were significantly lower. These biomarkers are related to glucose metabolism, lipid metabolism, amino acid metabolism, nucleic acid metabolism and so on.

CONCLUSIONS: The data suggest that psoriasis vulgaris patients may have disrupted lipid and amino acid metabolism, as well as inflammation and functional lesions in the liver and kidney. This study deepens the understanding of psoriasis vulgaris pathogenesis and proposes novel ideas and methods for auxiliary diagnosis and treatment of the disease.

Key Words
Psoriasis vulgaris, Biomarkers, Plasma metabolomic profiling, UPLC/Q-TOF MS.

Introduction

Psoriasis is a common and easy to relapse chronic inflammatory skin disease affecting up to 3% of the world's population[1]. The major histopathological changes of the disease include hyper-proliferation of keratinocytes in the epidermis, dilation and growth of dermal capillary vasculature, infiltration of T cells, dendritic cells, neutrophils and other inflammatory cells[2-4]. There are four types of clinical psoriasis, namely arthropathic, pustular, erythrodermic and vulgaris, among which psoriasis vulgaris is the most common and is typically characterized by circumscribed, scaling and erythematous plaques[1,4]. The duration of psoriasis vulgaris is longer, the lesions are extensive and the disease often relapses repeatedly. Scales, itching and visible plaques are the main problems frustrating the patients and the disease is hard to be cured. Therefore, it not only bring heavy economic burden to patients, but also increase the patients' mental pressure, which seriously affects their quality of life[5,6]. In addition,

Corresponding Author: Xiaosong Wu, MD; e-mail: twxs2010@126.com

psoriasis vulgaris can cause a variety of complications, such as psoriatic arthritis, cardiovascular disease and metabolic syndrome[5,7]. Hence, it is one of the dermal diseases for emphatic study at the moment.

At present, the clinical diagnosis of psoriasis vulgaris is mainly based on the clinical manifestations, lesion characteristics and location and histopathological changes[8]. Histopathological analysis of a skin biopsy specimen is currently the most common and efficient clinical identification method. Nonetheless, skin biopsy is invasive and the pathological alterations are not significant at early stages of psoriasis[9]. In addition, the specificity and sensitivity of routine laboratory examination indexes for psoriasis vulgaris are low, the clinical value and significance are limited. So there is still a lack of specific diagnostic criteria for psoriasis vulgaris. The pathogenesis of the disease is uncertain and the patients are often accompanied by a variety of endogenous metabolic disorders. Therefore, it is possible to establish specific diagnostic criteria for psoriasis vulgaris from the perspective of metabolites, and to provide a direction for the further study of the pathogenesis of the disease and new long-term, effective and safe treatments.

Metabolomics is a branch of systems biology following genomics, transcriptomics and proteomics[10], and it studies the metabolic network of the body by investigating the metabolite spectrum and its dynamic changes of a living organism stimulated or disturbed (such as genic mutation or pathophysiological status)[11,12]. It is a sensitive and unbiased analytical method[13] that focuses on the measurement of the relative concentrations of endogenous small molecules in biofluids[14]. Commonly used metabolomic methods include nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and so on[15]. Especially, LC/MS is increasingly used for analysis of complex samples, such as serum or plasma[16]. Compared with LC-MS metabolomics technique, ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) has been widely applied to metabolomic studies owing to its high sensitivity, high resolution and reliable reproducibility[17,18].

Because of its unique advantages, metabolomics has developed rapidly since its appearance. In recent years, it has been widely used in many fields, such as disease diagnosis, drug mechanism research and drug development[19-21]. At present, metabolomics has made phased progress in the research of tumor[22-24], circulatory system[25,26], digestive system[27,28], urinary system[29], and endocrine system[30,31]. There have also been reports on the metabolomics study of psoriasis; for example, Kamleh et al[32] carried out an LC-MS metabolomic study on plasma of psoriasis patients, and discovered that psoriasis patients reveal disease severity-dependent increases in circulating amino acids. Lu et al[33] investigated the metabolic profiles of the urine samples of psoriasis patients with Blood Stasis Syndrome using LC-MS, and their data showed that the psoriasis patients with Blood Stasis Syndrome had changes mainly in fat metabolism. In Kang et al[34] exploration of candidate biomarkers for human psoriasis based on GC-MS serum metabolomics, it appears that the glycolysis pathway and amino acid metabolic activity are increased in patients with psoriasis. At present, there is no report on plasma metabolomic study of psoriasis vulgaris using UPLC/Q-TOF MS metabolomic technology.

In this paper, we investigated plasma metabolites from patients with psoriasis vulgaris and healthy controls using UPLC/Q-TOF MS metabolomic technology combined with multivariate statistical analysis, to identify potential biomarkers for the discrimination of psoriasis vulgaris patients from healthy volunteers, and to carry out related metabolic pathway analysis. It will be helpful for the diagnosis of the disease the further investigate the pathogenesis and the formulation of the individualized dosage regimen.

Patients and Methods

Patients

Psoriasis vulgaris patients ($N = 12$) and healthy volunteers ($N = 12$) were recruited at the First Affiliated Hospital of Jinan University in Guangzhou, China. The age, body mass index (BMI) and gender ratio of the healthy volunteers were equivalent to those of the patients enrolled. The severity of psoriasis vulgaris were evaluated by psoriasis area and severity index (PASI) scores and body surface area (BSA). The clinical characteristics of the subjects are provided in Table I. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University, China. All participants signed an informed consent before the research.

Table I. Characteristics of the participants.

	Psoriasis vulgaris group	Control group (N=12)	*p*-value (N=12)
Gender (male/female)	7/5	5/7	0.684[a]
Age (mean ± SD, years)	44.42 ± 9.97	41.67 ± 15.74	0.614[b]
BMI(kg/m^2)	22.60±1.39	21.68±1.46	0.127[b]
Disease duration (mean ± SD, years)	9.08 ± 5.90	–	–
BSA (mean ± SD, %)	18.60 ± 3.94%	–	–
PASI (mean ± SD)	9.93 ± 2.06	–	–

[a]*p* value obtained by χ^2-test of four-fold table.
[b]*p* value obtained by independent t-test with the Mann-Whitney U-test.

The inclusion criteria of patients were as follows: 1) In accordance with the diagnosis of psoriasis vulgaris in stable phase referring to *2008 Clinical Guidelines for Psoriasis* reported by the Chinese Medical Association; 2) age between 18 and 65 years old; 3) a PASI score between 7 and 20, and a BSA < 30%.

The exclusion criteria were: 1) arthropathic, pustular, erythrodermic psoriasis, or psoriasis in areas such as face, scalp, nails, wrinkle, glans, mucous membrane, or palmoplantar patients; 2) women in pregnancy, lactation, or planning pregnancy within one year; 3) patients with a Self-Rating Anxiety Scale (SAS) of > 50 or Self-Rating Depression Scale (SDS) of > 53, or patients with other mental illness; 4) patients taking part in other drug clinical trials or those who have participated in other clinical trials in the recent one month; 5) patients who have been treated with topical drugs such as hormone or retinoic acid in the recent 2 weeks or with ultraviolet light therapy or system therapy in the recent 4 weeks or with biological preparations in the recent 12 weeks; 6) patients that have tumors or severe primary diseases such as circulatory, respiratory, digestive, urinary, endocrine or hematopoietic diseases, or severe infection, water, electrolyte and acid-base balance disorders or calcium metabolism imbalance.

Sample Collection and Preparation

Blood samples were collected into 5 mL EDTA-K2 anticoagulant tubes from fasting subjects, and plasma was obtained after centrifugation at 4000 rpm for 15 min at 4°C. The supernatants were frozen at -80°C until analysis. For analysis, the plasma samples were thawed at room temperature for 20 min, and then 600 µL acetonitrile was added into 200 µL plasma, the mixture was vortexed and centrifuged at 12,000 rpm for 10 min at 4°C to precipitate the proteins. Next, 750 µL supernatant was collected and dried with nitrogen gas at 37°C. The dried residue was reconstituted in 100 µL of acetonitrile-water (10:90, v/v), after centrifugation for 10 min at 12,000 rpm. An aliquot of 5 µL was injected for UPLC/Q-TOF MS analysis.

In addition, 100 µL of plasma was extracted from each sample of healthy group and thoroughly mixed, and then divided into six aliquots as the quality control (QC) samples, which was used to analyze the reproducibility and stability of the present method.

UPLC/Q-TOF MS Analysis

UPLC/Q-TOF MS analysis was performed on a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) coupled to a Waters Micromass Q-TOF Mass Spectrometer (Waters Corporation, Manchester, UK) both in the positive and negative ionization modes. The column was an ACQUITY UPLC BEH-C18 column (2.1 mm × 100 mm, i.d. 1.7 µm; Waters Corporation, Milford, MA, USA) and the temperature was maintained at 40°C. The mobile phases used were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient program was optimized as follows: the composition of B was 5% initially, and it increased to 10% in a linear gradient from 0 to 6 min, 10%-38% from 6 to 10 min, 38%-100% from 10 to 15 min, 100% from 15 to 18 min, then decreased to 5% in 1 min and maintained for 3 min until the next injection, each run time was 22 min. The flow rate was 0.4 mL/min and the injection volume was 5 µL, the autosampler was maintained at 4°C.

For MS analysis, the source temperature was set at 120°C with the cone gas flow at 50 L/h, and the desolvation gas flow was set to 600 L/h at 350°C. The capillary voltage was set at 3.0 kV in the positive mode and 2.8 kV in the negative mode, and the cone voltage was set at 30

V. The scan time and interscan delay were set to 0.6 s and 0.1 s, respectively. The MS data were collected in centroid mode from m/z 50 to 1000 and the MS/MS spectra of metabolites were obtained by a collision energy ramp from 10-30 eV. All analyses were performed using the lock spray to ensure accuracy and reproducibility. A lock-mass of leucine-enkephalin for positive ESI mode (m/z = 556.2771) and negative ESI mode (m/z = 554.2615) was used *via* a LockSpray™ interface, and the flow rate was set at 5 μL/min. The lock spray frequency was set at 5 s and the lock mass data were averaged over 10 scans for correction.

Data Processing and Analysis

The raw data were imported to the MarkerLynx software version 4.1 (Waters Corporation, Milford, MA, USA) for deconvolution, alignment and normalization to obtain a three-dimensional data matrix containing retention time, m/z, and ion intensity. MarkerLynx parameters were set as follow: a peak width at 5%, height 1 s, a noise elimination of 6, and an intensity threshold of 70. Data were aligned with a mass tolerance of 0.04 Da and a retention time window of 0.2 min. Then, the data matrix was imported into the SIMCA-P 12.0 software (Umetrics AB, Umea, Sweden) for multivariate statistical analysis. The data variables were mean-centered and pareto scaled before analysis, unsupervised principal component analysis (PCA) and supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) were then performed to acquire clustering information and differential metabolites for distinguishing between the psoriasis vulgaris group and the control group. The variables with variable importance in the projection (VIP) ≥ 1 in the OPLS-DA model were selected as biomarker candidates for further statistical analysis using SPSS (Statistical Package for the Social Sciences) version 17.0 (SPSS Inc., Chicago, IL, USA). A level of $p<0.05$ was considered as a threshold for statistical significance and subsequent selection as the potential biomarkers. The goodness of fit was quantified by R^2X and R^2Y, while the predictive ability was indicated by Q^2Y. In addition, a cross-validation was performed by a 200 times permutation test to avoid the over-fitting of the supervised models.

Identification of Potential Biomarkers

According to mass spectra (MS or MS/MS, mass error of <10 ppm) and retention time, the chemical structure of the potential biomarkers was determined by matching with standard metabolites and/or interpreting with available reference standard mass spectral databases, such as HMDB (http://www.hmdb.ca/), Mass Bank (http://www.massbank.jp/), KEGG (http://www.kegg. com/) and METLIN (http://metlin. scripps.edu/).

Results

Method Validation

QC samples analysis was conducted by evaluating the repeatability and stability of the present method. One QC sample was injected at the start of the analytical sequence, followed by the analysis of one QC sample after running every 4 plasma samples. Ten ions were randomly extracted from the Base Peak Intensity (BPI) chromatography in the positive and negative ion modes and selected for method validation. The overlapped performance of the spectral peak was evaluated. The relative standard deviations (RSD) of peak intensity ranged between 3.12% and 9.45% in the positive ESI mode (<10%), and between 2.08% and 8.75% in the negative ESI mode (<10%). The results revealed that the developed method had excellent reproducibility and stability for metabonomic analysis.

Plasma Metabolic Profiling

Typical BPI chromatograms of plasma metabolic profiles analyzed using UPLC/Q-TOF MS both in positive and negative ion modes are shown in Figure 1. As shown in the figure, there were significant differences in the intensity of chromatographic peaks in the positive ion modes at some chemical shifts between the psoriasis vulgaris patients and the healthy controls (Figure 1A, 1B). The chromatographic peaks in the negative ion modes between the psoriasis vulgaris patients and the healthy controls attained similar results (Figure 1C, 1D).

Multivariate Statistical Analysis

Pattern recognition analysis *via* PCA and OPLS-DA was performed on positive and negative ESI data. The PCA and OPLS-DA score plots of psoriasis vulgaris patients and healthy controls are shown in Figure 2A and Figure 2B (positive ion mode), Figure 2D and Figure 2E (negative ion mode). The score plot reflects the distribution of various samples in the coordinate system composed of t[1] and t[2], in which each point represents a sample, and t[1] and t[2] represent the first two principal components, respectively. In PCA score plots (Figure 2A and Figure 2D), clear separation between the patients and the controls could be observed (posi-

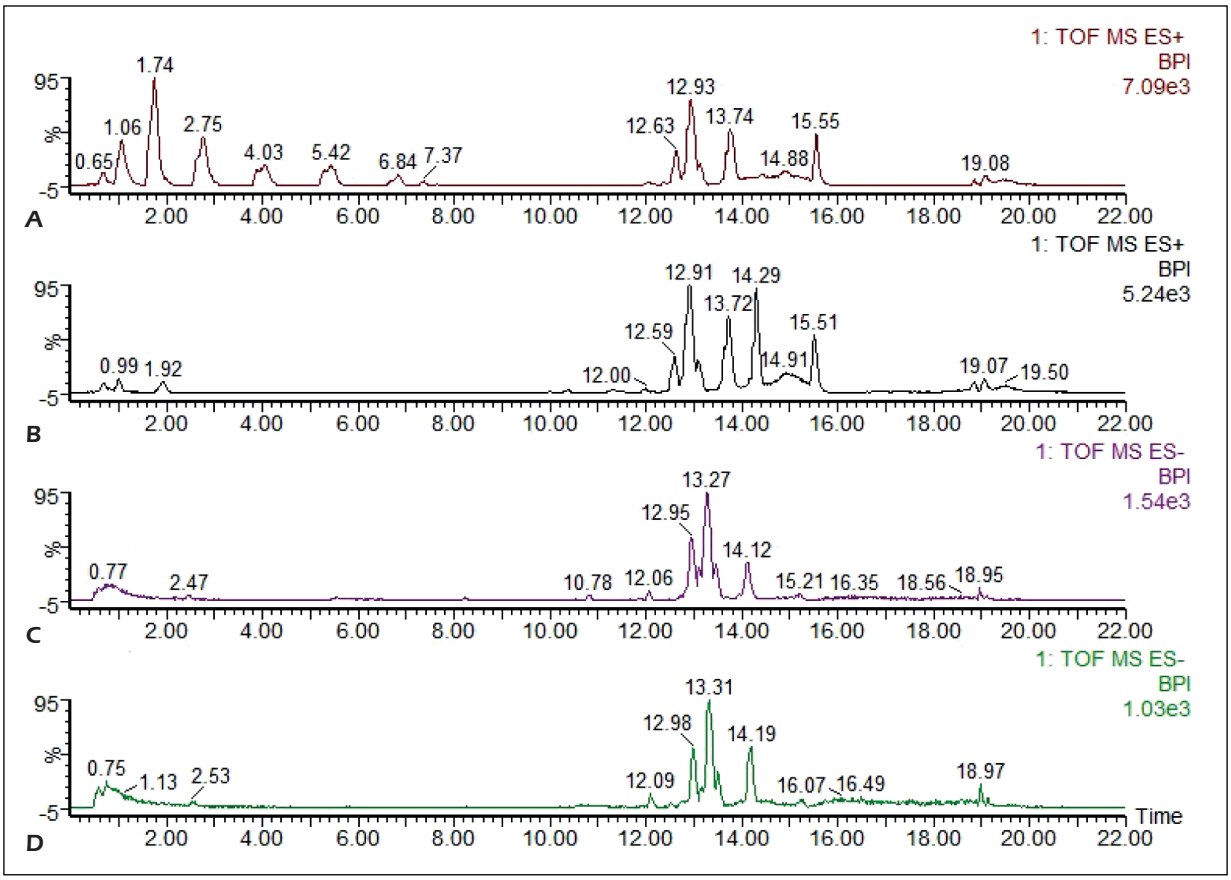

Figure 1. UPLC/Q-TOF MS BPI chromatograms obtained from the plasma samples of healthy control and psoriasis vulgaris patient. **A**, UPLC/Q-TOF MS BPI chromatograms in the positive ion modes obtained from the plasma samples of healthy control; **B**, UPLC/Q-TOF MS BPI chromatograms in the positive ion modes obtained from psoriasis vulgaris patient; **C**, UPLC/Q-TOF MS BPI chromatograms in the negative ion modes obtained from the plasma samples of healthy control; **D**, UPLC/Q-TOF MS BPI chromatograms in the negative ion modes obtained from psoriasis vulgaris patient.

tive ion mode: $R^2X = 70.6\%$, $Q^2 = 60.7\%$; negative ion mode: $R^2X = 73.0\%$, $Q^2 = 51.0\%$). In OPLS-DA score plots (Figure 2B. and Figure 2E), the separation between the two groups was more significant than that of the PCA score plots (positive ion mode: $R^2X = 67.1\%$, $R^2Y = 99.8\%$, $Q^2 = 99.5\%$; negative ion mode: $R^2X = 76.2\%$, $R^2Y = 98.5\%$, $Q^2 = 94.5\%$). The results suggest that these models have good practicability and predictability, and the separation between the patients and the controls reveal fundamental metabolic differences between the two groups. The permutation test with a permutation number of 200 was performed and indicated a ^{2} intercept value of 0.456 and a ^{2} intercept value of -0.060 in the positive ion mode, and a ^{2} intercept value of 0.850 and a ^{2} intercept value of -0.017 in the negative ion mode, indicating that there was no over-fitting of the supervised models.

The OPLS-DA S-plots of psoriasis vulgaris patients and healthy controls are shown in Figure 2C (positive ion mode) and Figure 2F (negative ion mode). The S plots of metabolites along the axes corresponding to the combined weight (w*) and reliability correlation [p(corr)] indicates the contribution of individual metabolites to the separation between the two groups, with each point in the figure representing a metabolite. The variables plotted farther away from the origin of the plot have a greater contribution to the model classification, and are identified as candidates for potential biomarkers. Potential biomarkers were eventually selected based on VIP values (VIP $\geq$ 1) and p values ($p<0.05$).

The structures of the differential variables were deduced by searching the databases and/or comparing against the standard samples based on their retention time, accurate m/z of the MS fragments and the MS/MS spectrum. A total of 22 different metabolites were identified from both the positive and negative modes and are listed in Table II. As seen from the table, threonine ($p<0.001$), leucine ($p<0.001$),

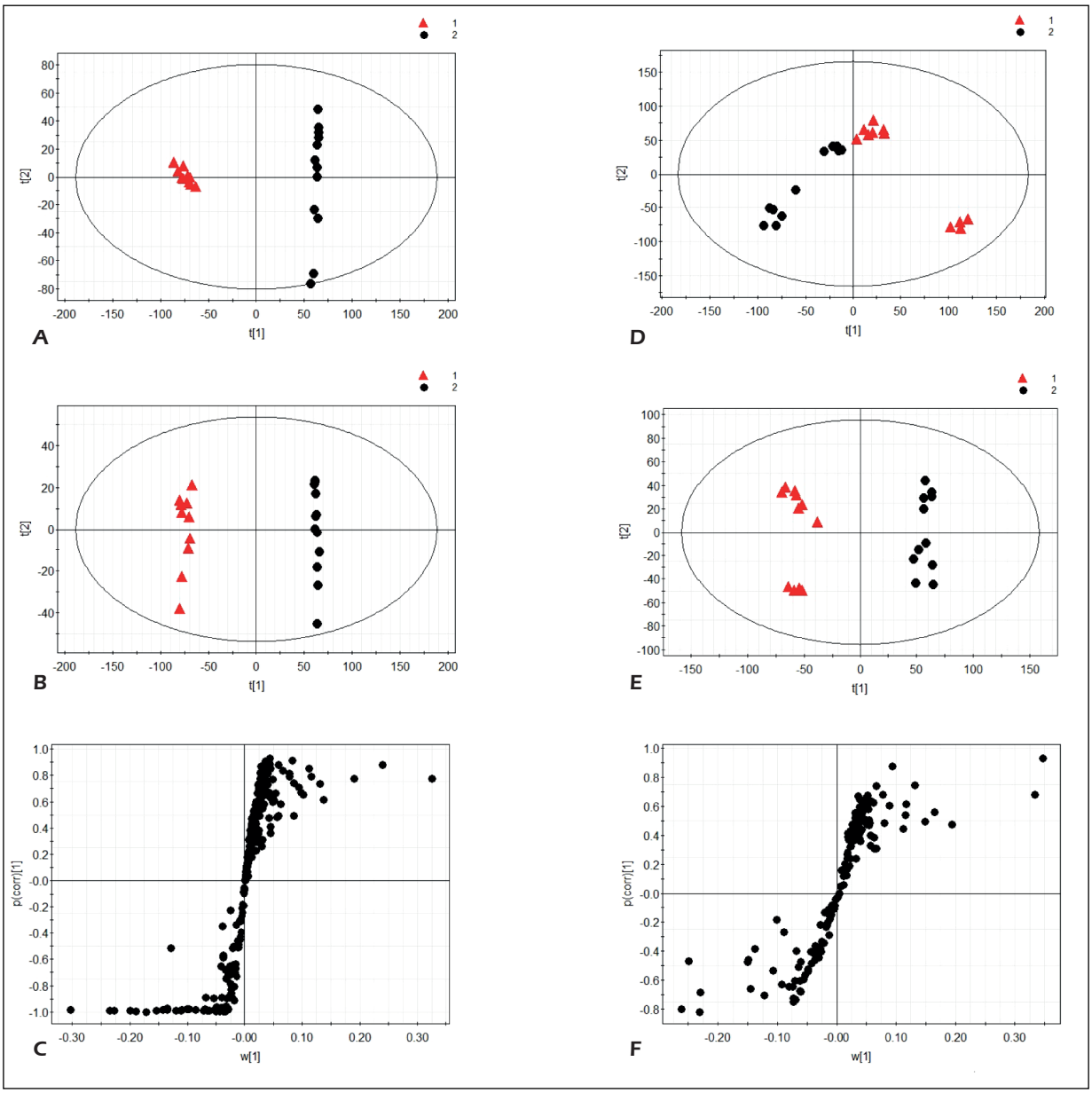

Figure 2. Score plot from multivariate statistical analysis of the two groups. In Panels **A**, **B**, **D**, and **E**, red triangles stand for healthy individuals and black circles stand for psoriasis vulgaris patient. Data for **A-C**, are from the positive mode, and data for **D-F**, are from the negative mode. **A**, and **D**, are score plots of psoriasis vulgaris patients and healthy controls by PCA, **B**, and **E**, are score plots of OPLS-DA model, and **C**, and **F**, are S-plots by OPLS-DA model. Outlying samples of the ellipse region with the 95% confidence interval were excluded by Hotelling's T^2-test, and R^2X, R^2Y, and Q^2Y are quality parameters of the models. The supervised model was validated by a permutation test (N=200) (*p*-values, Ri, and Qi).

phenylalanine (p<0.001), tryptophan (p=0.018), palmitamide (p<0.001), Linoleic amide (p<0.001), oleamide (p<0.001), stearamide (p<0.001), *cis*-11-eicosenamide (p<0.001), *trans*-13-Docosenamide (p<0.001), uric acid (p= 0.034), LysoPC (16:0) (p<0.001), LysoPC (18:3) (p<0.001), LysoPC (18:2) (p=0.024), LysoPC(18:1) (p=0.012) and LysoPC(18:0) (p=0.002) were significantly higher in the plasma of psoriasis vulgaris patients compared with the healthy controls, whereas oleic acid (p<0.001), arachidonic acid (p<0.001) and N-linoleoyl taurine (p<0.001) were significantly lower. These biomarkers are related to glucose metabolism, lipid metabolism, amino acid metabolism, nucleic acid metabolism and so on, the metabolic pathways of the potential biomarkers are shown in Figure 3.

Table II. The differential metabolites between psoriasis vulgaris group and control group↓.

No.	tR (min)	Identity	m/z	Adduct ion	Formula	trend	VIP[a]	*p*-value[b]	KEGG
1	0.99	Threonine	120.0840	$[M+H]^+$	$C_4H_9NO_3$	↑[d]	1.01	<0.001	Aminoacyl-tRNA biosynthesis (hsa00970)
2	0.69	Leucine	132.1041	$[M+H]^+$	$C_6H_{13}NO_2$	↑	1.32	<0.001	Aminoacyl-tRNA biosynthesis (hsa00970)
3	0.99	Phenylalanine	166.0894	$[M+H]^+$	$C_9H_{11}NO_2$	↑	1.91	<0.001	Aminoacyl-tRNA biosynthesis (hsa00970)
4	1.92	Tryptophan	188.0738	$[C11H10NO2]^+$	$C_{11}H_{12}N_2O_2$	↑	1.01	0.018	Aminoacyl-tRNA biosynthesis (hsa00970)
5	14.15	Palmitamide	256.2648	$[M+H]^+$	$C_{16}H_{33}NO$	↑	2.24	<0.001	
6	13.74	Linoleic amide	280.2657	$[M+H]^+$	$C_{18}H_{33}NO$	↑	1.45	<0.001	
7	14.28	Oleamide	282.2782	$[M+H]^+$	$C_{18}H_{35}NO$	↑	5.61	<0.001	
8	2.78	Oleic acid	283.2647	$[M+H]^+$	$C_{18}H_{34}O_2$	↓	4.09	<0.001	Biosynthesis of unsaturated fatty acid (hsa01040)
9	14.88	Stearamide	284.2967	$[M+H]^+$	$C_{18}H_{37}NO$	↑	1.98	<0.001	
10	1.78	Arachidonic acid	305.2473	$[M+H]^+$	$C_{20}H_{32}O_2$	↓	3.00	<0.001	Biosynthesis of unsaturated fatty acid (hsa01040)
11	14.94	cis-11-eicosenamide	310.3119	$[M+H]^+$	$C_{20}H_{39}NO$	↑	1.33	<0.001	
12	15.51	trans-13-Docosenamide	338.3409	$[M+H]^+$	$C_{22}H_{43}NO$	↑	3.28	<0.001	
13	5.41	N-linoleoyl taurine	388.2535	$[M+H]^+$	$C_{20}H_{37}NO_4S$	↓	2.35	<0.001	
14	12.89	LysoPC(16:0)[c]	496.3342	$[M+H]^+$	$C_{24}H_{50}NO_7P$	↑	4.13	<0.001	
15	12.88	LysoPC(18:3)	518.3229	$[M+H]^+$	$C_{26}H_{48}NO_7P$	↑	1.42	<0.001	
16	12.59	LysoPC(18:2)	520.3379	$[M+H]^+$	$C_{26}H_{50}NO_7P$	↑	1.46	0.024	
17	13.72	LysoPC(18:0)	524.3675	$[M+H]^+$	$C_{26}H_{54}NO_7P$	↑	2.36	0.002	
18	0.75	Uric acid	167.0222	$[M-H]^-$	$C_5H_4N_4O_3$	↑	1.03	0.034	
19	13.31	LysoPC(16:0)	540.3296	$[M+HCOO]^-$	$C_{24}H_{50}NO_7P$	↑	4.31	0.002	
20	12.96	LysoPC(18:2)	564.3275	$[M+HCOO]^-$	$C_{26}H_{50}NO_7P$	↑	1.92	0.033	
21	13.50	LysoPC(18:1)	566.3463	$[M+HCOO]^-$	$C_{26}H_{52}NO_7P$	↑	2.13	0.012	
22	14.24	LysoPC(18:0)	568.3598	$[M+HCOO]^-$	$C_{26}H_{54}NO_7P$	↑	4.48	<0.001	

[a]VIP stands for variable importance in the project, and a value of greater than 1.00 indicates high relevance for explaining the differences between sample groups.
[b]*p* value was analyzed by independent t-test with the Mann-Whitney U-test.
[c]LysoPC stands for lysophosphatidylcholine.
[d]↑ indicates an increase compared with the control group.
[e]↓ indicates a decrease compared with the control group.

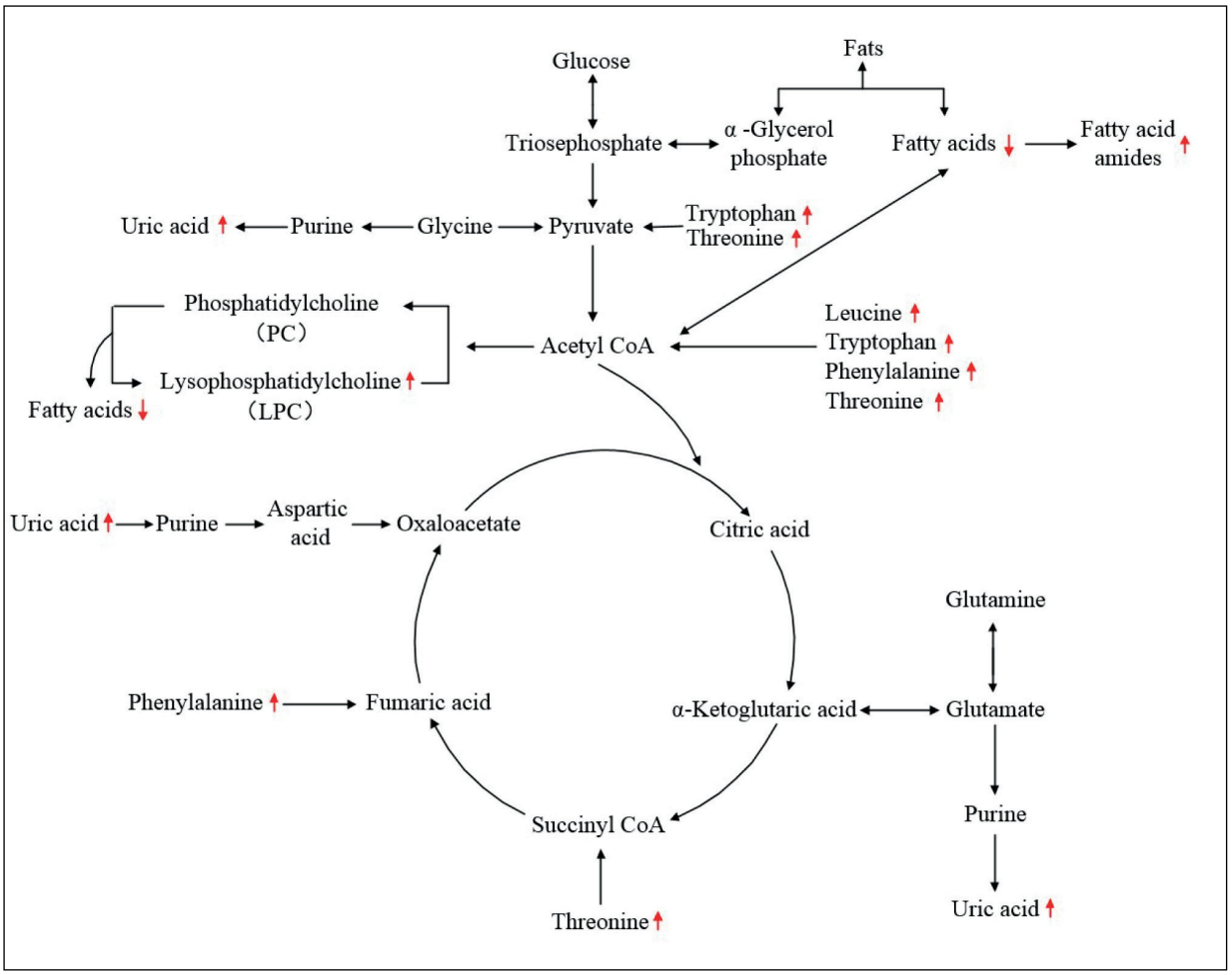

Figure 3. The metabolic pathways of the potential biomarkers.

Discussion

In this study, UPLC/Q-TOF MS was used to analyze the metabolic profile of psoriasis vulgaris patients and healthy controls. The results showed that the two groups can be clearly distinguished, and there were significant differences in the levels of endogenous metabolites between the two groups. 22 potential biomarkers were identified by UPLC/Q-TOF MS technique combined with multivariate statistical analysis, suggesting that related biological metabolic pathways of psoriasis vulgaris patients were altered remarkably. Among these biomarkers, some are consistent with previous reports[35,36], particularly increased amino acid levels and uric acid levels.

Compared to healthy controls, plasma levels of amino acids such as threonine, leucine, phenylalanine and tryptophan were significantly increased in psoriasis vulgaris patients, indicating dysfunctional amino acid metabolism. These amino acids are essential amino acids that the human body cannot synthesize or cannot synthesize efficiently and therefore they must be absorbed from diet. As the main site of amino acid metabolism in human body[35], the liver plays a major role in the intake, synthesis and secretion of amino acids. Previous studies[35,36] have shown that the level of amino acid metabolism directly reflects the state of liver cell metabolism. In case of a liver injury or disease, the amino acid metabolism in the liver cell decreases due to the disorder of liver function, resulting in increased levels of amino acids in the plasma. Moreover, the elevated amino acids may also be due to keratinocyte hyperproliferation, increased proteolysis due to cachexia[32,34], so it is very important to identify amino acids as biomarkers of psoriasis vulgaris.

Other than amino acids, differential metabolites of psoriasis vulgaris identified also included lysophosphatidylcholines (LPCs), fatty acids and

fatty acid amides, indicating disorders of lipid metabolism. Abnormal lipid metabolism has been considered to be an important factor in the etiopathogenesis of psoriasis[33].

Plasma levels of LPCs in the psoriasis vulgaris group were increased compared to the healthy controls. LPCs are known to account for 5-20% of all phospholipids in the serum and are formed mainly by hydrolysis of phosphatidylcholines (PC)[37,38], which have combinations of fatty acids of varying lengths and saturation attached at the glycerol molecular skeleton C-1 position[13]. LPCs are fundamental components of cellular membranes and play key roles in the progress of atherosclerosis and inflammatory diseases generated by pathological activities[16,38,39]. They are also an inflammatory factor[40], and are capable of aggregating mononuclear cells and promoting production of inflammatory cytokines in macrophages[41] at millimolar concentrations. Psoriasis vulgaris is a disease characterized by substantial inflammatory cell infiltration and excessive keratinocyte proliferation, so the increased of LPCs may be clinically relevant to the occurrence and progression of psoriasis vulgaris.

Fatty acids are a category of metabolites that have been found to be down-regulated in psoriasis vulgaris patients. Among them, arachidonic acid (AA) is a highly unsaturated fatty acid, and it is not only an abundant component of cell membranes (incorporated into phospholipids) but also the direct precursor of many circulating eicosanoids derivatives. It is mainly catalyzed by cyclooxygenase or lipoxygenase pathway[42,43], and its derivative products such as prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs) are closely related to pathological processes such as inflammation and immune responses[33,44]. Oleic acid can be converted to arachidonic acid in the human body, so it can be speculated that the downregulation of oleic acid and arachidonic acid may be relevant to up-expression of cyclooxygenase or lipoxygenase in psoriasis vulgaris patients. The levels of fatty acid amides were increased in patients; fatty acid amides are mainly divided into two categories including fatty acid ethanolamides and fatty acid primary amides[45]. They serve as endogenous lipid signaling molecules and play an important role in cell signal transduction pathways involving anxiety, inflammation and appetite[16,45].

Uric acid is another metabolite whose plasma level was up-regulated in psoriasis vulgaris patients. Mainly excreted by the kidney, uric acid is the final product of purine metabolism in human body[37]. Renal disease likely leads to a reduction in uric acid excretion, thereby increasing the level of uric acid in the plasma, so increased plasma levels of uric acid may serve as a biomarker for kidney damage[46]. In addition, it is reported that elevated uric acid levels are closely related to diseases such as endothelial dysfunction, subclinical atherosclerosis, and inflammation[37,47] . Therefore uric acid is an important biomarker for psoriasis vulgaris. At last, the plasma levels of N-linoleoyl taurine in patients was decreased, but due to the lack of literature reports, the relationship between N-linoleoyl taurine and psoriasis vulgaris still needs to be investigated.

Our results also proved that UPLC/Q-TOF MS-based metabonomic approach is a powerful tool for the identification of potential metabolite biomarkers for auxiliary diagnosis and clinical applications. Changes in the levels of these differential metabolites may provide more biochemical information about the occurrence and development of psoriasis vulgaris, and deepen the understanding of the pathogenesis of the disease. It offers a different perspective in psoriasis vulgaris research, which is of great significance to the comprehensive prevention and treatment of the disease.

Conclusions

We suggest that psoriasis vulgaris patients may have disrupted lipid and amino acid metabolism, inflammation and functional lesions in the liver and kidney. The study is based on the evidence of psoriasis vulgaris pathogenesis, and confers novel ideas and methods for auxiliary diagnosis and treatment of the disease.

Acknowledgements

This work was supported by Science and Technology Planning Project of Guangdong Province, China (2014A020221063), Natural Science Foundation of Guangdong Province, China (2017A030310020) and Scientific Research Cultivation Foundation of The First Clinical Medical College of Jinan University, China (2016102).

Conflict of Interests

The authors declare no conflict of interest.

References

1) Gupta R, Debbaneh MG, Liao W. Genetic epidemiology of psoriasis. Curr Dermatol Rep 2014; 3: 61-78.
2) Kim J, Krueger JG. The immunopathogenesis of psoriasis. Dermatol Clin 2015; 33: 13-23.

3) Colombo E, Galleri G, Erre GL, Piras C, Biondi G, Taras L, Zinellu A, Mangoni AA, Manetti R, Montesu M, Passiu G. Peripheral blood CD8+ T-cell profiles in patients with psoriatic arthritis: a cross-sectional case-control study. Eur Rev Med Pharmacol Sci 2017; 21: 5166-5171.

4) Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet 2007; 370: 263-271.

5) Kimball AB, Gieler U, Linder D, Sampogna F, Warren RB, Augustin M. Psoriasis: is the impairment to a patient's life cumulative? J Eur Acad Dermatol Venereol 2010; 24: 989-1004.

6) de Arruda LH, De Moraes AP. The impact of psoriasis on quality of life. Br J Dermatol 2001; 144 Suppl 58: 33-36.

7) Lebwohl M, Tyring S, Bukhalo M, Alonso-Llamazares J, Olesen M, Lowson D, Yamauchi P. Fixed combination aerosol foam calcipotriene 0.005% (Cal) plus betamethasone dipropionate 0.064% (BD) is more efficacious than Cal or BD aerosol foam alone for psoriasis vulgaris: a randomized, double-blind, multicenter, three-arm, phase 2 study. J Clin Aesthet Dermatol 2016; 9: 34-41.

8) McHugh NJ, Balachrishnan C, Jones SM. Progression of peripheral joint disease in psoriatic arthritis: a 5-yr prospective study. Rheumatology (Oxford) 2003; 42: 778-783.

9) Jiang S, Hinchliffe TE, Wu T. Biomarkers of an autoimmune skin disease--psoriasis. Genomics Proteomics Bioinformatics 2015; 13: 224-233.

10) Guan Q, Liang S, Wang Z, Yang Y, Wang S. ^{1}H NMR-based metabonomic analysis of the effect of optimized rhubarb aglycone on the plasma and urine metabolic fingerprints of focal cerebral ischemia-reperfusion rats. J Ethnopharmacol 2014; 154: 65-75.

11) Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999; 29: 1181-1189.

12) Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. Nat Rev Drug Discov 2002; 1: 153-161.

13) Wang C, Feng R, Li Y, Zhang Y, Kang Z, Zhang W, Sun DJ. The metabolomic profiling of serum in rats exposed to arsenic using UPLC/Q-TOF MS. Toxicol Lett 2014; 229: 474-481.

14) Kim JY, Park JY, Kim OY, Ham BM, Kim HJ, Kwon DY, Jang Y, Lee JH. Metabolic profiling of plasma in overweight/obese and lean men using ultra performance liquid chromatography and Q-TOF mass spectrometry (UPLC-Q-TOF MS). J Proteome Res 2010; 9: 4368-4375.

15) Blow N. Metabolomics: biochemistry's new look. Nature 2008; 455: 697-700.

16) Li Y, Song X, Zhao X, Zou L, Xu G. Serum metabolic profiling study of lung cancer using ultra high performance liquid chromatography/quadrupole time-of-flight mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 966: 147-153.

17) Dong F, Deng D, Chen H, Cheng W, Li Q, Luo R, Ding S. Serum metabonomics study of polycystic ovary syndrome based on UPLC-QTOF-MS coupled with a pattern recognition approach. Anal Bioanal Chem 2015; 407: 4683-4695.

18) Zhao YY, Cheng XL, Wei F, Xiao XY, Sun WJ, Zhang Y, Lin RC. Serum metabonomics study of adenine-induced chronic renal failure in rats by ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Biomarkers 2012; 17: 48-55.

19) Liu T, Li J, Xu F, Wang M, Ding S, Xu H, Dong F. Comprehensive analysis of serum metabolites in gestational diabetes mellitus by UPLC/Q-TOF-MS. Anal Bioanal Chem 2016; 408: 1125-1135.

20) Gika HG, Theodoridis GA, Plumb RS, Wilson ID. Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics. J Pharm Biomed Anal 2014; 87: 12-25.

21) Haoula Z, Ravipati S, Stekel DJ, Ortori CA, Hodgman C, Daykin C, Raine-Fenning N, Barrett DA, Atiomo W. Lipidomic analysis of plasma samples from women with polycystic ovary syndrome. Metabolomics 2015; 11: 657-666.

22) Zhang Y, Ren H, Jiang Y, Gao YF, Liu SY. Urinary metabolomics of stomach cancer assessed by rapid resolution liquid chromatography/time-of-fight mass spectrometry. Chin Med J (Engl) 2013; 126: 1930-1933.

23) Wu M, Xu Y, Fitch WL, Zheng M, Merritt RE, Shrager JB, Zhang W, Dill DL, Peltz G, Hoang CD. Liquid chromatography/mass spectrometry methods for measuring dipeptide abundance in non-small-cell lung cancer. Rapid Commun Mass Spectrom 2013; 27: 2091-2098.

24) Qiu Y, Zhou B, Su M, Baxter S, Zheng X, Zhao X, Yen Y, Jia W. Mass spectrometry-based quantitative metabolomics revealed a distinct lipid profile in breast cancer patients. Int J Mol Sci 2013; 14: 8047-8061.

25) Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nat Med 2002; 8: 1439-1444.

26) Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, Berriz GF, Roth FP, Gerszten RE. Metabolomic identification of novel biomarkers of myocardial ischemia. Circulation 2005; 112: 3868-3875.

27) Yang J, Xu G, Zheng Y, Kong H, Pang T, Lv S, Yang Q. Diagnosis of liver cancer using HPLC-based metabonomics avoiding false-positive result from hepatitis and hepatocirrhosis diseases. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 813: 59-65.

28) Yin P, Zhao X, Li Q, Wang J, Li J, Xu G. Metabonomics study of intestinal fistulas based on ultra-performance liquid chromatography coupled with Q-TOF mass spectrometry (UPLC/Q-TOF MS). J Proteome Res 2006; 5: 2135-2143.

29) Psihogios NG, Kalaitzidis RG, Dimou S, Seferiadis KI, Siamopoulos KC, Bairaktari ET. Evaluation of tubulointerstitial lesions' severity in patients with glomerulonephritides: an NMR-based metabonomic study. J Proteome Res 2007; 6: 3760-3770.

30) Yang J, Xu G, Hong Q, Liebich HM, Lutz K, Schmulling RM, Wahl HG. Discrimination of Type 2 diabetic patients from healthy controls by using metabonomics method based on their serum fatty acid profiles. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 813: 53-58.

31) Wang C, Kong H, Guan Y, Yang J, Gu J, Yang S, Xu G. Plasma phospholipid metabolic profiling and biomarkers of type 2 diabetes mellitus based on high-performance liquid chromatography/electrospray mass spectrometry and multivariate statistical analysis. Anal Chem 2005; 77: 4108-4116.

32) Kamleh MA, Snowden SG, Grapov D, Blackburn GJ, Watson DG, Xu N, Stahle M, Wheelock CE. LC-MS metabolomics of psoriasis patients reveals disease severity-dependent increases in circulating amino acids that are ameliorated by anti-TNFalpha treatment. J Proteome Res 2015; 14: 557-566.

33) Lu C, Deng J, Li L, Wang D, Li G. Application of metabolomics on diagnosis and treatment of patients with psoriasis in traditional Chinese medicine. Biochim Biophys Acta 2014; 1844: 280-288.

34) Kang H, Li X, Zhou Q, Quan C, Xue F, Zheng J, Yu Y. Exploration of candidate biomarkers for human psoriasis based on gas chromatography-mass spectrometry serum metabolomics. Br J Dermatol 2017; 176: 713-722.

35) Rosen HM, Yoshimura N, Hodgman JM, Fischer JE. Plasma amino acid patterns in hepatic encephalopathy of differing etiology. Gastroenterology 1977; 72: 483-487.

36) Morgan MY, Marshall AW, Milsom JP, Sherlock S. Plasma amino-acid patterns in liver disease. Gut 1982; 23: 362-370.

37) Kim MJ, Yang HJ, Kim JH, Ahn CW, Lee JH, Kim KS, Kwon DY. Obesity-related metabolomic analysis of human subjects in black soybean peptide intervention study by ultraperformance liquid chromatography and quadrupole-time-of-flight mass spectrometry. J Obes 2013; 2013: 874981.

38) Liu YT, Peng JB, Jia HM, Cai DY, Zhang HW, Yu CY, Zou ZM. UPLC-Q/TOF MS standardized Chinese formula Xin-Ke-Shu for the treatment of atherosclerosis in a rabbit model. Phytomedicine 2014; 21: 1364-1372.

39) Fukushima N, Ishii I, Contos JJ, Weiner JA, Chun J. Lysophospholipid receptors. Annu Rev Pharmacol Toxicol 2001; 41: 507-534.

40) Radu CG, Yang LV, Riedinger M, Au M, Witte ON. T cell chemotaxis to lysophosphatidylcholine through the G2A receptor. Proc Natl Acad Sci U S A 2004; 101: 245-250.

41) Olofsson KE, Andersson L, Nilsson J, Bjorkbacka H. Nanomolar concentrations of lysophosphatidylcholine recruit monocytes and induce pro-inflammatory cytokine production in macrophages. Biochem Biophys Res Commun 2008; 370: 348-352.

42) Das UN. Essential Fatty acids – a review. Curr Pharm Biotechnol 2006; 7: 467-482.

43) Gachet MS, Rhyn P, Bosch OG, Quednow BB, Gertsch J. A quantitiative LC-MS/MS method for the measurement of arachidonic acid, prostanoids, endocannabinoids, N-acylethanolamines and steroids in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2015; 976-977: 6-18.

44) Samuelsson B. Arachidonic acid metabolism: role in inflammation. Z Rheumatol 1991; 50 Suppl 1: 3-6.

45) Ezzili C, Otrubova K, Boger DL. Fatty acid amide signaling molecules. Bioorg Med Chem Lett 2010; 20: 5959-5968.

46) Obermayr RP, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, Klauser-Braun R. Elevated uric acid increases the risk for kidney disease. J Am Soc Nephrol 2008; 19: 2407-2413.

47) Kanellis J, Kang DH. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. Semin Nephrol 2005; 25: 39-42.