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Review

UPLC-based metabonomic applications for discovering biomarkers of diseases in clinical chemistry



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ABSTRACT

**Objectives:** Metabonomics is a powerful and promising analytic tool that allows assessment of global low-molecular-weight metabolites in biological systems. It has a great potential for identifying useful biomarkers for early diagnosis, prognosis and assessment of therapeutic interventions in clinical practice. The aim of this review is to provide a brief summary of the recent advances in UPLC-based metabonomic approach for biomarker discovery in a variety of diseases, and to discuss their significance in clinical chemistry.

**Design and methods:** All the available information on UPLC-based metabonomic applications for discovering biomarkers of diseases were collected via a library and electronic search (using Web of Science, Pubmed, ScienceDirect, Springer, Google Scholar, etc.).

**Results:** Metabonomics has been used in clinical chemistry to identify and evaluate potential biomarkers and therapeutic targets in various diseases affecting the liver (hepatocarcinoma and liver cirrhosis), lung (lung cancer and pneumonia), gastrointestinal tract (colorectal cancer) and urogenital tract (prostate cancer, ovarian cancer and chronic kidney disease), as well as metabolic diseases (diabetes) and neuropsychiatric disorders (Alzheimer's disease and schizophrenia), etc.

**Conclusions:** The information provided highlights the potential value of determination of endogenous low-molecular-weight metabolites and the advantages and potential drawbacks of the application of UPLC-based metabonomics in clinical setting.

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## Introduction

Metabonomics is a powerful new technology that allows assessment of global low-molecular-weight metabolites in biological systems and holds great potential in biomarker discovery. Analysis of the key metabolites in the body fluids has become an important part of the diagnosis, prognosis, and assessment of therapeutic interventions in clinical application [1]. This review is intended to provide an overview of the main applications of ultra-performance liquid chromatography (UPLC) in metabonomics and the current utility of the UPLC-based metabonomics in the fields of oncology, metabolic, neuropsychiatric, cardiovascular, infectious, and other diseases. Especial emphasis is placed on the potential use of endogenous low-molecular-weight metabolites in clinical chemistry.

## Metabonomics

Metabonomics is defined as the “quantitative measurement of the dynamic multi-parametric metabolic responses of living systems to pathophysiological stimuli or genetic modifications” [2]. It is used to characterize the biochemical patterns of the endogenous metabolites in cells, body fluids or tissues for physiological evaluation, disease diagnosis and disease prognosis [3]. In contrast to classical biochemical approaches that often focus on a single metabolite, metabonomics reveals a collection of molecules which covers a broad range of small molecules such as lipids, amino acids, sterols, nucleic acids, peptides, organic acids, carbohydrates and vitamins and as such provides a comprehensive overview of the impact of the pathophysiological processes or pharmacological interventions of interest on metabolism and metabolic dynamics.

## Metabonomic analytical technologies

Various analytical techniques are used in metabonomics which can be classified into two categories: I—nuclear magnetic resonance (NMR) and II—mass spectrometry (MS) [4]. Although other spectral approaches including Fourier transform ion cyclotron resonance, Raman and ultraviolet spectrum are employed for metabonomics studies, they are generally less sensitive than MS [5]. An increasing number of publications have described metabonomics using analytical techniques including  $^1\text{H}$  NMR, gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (UPLC–MS) [6].  $^1\text{H}$  NMR, which is as one of the first methods used for metabonomics, represents a rapid, non-destructive and highly robust technology that provide highly informative structural information [7].  $^1\text{H}$  NMR is often used without any pre-separation process and unlike chromatography it does not require development. However, as each metabolite participates in the  $^1\text{H}$  NMR spectra, the deconvolution of the signals is often quite tedious [8]. GC–MS based metabonomics can resolve hundreds

of metabolite peaks, with metabolite identification performed by matching the fragmentation ion spectra and retention indices to the established database. The use of GC–MS is limited to the analysis of thermally stable, volatile and relatively non-polar components. Components' volatility can be increased using derivatization, which is laborious and increases annotation complexity [9]. UPLC–MS can analyze volatile, non-volatile and polar compounds, over a wider mass range than GC–MS and does not require sample derivatization [6]. However, UPLC–MS based metabonomics is hindered by the lack of established spectral database. Among the analytical techniques in metabonomics research, it is generally accepted that LC–MS is superior to NMR in terms of selectivity and sensitivity, while  $^1\text{H}$  NMR and GC–MS based metabonomics are characterized by high reproducibility. Therefore use of UPLC–MS combined with  $^1\text{H}$  NMR and GC–MS can provide a superior approach to study metabonomics.

## UPLC–MS technique

The recently introduced UPLC technique is considered to be suitable for metabonomics, especially for large-scale untargeted metabonomics due to its high sensitivity in detecting metabolites [6]. UPLC operates with  $1.7\ \mu\text{m}$  chromatographic particles and a fluid system capable of operating at pressures in the 6000–15000 psi range, providing an increased chromatographic selectivity compared with conventional high performance liquid chromatography (HPLC) which uses larger particles [10]. Due to a reduction of peak width, there will also be a greater S/N ratio and an increasing sensitivity compared with the conventional HPLC. This can provide better peak resolution and higher sensitivity and speed for complex mixture separation. Because of the superior UPLC resolution, the problem of ion suppression is greatly reduced [10].

Mass spectrometry<sup>Elevated Energy</sup> ( $\text{MS}^E$ ) technique was first applied to metabonomics by Plumb and co-workers [11]. Two scanning functions are simultaneously used for data collection. In the first function, Q1 is scanned from  $m/z$  50–1000, and Q2 (collision cell) uses a normal low collision energy that provides for the transmission of intact ions through the cell collisions. These ions are then pushed into the TOF analyzer and detected with high resolution and mass accuracy. The second scan function also scans Q1 over the same mass range; however, Q2 has a high collision energy that fragments all of the ions transmitted through Q1. The resulting ions are again detected in the TOF analyzer. In this way, two mass chromatograms are generated, one with information on the intact molecules from the first function, and the other with the fragmented ion information from the second function. A variety of data-processing algorithms can be used to extract metabolite information from these data [12,13]. In other words,  $\text{MS}^E$  can provide parallel alternating scans for acquisition at either low collision energies to obtain precursor ion information or high collision energies to obtain full-scan accurate mass fragment, precursor ion, and small neutral molecules.

MS<sup>E</sup> involves a simultaneous acquisition through alternating between high and low collision energies during a single chromatographic run. This ability is of major importance, as it offers the structural information required for the identification of the unknown biomarkers in the context of untargeted analyses. Recently, the MS<sup>E</sup> technique has proved to be a powerful tool for the identification of trace components of complex mixtures and for confirming their presence [14–19].

### Data analysis of the UPLC-based metabolomics

The detection of biomarkers includes the use of various methods for taking the acquired mass values, retention time, and peak intensity and performing pattern recognition by the multivariate statistics including principal component analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), and partial least squares-discriminant analysis (PLS-DA). PCA is the most commonly used statistical method in metabolomics. PCA is an unsupervised multivariate data analysis method which provides a comprehensive view of the clustering trend for the multi-dimensional data. PCA can visualize correlated variations in more than two dimensions. This method represents data in the form of a linear combination of scores containing information on the tested samples and loadings containing information on the variables. The advantage of PCA is that the results are intuitively understandable owing to the graphical representation [20]. However, PCA is limited by the fact that it is not based on a statistical analytical model [21]. PLS-DA has gained wide applications in metabolomics and bioinformatics. PLS-DA is a PLS regression of a set Y of binary variables representing the kinds of a categorical variable on a set X of predictor variables [22]. It is a compromise between the usual discriminant analysis and a discriminant analysis on the significant principal components of the predictor variables. This method is suitable for processing plenty of predictors [22]. The OPLS-DA method is an extension of the PLS-DA method which integrates an orthogonal signal correction filter to distinguish variations that are suited to predict a quantitative response from variations that are orthogonal to prediction. OPLS-DA was shown to be a powerful tool for the analysis of qualitative data structures. The OPLS-DA score plot revealed good fitness and high predictability of the OPLS-DA model with high statistical values of R<sup>2</sup> and Q<sup>2</sup>[23]. OPLS-DA method was used as a complement to the PLS-DA to discriminate two or more groups using multivariate data [24,25]. OPLS-DA model is calculated between the multivariate data and a response variable that only contains class information. The advantage of OPLS-DA compared to PLS-DA is that a single component is used as a predictor for the class, while the other variables describe the variation orthogonal to the first predictive component. UPLC-MS based metabolomics produces large amounts of raw data. The handling of such complex data sets manually is practically impossible. Hence several software tools and methods have been developed for processing and advanced statistical analysis of the raw data. A number of software tools from MS manufacturers and researchers have been developed to process metabolomics MS data. The software packages are linked to the corresponding analytical platform such as MarkerLynx from Waters to process the raw data. MS software packages apply special algorithms that filter and bin the raw data and then assign as a pair of retention time and *m/z* ratio. The software next aligns and normalizes the features found in the sample set, finally producing a large data matrix, which is then subjected to PCA, OPLS-DA, PLS-DA and other statistical analysis tools. In a comprehensive review article, Katajamaa and Oresic have described in detail the data processing methods for the MS-based metabolomics [26]. The final goal is to identify ions of interest on which the investigations can focus as a possible source of biomarker information. Biomarker identification employs a range of mass spectral techniques including MS, MS/MS, MS<sup>E</sup>,

isotope patterns and neutral losses, and searches in HMDB, Chemspider and KEGG. A sample workflow of UPLC-QTOF/MS is shown in Fig. 1.

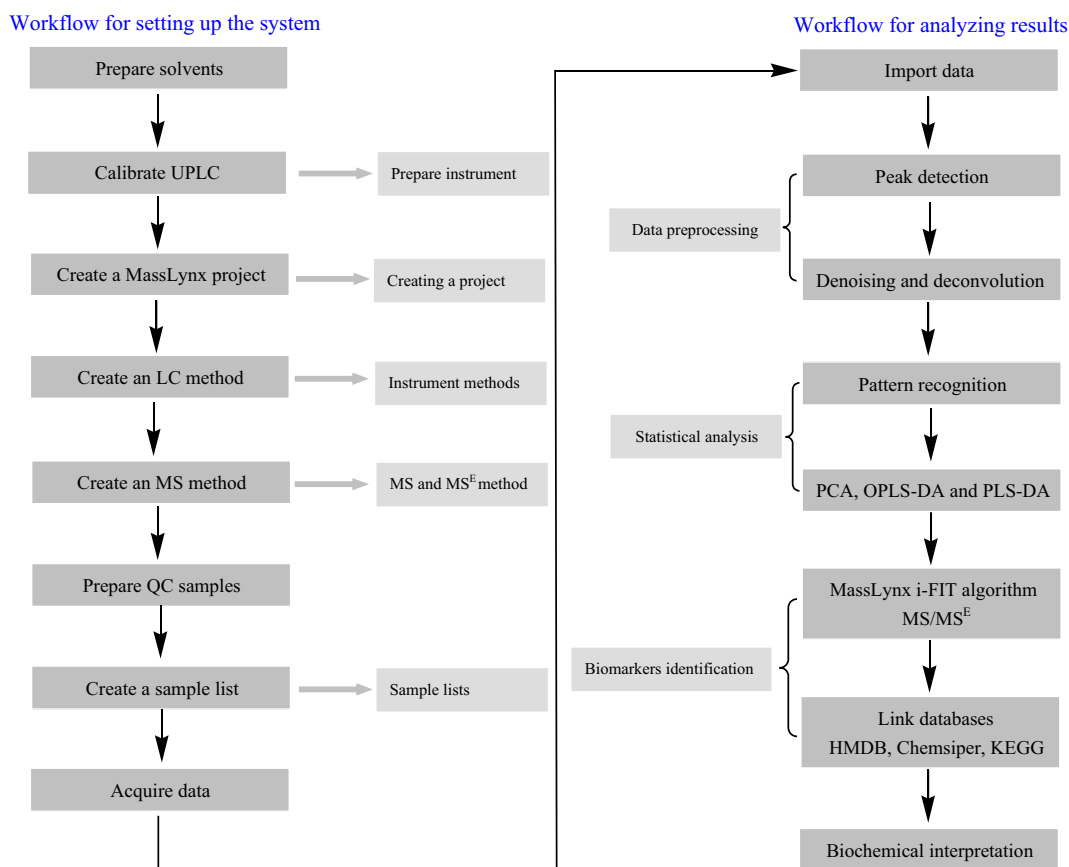
### UPLC-based metabolomics and biomarker discovery in clinical chemistry

Clinical chemistry deals with any analysis performed on the body fluids for a medical purpose including disease diagnosis, prognosis and treatment. Nowadays, most clinical tests still use the old method including single biomarker test, histopathology and immunohistochemistry. Current test methods are usually neither specific nor sensitive for a particular disease, and traditional biomarkers only change significantly after substantial disease injury or dysfunction has occurred. For example, serum creatinine (Scr) is the most commonly used biomarker of renal function. However, Scr concentrations may not change until a significant amount of renal function has been lost, meaning that renal injury is already present or occurs before Scr is elevated. In addition, the amount of tubular secretion of creatinine results in overestimation of renal function at lower glomerular filtration rates. Moreover, inter-individual differences in the body's muscle mass significantly alter Scr independent of renal function. Therefore, novel and more sensitive biomarkers are urgently needed for early detection and diagnosis of the disease. The UPLC-based metabolomic approach is now increasingly considered as a novel diagnostic approach in clinical studies including liver, lung, gastrointestinal, urogenital and other diseases. Table 1 displays UPLC-based metabolomic applications for discovering biomarkers of various diseases in clinical chemistry.

#### *Hepatocarcinoma (HCC), liver cirrhosis and chronic liver diseases*

##### HCC

Late diagnosis of HCC is one of the primary reasons for poor survival of patients. Identification of sensitive and specific biomarkers is of great importance in early diagnosis of HCC. Resson et al. studied serum metabolites in HCC patients and cirrhotic controls. They found increased sphingosine-1-phosphate and LPC (17:0) and decreased glycochenodeoxycholic acid (GCDCA) 3-sulfate, glycocholic acid (GCA), glycodeoxycholic acid (GDCA), taurocholic acid (TCA), and taurochenodeoxycholate which are involved in bile acid biosynthesis and cholesterol metabolism in HCC patients compared to patients with liver cirrhosis [27]. Another study identified serum 1-methyladenosine as a characteristic metabolite in HCC [28]. Serum and urinary metabolomics were performed on patients with HCC and benign liver tumor as well as healthy controls. 43 serum metabolites and 31 urinary metabolites involved in bile acids, free fatty acids, glycolysis, and methionine metabolism as well as urea cycle were identified in HCC patients. Bile acids, histidine and inosine were markedly elevated in HCC patients. However, liver cirrhosis and hepatitis were associated with alterations of several bile acids including GCDCA, GCA, TCA and chenodeoxycholic acid (CDCA). The HCC patients with  $\alpha$  fetoprotein were successfully differentiated from healthy controls using metabolite biomarkers [29]. In addition, UPLC-QTOF/MS and UPLC-MS/MS approaches were used for qualitative and quantitative analyses of serum biomarkers for patients with HCC. The results indicated that patients with HCC had decreased LPCs, increased long-chain and decreased medium-chain acylcarnitines, and increased aromatic and decreased branched-chain amino acid [30]. UPLC-QTOF/MS and UPLC triple quadrupole linear ion trap MS approaches were performed on qualitative and quantitative comparisons of metabolite levels in sera of HCC patients and cirrhosis patients from Egypt [31]. The metabolites including GCA, GDCA, 3 $\beta$ ,6 $\beta$ -dihydroxy-5 $\beta$ -cholan-24-oic acid, oleoyl carnitine and Phe-Phe were identified by UPLC-QTOF/MS. UPLC triple quadrupole linear ion trap MS-based quantitation confirmed significant differences between HCC and cirrhotic controls



**Fig. 1.** A typical example workflow of metabolomics using UPLC Q-TOF/MS with MS<sup>E</sup> data collection technique as research tools for discovering and quantifying metabolites in complex mixtures.

in the metabolite levels of bile acid metabolites, long chain carnitines and small peptide.

To discriminate HCC from liver cirrhosis, UPLC-based metabolomics has been conducted to characterize serum profiles from HCC patients, liver cirrhosis patients and healthy subjects. Metabolic profiles were capable of discriminating not only HCC patients from the controls but also HCC from liver cirrhosis. Thirteen biomarkers were identified and suggested that there were significant disturbances of organic acids, phospholipids, fatty acids, bile acids and gut flora metabolism in HCC patients. Canavaninosuccinate was first identified as a metabolite that is significantly reduced in liver cirrhosis and increased in HCC. In addition, GCDCA was suggested to be an important indicator for HCC diagnosis and disease prognosis [32]. Chronic liver diseases including chronic hepatitis B and hepatic cirrhosis are the major risk factors for development of HCC. The differential diagnosis between chronic liver diseases and HCC is a challenge. Serum metabolomics showed that long-chain acylcarnitines accumulated, whereas free carnitine, medium and short-chain acylcarnitines decreased with the severity of the non-malignant liver diseases, accompanied by the corresponding alterations of the enzyme activities. However, the magnitude of the changes was smaller in HCC than in hepatic cirrhosis, possibly due to the differences in energy metabolism in the tumor cells [33].

Urinary metabolome of patients suffering from HCC was studied using UPLC–MS (Fig. 2) and 21 metabolites were considered as potential biomarkers. Urinary metabolites related to arginine and proline metabolism, alanine and aspartate metabolism, lysine degradation, fatty acid oxidation, nicotinate and nicotinamide metabolism were significantly changed in HCC patients [34]. UPLC-based urinary metabolomics was used to explore common and specific metabolites in HCC patients with hepatitis B virus (HBV) or hepatitis C virus (HCV) infections. Increased arachidonic acid and decreased lysophosphatidylcholines

(LPCs) were observed in the HCC and cirrhosis patients compared with the healthy control, which may partly contribute to chronic inflammation and the initiation and progression of the malignant hepatoma. Decreased ratios of polyunsaturated to saturated LPCs in patients with HCC compared with chronic liver disease patients with HBV or HCV infection and healthy controls further demonstrated the profound influence of the malignant liver tumor independent of viral infection. Significant increases in serum endocannabinoids, anandamide and palmitylethanolamide, were found in the HCC compared with the healthy control and in HCC patients with HCV compared with corresponding patients with chronic liver disease. Endocannabinoids anandamide and palmitylethanolamide showed better sensitivity and specificity as potential biomarkers to distinguish the HCC from cirrhosis associated with HCV infection [35]. The UPLC metabolomics was also used to identify and measure the metabolic profile of GCA in HCC patients. HCC patients had increased urinary GCA which was associated with changes in primary bile acid biosynthesis, secondary bile acid biosynthesis and bile secretion [36]. UPLC-based fecal metabolomics were performed on the liver cirrhosis and HCC patients and healthy volunteers. CDCA dimeride, urobilin, urobilinogen, 7-ketolithocholic acid, LPC(18:0) and LPC(16:0) were considered as potential biomarkers with a strong increase in LPCs and a dramatic decrease in bile acids and bile pigments in patients with liver cirrhosis and HCC compared with the healthy volunteers [37]. In addition, UPLC and linear trap quadrupole-Orbitrap XL-MS platform were used to analyze endogenous metabolites in the homogenate of central tumor tissue, adjacent tissue, and distant tissue obtained from 10 HBV-related HCC patients [38]. Five metabolites quinaldic acid,  $\beta$ -sitosterol, arachidyl carnitine, oleamide and tetradecanal were observed for the first time. Nine metabolite lysophosphatidylethanolamines,



**Table 1**  
UPLC-based metabolomic applications for discovering biomarkers of diseases in clinical chemistry.

Experimental conditions	Application	Biological medium	Reference
UPLC-QTOF/MS UPLC-QqQLIT/MS UPLC-QqQ/MS	HCC with liver cirrhosis	Serum	[27]
UPLC-MS UPLC-QTOF/MS GC-TOF/MS	HCC HCC	Serum Serum, Urine	[28] [29]
UPLC-QTOF/MS UPLC-MS/MS UPLC-QTOF/MS UPLC-QqQLIT/MS	HCC HCC	Serum Serum	[30] [31]
UPLC-QTOF/MS RRLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS HPLC-TQ/MS	HCC from liver cirrhosis HCC and CLD HCC HCC with HBV or HCV	Serum Serum Urine Serum	[32] [33] [34] [35]
UPLC-QTOF/HDMS/MS <sup>E</sup> UPLC-QTOF/MS UPLC-LTQ Orbitrap XL-MS	HCC HCC and liver cirrhosis HCC	Urine Feces Liver tissue	[36] [37] [38]
UPLC-QTOF/MS UPLC-TQ/MS GC-TOF/MS UPLC-QTOF/MS GC-TOF/MS UPLC-QTOF/MS	HCC HCC Hepatitis B cirrhosis Acute and chronic liver failure	Plasma Urine Plasma	[41] [42] [43]
UPLC-QTOF/MS UPLC-QTOF/HDMS/MS <sup>E</sup> UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS RRLC-QTOF/MS UPLC-HILIC-QTOF/MS UPLC-Orbitrap MS GC-MS	Liver cirrhosis HBV Primary biliary cirrhosis Liver transplantation Lung cancer Lung cancer Lung cancer Lung cancer Lung cancer	Serum Urine Serum Bile Plasma Plasma Urine Plasma Serum, Plasma	[44] [45] [46] [47] [53] [54] [55] [58] [59]
UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS GC-TOF/MS UPLC-QTOF/MS GC-TOFMS UPLC-QTOF/MS SPE-HPLC UPLC-QTOF/MS UPLC-QTOF/MS UPLC-LTQ/MS GC-MS	Pneumonia Colorectal cancer Colorectal cancer Colorectal cancer Colorectal cancer Colorectal cancer Prostate cancer	Plasma, Urine Urine Serum Serum Urine Colon Urine Plasma	[60] [64] [65] [66] [67] [68] [69] [70]
UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS GC × GC-TOF/MS UPLC-MS/MS <sup>1</sup> H NMR UPLC-QTOF/MS UPLC-QTOF/MS <sup>1</sup> H NMR UPLC-QTOF/MS GC-MS <sup>1</sup> H NMR HILIC/UHPLC-MS RP/UHPLC-MS HILIC/UHPLC-MS UPLC-QTOF/MS UPLC-QTOF/MS UPLC-QTOF/MS GC × GC-TOF/MS UPLC-QTOF/MS <sup>1</sup> H NMR	Ovarian cancer Ovarian cancer Chronic renal failure Acute kidney injury Renal nephrolithiasis Autoimmune diabetes Type 1 diabetes Type 2 diabetes Type 2 diabetes Type 2 diabetes Alzheimer's disease Alzheimer's disease Cerebral infarction Acute cerebral infarction Schizophrenia Schizophrenia	Serum Plasma Serum Urine Urine Serum Plasma Serum Serum Cerebrospinal fluid CSF, Plasma Serum Plasma Serum Plasma	[72] [73] [74] [75] [78] [80] [81] [85] [86] [87] [94] [95] [99] [101] [107] [111]

glycerophosphocholine, LPCs, CDCA glycine conjugate and L-phenylalanine had been reported as serum metabolite biomarkers for HCC diagnosis in previous research [39–41].

Urinary metabolomics using GC-TOF/MS and UPLC-QTOF/MS was carried out to study post-hepatitis B cirrhosis patients. The study showed significant changes in  $\alpha$ -hydroxyhippurate, tyrosine-betaxanthin, 3-hydroxyisovalerate, canavaninosuccinate, estrone, and glycooursodeoxycholate among cirrhotic patients reflecting disturbance of amino acid, bile acids, hormonal and intestinal microbial metabolism [42].

#### Liver cirrhosis and chronic liver diseases

Liver failure induced by HBV is a severe disease with a high mortality rate. Plasma was employed to investigate metabolomics in acute-on-chronic liver failure patients. LPCs, primary fatty acid amides and conjugated bile acids were identified. LPCs and conjugated bile acids were found to be associated with survival whereas primary fatty acid amides represented risk factors [43]. Serum metabolomics was analyzed from control subjects and patients with alcoholic cirrhosis or HBV-induced cirrhosis. Decreased serum LPCs and increased serum GCA, GCDCA, hypoxanthine and stearamide were observed in cirrhosis patients and are considered common biomarkers for hepatic cirrhosis. Oleamide and myristamide were increased in patients with alcoholic cirrhosis but were decreased in those with HBV-induced cirrhosis. These could be specific biomarkers for differential diagnosis between alcohol- and HBV-induced hepatic cirrhosis [44]. Eleven urinary metabolites were identified potential predictors of progression of HBV-related liver disease. The biotin sulfone, 5-oxo-heneicosanoic acid, D-glucosaminide and 2-methylhippuric acid were effective for the diagnosis of human HBV [45].

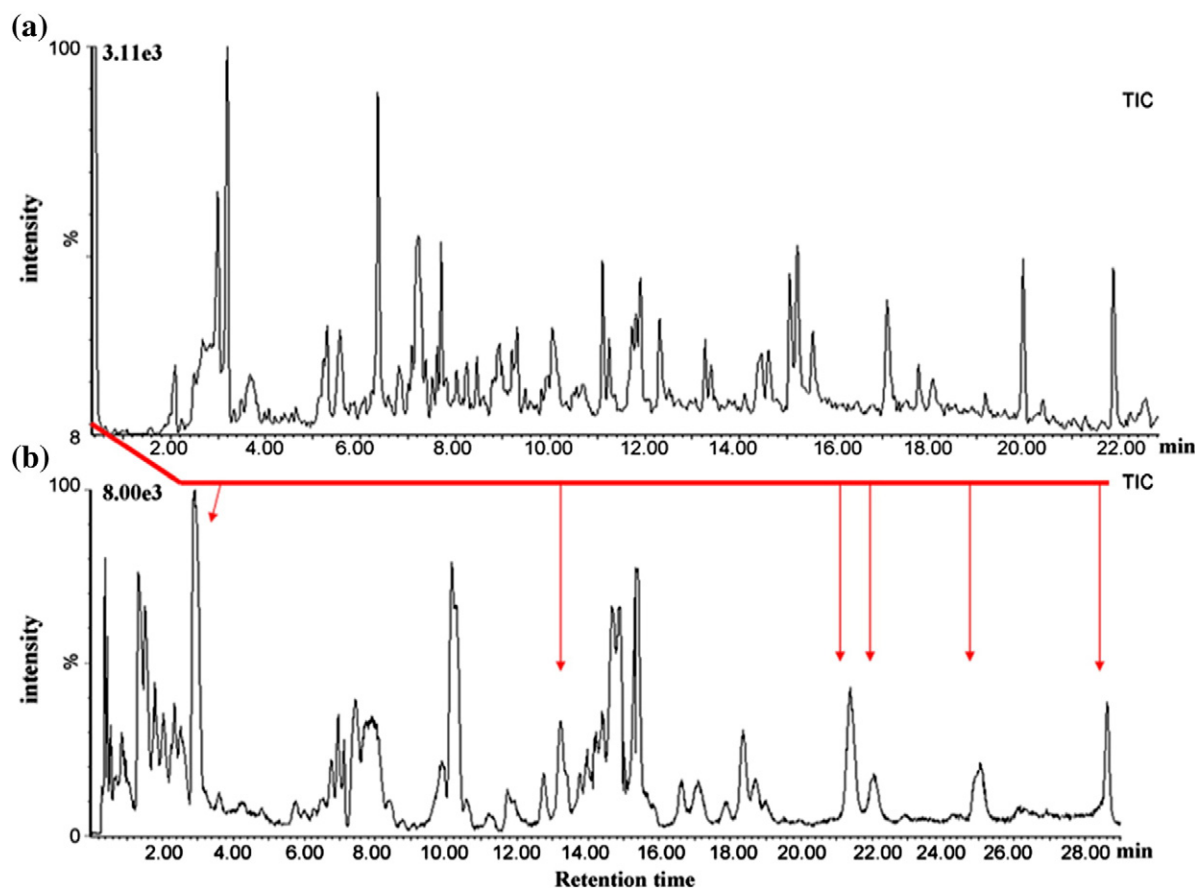
Primary biliary cirrhosis and primary sclerosing cholangitis are two cholestatic diseases characterized by hepatic accumulation of bile acids. Serum metabolomics was carried out to explore patients with primary biliary cirrhosis, with severe pruritus, and without pruritus and healthy controls. More than 400 serum metabolites were identified from patients with primary biliary cirrhosis. The metabolic profile of patients with primary biliary cirrhosis and pruritus was characterized by a significant change in the lipid metabolites, particularly in the ceramides, sphingomyelins and LPCs [46]. Bile flow restoration is a crucial step in the recovery process following liver transplantation. UPLC metabolomics has been conducted to monitor total bile fingerprint during human liver transplantation. Ten major conjugated bile acid salts were measured and significantly increased TCA and taurochenodeoxycholic acid (TCDCA) were observed after transplantation. Bile acid ratios in the donor liver at the pre-transplant and post-transplant stage may be important and that profiling of secreted bile after transplantation may aid clinical assessment and progress post-transplantation [47].

It has been reported that the abnormal bile acids and lysophospholipids are associated with liver cirrhosis and hepatitis [48]. Conjugated bile acids can bind lipids, cholesterol and fat-soluble vitamins. GDCA was reported to play an important role in the detoxification of lipophilic compounds [49]. Decreased serum bile acids have been related to the accumulation of toxic and even carcinogenic bile acid in liver that may be caused by the alteration in the bile acid transport pathway [50]. GCDCA has been reported as an inducer of apoptosis in human hepatocyte [51]. Abnormality of bile acid biosynthesis leads to development and progression of liver cancer [52].

#### Lung cancer and pneumonia

##### Lung cancer

Lung cancer is one of the most common cancers in the world, but reliable clinical biomarkers that can help to diagnose and assess prognosis of the disease at an early stage are lacking and urgently needed. UPLC-based metabolomics was used to find potential biomarkers by



**Fig. 2.** Typical urinary total ion chromatograms separated on (a) reversed-phase liquid chromatography and (b) hydrophilic interaction chromatography from patients with liver cancer. Several metabolites marked with an arrow were not retained on reversed-phase column but well separated on a hydrophilic interaction column.

comparing serum samples from lung cancer patients with healthy volunteers. LPC(16:0), isomer of LPC(16:0), LPC(18:0), LPC(18:1) and LPC(18:2) were identified as specific biomarkers [53,54]. Decreased LPCs may be explained by Lands' cycle pathway. In lung cancer, cell proliferation is accompanied by a high metabolic state, and abnormal LPCAT1 results in the reduction of LPCs. Other investigators developed a rapid resolution liquid chromatography–mass spectrometry (RRLC–MS) for global metabolic profiling of the urine in lung cancer patients. Eleven potential biomarkers including amino acids, nucleosides and indole were identified. The study revealed elevated amino acid and nucleoside metabolism as well as protein degradation in patients with lung cancer [55]. Previous studies indicated increased urinary aromatic amino acids that might be caused by derangement of protein metabolism in cancer patients [56]. Indoxyl is metabolic end-products of the tryptophan's metabolite indole, both of which have been implicated as etiological factors in development and growth of cancer. Significant variations of modified nucleosides have been demonstrated in various types of cancer due to the regulated cell turnover rate, activity of modifying enzymes, and RNA/DNA modifications [57]. In addition, highly polar metabolites were also compared in the plasma from lung cancer patients and healthy volunteers. Nineteen metabolites showed a significant difference between lung cancer patients and healthy controls. This method was also applied to the effect of radiotherapy on highly polar metabolites. Nineteen metabolites were altered at different points during the course of radiotherapy [58]. Serum and plasma metabolomics were developed and tested in patients with small-cell lung cancer. Plasma glycerophosphocholines, erythritol, creatinine, hexadecanoic acid and glutamine were associated with life expectancy and response to the clinical management in small-cell lung cancer patients [59].

#### *Pneumonia*

Pneumonia remains the leading cause of death in young children worldwide. Increased plasma uric acid, hypoxanthine and glutamic acid and decreased L-tryptophan and adenosine-5'-diphosphate were observed in pneumonia patients. This was associated with decreased urinary uric acid and L-histidine in these patients [60]. Based on the previous studies, the identified metabolites are important for the host's response to infection through antioxidant, inflammatory, and antimicrobial pathways and energy metabolism [61–63].

#### *Gastrointestinal diseases*

Colorectal cancer (CRC) is the third most common cancer worldwide, and its prognosis if not detected at early stages is poor. Both targeted and untargeted metabolomics have been used to identify biomarkers of CRC. UPLC–MS was applied to explore urinary metabolic profile in patients with CRC undergoing colorectal resection. The study showed a significant increase in two compounds with molecular weights of 283 and 234 in patients before surgery compared with healthy volunteers. The levels of these metabolites significantly decreased after the surgical resection of the tumor [64].

GC–MS and UPLC–MS-based metabolomics developed and applied on the serum from CRC patients revealed perturbation of glycolysis, arginine and proline metabolism, fatty acid metabolism and oleamide metabolism and its association with CRC morbidity [65]. Tricarboxylic acid cycle, urea cycle, glutamine, fatty acids, and gut flora metabolism were disturbed [66], which are consistent with previous findings [67]. Other studies showed that identified metabolites form CRC patients are related to glutamine metabolism, fatty acid oxidation, nucleotide biosynthesis and protein metabolism [68]. UPLC–MS method was

developed and validated for the targeted profiling of eight relevant eicosanoids and the major metabolic precursor, arachidonic acid in the human colon. Arachidonic acid, prostaglandin E<sub>2</sub>, prostacyclin and 12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid were found to be significantly different between cancerous and normal mucosae [69]. The previous studies also showed that eicosanoids such as prostaglandins, leukotrienes, thromboxanes and hydroxy eicosatetraenoic acids play an important role in promoting inflammation in CRC patients.

### *Urogenital diseases*

#### *Prostate cancer*

UPLC-based metabolomics has been applied to identify biomarkers for non-invasive diagnosis and prognosis, and aggressiveness of urogenital cancers. One of the major biomarker discovery studies in this field was the unbiased metabolomics performed using plasma, tissue and urine from patients with biopsy proven prostate cancer and biopsy negative controls [70]. More than 1126 metabolites were identified to be related to prostate cancer using two complementary GC-MS and UPLC-MS methods. The tissue metabolomics was able to distinguish normal prostate, localized prostate cancer, and metastatic prostate cancer. For 628 tissue metabolites, sixty metabolites were found in localized and/or metastatic tumors but not in normal prostate. Significantly increased sarcosine, uracil, kynurenine, glycerol-3-phosphate, leucine and proline levels were observed from benign to clinically localized prostate cancer and metastatic prostate cancer. Sarcosine was highly increased in tissues during prostate cancer progression to metastasis. Sarcosine was identified as a potential candidate for early disease detection and marker of aggressiveness of prostate cancer. However, monitoring of prostatic tissue sarcosine is of limited interest since histological examination of the available tissue is a more powerful tool for the diagnosis and the prognosis of cancer. For this reason the authors focused on urine sarcosine. They found that sarcosine was detectable in the urine at trace levels and had a modest but significant predictive value for prostate cancer diagnosis and disease progression. In addition to being a biomarker of prostate cancer, Sarcosine serves as a cytokine that contributes to the progression of the disease. In fact using molecular approaches and targeting metabolic pathways, the authors demonstrated the role of sarcosine in modulating cancer cell invasion and migration, making it a potential target for development of novel therapeutic interventions. Another study demonstrated that lipid metabolism and insulin resistance were decreased in this condition [71].

#### *Ovarian cancer*

The UPLC-based untargeted metabolomics has been conducted to identify and validate novel metabolic biomarkers for the epithelial ovarian cancer (EOC) and benign ovarian tumors (BOT). The study revealed increased 27-nor-5 $\beta$ -cholestane-3,7,12,24,25 pentol glucuronide (CPG), phenylalanine, GCA, propionylcarnitine, Phe-Phe and LPC(18:2) levels in EOC compared to the BOT specimens and as such could be considered as potential biomarkers. In particular tissue CPG level was significantly higher in EOC tissue compared with BOT tissue and increased CPG level was found in early stages of EOC and in all of its three histological types. For this reason CPG was considered to be complementary to CA125 and a relevant biomarker for detection of early stages of EOC [72]. UPLC-MS metabolomics has also been applied to differentiate between EOC and BOT. Decreased plasma L-tryptophan, LPC(18:3), LPC(14:0) and 2-piperidinone were observed in EOC patients when compared to the BOT patients. Tryptophan and LPCs have been suspected to participate in cancer progression, and 2-piperidinone might be a novel biomarker for EOC [73]. Accelerated L-tryptophan degradation has been observed in the blood of EOC patients when compared to the healthy controls [74]. A similar phenomenon has been observed in other malignant tumors. The abnormal levels of LPCs are due to binding and activation by the specific cell-

surface G protein-coupled receptors, which initiate cell growth, proliferation, and survival pathways [75].

#### *Chronic kidney disease (CKD)*

UPLC-based metabolomics was employed to investigate the serum from chronic renal failure patients. Increased LPC(18:0), phenylalanine and kynurenine and decreased LPC(16:0), LPC(18:1) and tryptophan were observed in chronic renal failure patients [76]. UPLC-based metabolomics was developed to analyze the plasma samples from end-stage renal disease patients. 1-Methylinosine was found to be an effective candidate biomarker to estimate adequacy of a hemodialysis regimen [77]. One study reported that urinary hypoxanthine was the most significant metabolite in children with nephrolithiasis. However, other investigator demonstrated that proline and 5C-aglycone were barely detected in the urine of these patients but were abundant in the healthy controls [78]. Based on the <sup>1</sup>H NMR and UPLC-MS/MS techniques, urine metabolome was analyzed from 15 patients with CKD and 15 healthy controls to find a classification pattern clearly indicative of CKD. Seven urinary metabolites glutamate, 5-oxoproline, guanidoacetate, taurine, phenylacetylglutamine, trimethylamine N-oxide and citrate differed between CKD and non-CKD urine samples [79].

### *Metabolic diseases*

#### *Type 1 diabetic (T1D)*

Insulin is as a major postprandial hormone with profound effects on carbohydrate, lipid, and protein metabolism. Serum metabolomics indicated that specific metabolic disturbances precede  $\beta$ -cell autoimmunity in humans and can be used to identify T1D children [80]. These findings suggest alternative metabolism-related pathways as therapeutic targets to prevent diabetes. Another UPLC-based metabolomic study has revealed significant perturbations in plasma amino acids and amino acid metabolites during insulin deprivation in T1D. Several known metabolic pathways are perturbed in acute insulin deprivation T1D [81]. Plasma branched chain amino acids are increased in untreated T1D [82] and have been attributed to the breakdown of muscle protein and release of amino acids in the circulation [83] and liver [84].

#### *Type 2 diabetes (T2DM)*

T2DM and its attendant complications have emerged as a major public health problem worldwide. T2DM is a typical metabolic disorder characterized by insulin resistance and relative deficiency of insulin production. Fatty acid metabolism and free fatty acid levels are markedly altered in diabetic patients [85]. Increased plasma acylcarnitines and tryptophan and decreased plasma LPC(16:0), LPC(18:0), LPC(18:2) and phenylalanine were reported in treated T2DM patients [86]. Another study has shown changes in plasma amino acid and perturbation of metabolic pathways linked to 3-indoxyl sulfate, glycerophospholipids, free fatty acids and interaction with the bile acids in diabetic patients [87]. Fatty acids can improve insulin secretion in the basal or glucose stimulated states and fatty acids are essential for stimulus-secretion coupling in  $\beta$ -cells [88]. However long-term elevation of free fatty acids can induce or aggravate insulin resistance and contribute to the development and progression of type 2 diabetes [89]. Extensive studies have shown that high level of circulating free fatty acids can inhibit insulin receptor substrates (IRSs) function [90,91].

### *Neuropsychiatric diseases*

#### *Alzheimer's disease (AD)*

AD is a neurodegenerative disorder which is characterized by progressive loss of cognitive functions and is the most common cause of dementia among older people. The results of the UPLC-based metabolomic studies in AD have been summarized in several published



reviews [92,93]. UPLC–MS metabolomics used to investigate cerebrospinal fluid (CSF) has revealed significant metabolic differences in subjects during the course of AD progression [94]. Mild cognitive impairment (MCI) is considered as a transition phase between normal aging and AD and its presence increases the risk of developing AD. UPLC–MS was applied to plasma and CSF from patients with different AD severity. Plasma lysine and CSF Krebs cycle were significantly affected in individuals with MCI compared to those with normal cognitive function. Cholesterol and sphingolipids metabolisms were altered in both CSF and plasma of AD patients. Other metabolic pathways including energy metabolism, Krebs cycle, mitochondrial function, neurotransmitter and amino acid metabolism and lipid biosynthesis were disturbed in MCI and AD patients. Plasma polyamine and lysine, and tryptophan metabolism and aminoacyl-tRNA biosynthesis and CSF cortisone and prostaglandin 2 biosynthesis and metabolism could discriminate between different groups [95]. Abnormal neuronal networks and neurotransmitter systems are one of the main dysfunctions in AD. Many studies have demonstrated that synaptic malfunction and synaptic loss occur prior to the development of amyloid  $\beta$ -plaques and neurofibrillary tangles [96]. These alterations of synaptic function are directly related to deterioration of synaptic strength and synaptic plasticity [96]. Acetylcholine, dopamine, serotonin and noradrenalin are primarily affected in AD with subsequent loss of the associated neurons [97]. Lysine, is a strictly ketogenic amino acid that is required for the synthesis of L-carnitine. L-Carnitine is the sole transporter of fatty acids to mitochondria for energy production and carnitine level has been shown to be lower in CSF of MCI-AD and AD patients than in CSF from non-AD subjects [98].

#### Stroke

Cerebral infarction is an acute neurological disorder which has serious consequences. Serum metabolomics was obtained from cerebral infarction patients and healthy controls. Folic acid, cysteine, S-adenosyl homocysteine and oxidized glutathione were identified as potential biomarkers of cerebral infarction [99]. These biomarkers are associated with the conjoined activated one-carbon and the folate cycle which are involved in protein and DNA stabilization, synthesis of various molecules, and protection against toxins and reactive oxygen metabolites [100].

UPLC-based metabolomics was employed to investigate non-dampness-phlegm and dampness-phlegm patients. LPC(18:2) and LPC(20:3) were lower in dampness-phlegm than in non-dampness-phlegm stroke pattern. However, increased LPC(18:0) and LPC(16:0) were observed in dampness-phlegm pattern [101]. The results demonstrated that plasma LPCs with polyunsaturated fatty acid were associated with dampness-phlegm pattern and suggested that variation of plasma lipid profiles could serve as potential biomarker for diagnosis of dampness-phlegm pattern [102]. Previous reports suggested the possibility of relationships between plasma LPC levels and dampness-phlegm pattern. It was known that dampness-phlegm pattern was related to obesity and hyperlipidemia [103,104], and some metabolomic analyses showed that plasma LPC levels were also associated with obesity [105,106].

#### Schizophrenia

UPLC–MS and two-dimensional GC–MS serum metabolomics were applied to schizophrenia patients who had significantly higher lipid and amino acid levels compared with the health controls [107]. Previous studies demonstrated that metabolic abnormalities of schizophrenia were related to gluco-regulatory processes and proline metabolism [108–110]. A combined UPLC–MS and  $^1\text{H}$  NMR-based metabolomics was used to study patients with new-onset neuroleptic-naïve schizophrenia before and after a 6-week risperidone monotherapy. A group of healthy control individuals served as controls. The study revealed a disturbance in neurotransmitters and their metabolites together with 32 identified biomarkers that underpin pathways involved in

neurotransmitters, amino acids, glucose, lipids, and energy metabolism, as well as antioxidant defense system, bowel microflora and endocrine system in schizophrenic patients. Bonferroni analysis of the data showed that among these metabolites pregnanediol, citrate and  $\alpha$ -ketoglutarate were significantly associated with symptomatology of schizophrenia and may be useful biomarkers for monitoring therapeutic efficacy [111].

#### Concluding remarks and perspectives

Metabolomics is a potent and promising new approach that allows the assessment of global low-molecular-weight endogenous and exogenous metabolites in a biological system and which shows great potential in investigation of physiological status, discovery of biomarkers, identification of metabolic pathways, and diagnosis of diseases and assessment of drug therapy and safety. The use of UPLC–QTOF/MS with a  $\text{MS}^E$  data collection technique has progressed and is now very popular because it is versatile, sensitive and provides comprehensive information about low-molecular-weight metabolites. The aim of this review was to introduce UPLC-based metabolomics and to present and discuss its key applications focusing on disease biomarkers in clinical chemistry. The clinical chemistry of application of metabolomics for study of the above-mentioned diseases has great potential for disease diagnosis and new biomarker discovery. Analysis of the key endogenous metabolites in the body fluids has become an important part of improving the diagnosis, prognosis, and therapy of human diseases. Metabolomics could help to discover early biochemical changes of disease and understand the mechanism of disease occurrence and progression on the metabolic level and provide information for the identification of early and differential metabolic markers. The clinical application of metabolomics could provide comprehensive information and improve the feasibility of high-throughput patient screening for diagnosis of disease status or risk evaluation. Indeed, identification of clinically relevant metabolites that may be regarded as potential new biomarkers will also help with the evaluation of prognosis and contribute to the development of new therapeutic strategies.

The metabolome is characterized by a large diversity of chemical structures requiring diverse analytical platforms ( $^1\text{H}$  NMR, UPLC, GC, MS, etc.) to reach its comprehensive coverage. Despite recent technological and conceptual improvements, metabolomics appears to be still in its infancy and sample preparation, acquisition of metabolic profiling, metabolite detection, statistical analyses and biomarker identification is a bottleneck in itself. How to accelerate metabolomics studies is, therefore, a challenging issue. The published papers in the field of clinical metabolomics have remained in the discovery phase and most of the identified potential biomarkers have not been adopted in routine clinical practice. Due to the complexity and various factorial interactions of diseases, the situation seems difficult in the field of clinical chemistry for which multiplexed targeted approaches provide the clinician with information on a limited number of metabolites by using MS/MS analysis performed on triple quadrupole MS. Furthermore, recent improvements in UPLC–QTOF/MS/ $\text{MS}^E$  have improved the efficacy of global approaches by facilitating the identification of metabolites of interest thanks to high-resolution and accurate mass measurements, which Q-TOF/MS/ $\text{MS}^E$  can simultaneously acquire MS and MS/MS ( $\text{MS}^E$ ) data of all the metabolites through alternating between high and low collision energies during a single chromatographic run [112–120].

Despite significant advances there are several limitations of current technology including lack of a single method for extensive analysis of the entire metabolome, limited spectral libraries and databases, and disadvantages of current metabolomic software for data processing and biomarker extraction. Further research is needed before finding a reasonable method for metabolite analysis that can replace or complement the traditional and non-specific diagnostic method or technology in clinical chemistry. Future technological development combined with more robust data analysis and bioinformatic tools will help to overcome

the current limitations and fully integrate small molecule biochemistry with systems biology. Because metabolomics is complementary to genomics, transcriptomics and proteomics, full integration of four omics technologies will ultimately improve personalized molecular diagnosis and disease treatment. Further improvements in the sensitivity and selectivity of analytical techniques and routine use of novel methods are certain to find novel targets in the future.

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## References

- Mamas M, Dunn WB, Neyses L, Goodacre R. The role of metabolites and metabolomics in clinically applicable biomarkers of disease. *Arch Toxicol* 2011;85:5–17.
- 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;29:1181–9.
- Lindon JC, Holmes E, Bolland ME, Stanley EG, Nicholson JK. Metabonomics technologies and their applications in physiological monitoring, drug safety assessment and disease diagnosis. *Biomarkers* 2004;9:1–31.
- Zhang A, Sun H, Wang P, Han Y, Wang X. Modern analytical techniques in metabolomics analysis. *Analyst* 2012;137:293–300.
- Griffin JL, Shockcor JP. Metabolic profiles of cancer cells. *Nat Rev Cancer* 2004;4:551–61.
- Zhao YY. Metabolomics in chronic kidney disease. *Clin Chim Acta* 2013;422:59–69.
- Brown FF, Campbell ID, Kuchel PW, Rabenstein DC. Human erythrocyte metabolism studies by <sup>1</sup>H spin echo NMR. *FEBS Lett* 1977;82:12–6.
- Reo NV. NMR-based metabolomics. *Drug Chem Toxicol* 2002;25:375–82.
- Draper J, Enot DP, Parker D, Beckmann M, Snowdon S, Lin W, et al. Metabolite signal identification in accurate mass metabolomics data with MZedDB, an interactive *m/z* annotation tool utilising predicted ionisation behaviour 'rules'. *BMC Bioinformatics* 2009;10:227.
- Wilson ID, Nicholson JK, Castro-Perez J, Granger JH, Johnson KA, Smith BW, et al. High resolution "ultra performance" liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies. *J Proteome Res* 2005;4:591–8.
- Plumb RS, Johnson KA, Rainville P, Smith BW, Wilson ID, Castro-Perez JM, et al. UPLC/MS<sup>E</sup>: a new approach for generating molecular fragment information for biomarker structure elucidation. *Rapid Commun Mass Spectrom* 2006;20:1989–94.
- Bateman KP, Castro-Perez J, Wrona M, Shockcor JP, Yu K, Oballa R, et al. MS<sup>E</sup> with mass defect filtering for in vitro and in vivo metabolite identification. *Rapid Commun Mass Spectrom* 2007;21:1485–96.
- Rainville PD, Stumpf CJ, Shockcor JP, Plumb RS, Nicholson JK. Novel application of reversed-phase UPLC-oaTOFMS for lipid analysis in complex biological mixture: a new tool for lipidomics. *J Proteome Res* 2007;6:552–8.
- Zhao YY, Lin RC. UPLC-MS<sup>E</sup> application in disease biomarker discovery: the discoveries in proteomics to metabolomics. *Chem Biol Interact* 2014;215:7–16.
- Zhao YY, Liu J, Cheng XL, Bai X, Lin RC. Urinary metabolomics study on biochemical changes in an experimental model of chronic renal failure by adenine based on UPLC Q-TOF/MS. *Clin Chim Acta* 2012;413:642–9.
- Zhao YY, Cheng XL, Wei F, Bai X, Lin RC. Application of faecal metabolomics on an experimental model of tubulointerstitial fibrosis by ultra performance liquid chromatography/high-sensitivity mass spectrometry with MS<sup>E</sup> data collection technique. *Biomarkers* 2012;17:721–9.
- Zhao YY, Feng YL, Bai X, Tan XJ, Lin RC, Mei Q. Ultra performance liquid chromatography-based metabolomic study of therapeutic effect of the surface layer of *Poria cocos* on adenine-induced chronic kidney disease provides new insight into anti-fibrosis mechanism. *PLoS One* 2013;8:e59617.
- Zhao YY, Li HT, Feng YL, Bai X, Lin RC. Urinary metabolomic study of the surface layer of *Poria cocos* as an effective treatment for chronic renal injury in rats. *J Ethnopharmacol* 2013;148:403–10.
- Zhao YY, Cheng XL, Wei F, Xiao XY, Sun WJ, Zhang Y, et al. Serum metabolomics study of adenine-induced chronic renal failure rat by ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Biomarkers* 2012;17:48–55.
- Dillon WR, Goldstein M. *Multivariate Analysis, Methods and Application*. UK: John Wiley and Sons Chichester; 1989.
- Nyamundanda G, Brennan L, Gormley IC. Probabilistic principal component analysis for metabolomic data. *BMC Bioinformatics* 2010;11:571.
- Pérez-Enciso M, Tenenhaus M. Prediction of clinical outcome with microarray data: a partial least squares discriminant analysis (PLS-DA) approach. *Hum Genet* 2003;112:581–92.
- Miao H, Chen H, Zhang X, Yin L, Chen DQ, Cheng XL, et al. Urinary metabolomics on the biochemical profiles in diet-induced hyperlipidemia rat using ultraperformance liquid chromatography coupled with quadrupole time-of-flight SYNAPT high-definition mass spectrometry. *J Anal Methods Chem* 2014;2014:184162.
- Bylesjo M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. *J Chemom* 2006;20:341–51.
- Trygg J, Wold S. Orthogonal projections to latent structures (O-PLS). *J Chemom* 2002;16:119–28.
- Katajamaa M, Oresic M. Data processing for mass spectrometry-based metabolomics. *J Chromatogr A* 2007;1158:318–28.
- Ressom HW, Xiao JF, Tuli L, Varghese RS, Zhou B, Tsai TH, et al. Utilization of metabolomics to identify serum biomarkers for hepatocellular carcinoma in patients with liver cirrhosis. *Anal Chim Acta* 2012;743:90–100.
- Chen F, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocellular carcinoma through liquid chromatography/mass spectrometry-based metabolomic method. *Anal Bioanal Chem* 2011;401:1899–904.
- Chen T, Xie G, Wang X, Fan J, Qiu Y, Zheng X, et al. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics* 2011;10 [M110.004945].
- Chen S, Kong H, Lu X, Li Y, Yin P, Zeng Z, et al. Pseudotargeted metabolomics method and its application in serum biomarker discovery for hepatocellular carcinoma based on ultra high-performance liquid chromatography/triple quadrupole mass spectrometry. *Anal Chem* 2013;85:8326–33.
- Xiao JF, Varghese RS, Zhou B, Nezami Ranjbar MR, Zhao Y, Tsai TH, et al. LC-MS based serum metabolomics for identification of hepatocellular carcinoma biomarkers in Egyptian cohort. *J Proteome Res* 2012;11:5914–23.
- Wang B, Chen D, Chen Y, Hu Z, Cao M, Xie Q, et al. Metabonomic profiles discriminate hepatocellular carcinoma from liver cirrhosis by ultra performance liquid chromatography-mass spectrometry. *J Proteome Res* 2012;11:1217–27.
- Zhou L, Wang Q, Yin P, Xing W, Wu Z, Chen S, et al. Serum metabolomics reveals the deregulation of fatty acids metabolism in hepatocellular carcinoma and chronic liver diseases. *Anal Bioanal Chem* 2012;403:203–13.
- Chen J, Wang W, Lv S, Yin P, Zhao X, Lu X, et al. Metabonomics study of liver cancer based on ultra performance liquid chromatography coupled to mass spectrometry with HILIC and RPLC separations. *Anal Chim Acta* 2009;650:3–9.
- Zhou L, Ding L, Yin P, Lu X, Wang X, Niu J, et al. Serum metabolic profiling study of hepatocellular carcinoma infected with hepatitis B or hepatitis C virus by using liquid chromatography-mass spectrometry. *J Proteome Res* 2012;11:5433–42.
- Zhang A, Sun H, Yan G, Han Y, Ye Y, Wang X. Urinary metabolic profiling identifies a key role for glycocholic acid in human liver cancer by ultra-performance liquid-chromatography coupled with high-definition mass spectrometry. *Clin Chim Acta* 2013;418:86–90.
- Cao H, Huang H, Xu W, Chen D, Yu J, Li J, et al. Fecal metabolome profiling of liver cirrhosis and hepatocellular carcinoma patients by ultra performance liquid chromatography-mass spectrometry. *Anal Chim Acta* 2011;691:68–75.
- Liu SY, Zhang RL, Kang H, Fan ZJ, Du Z. Human liver tissue metabolic profiling research on hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2013 Jun 14;19(22):3423–32.
- Yang Y, Li C, Nie X, Feng X, Chen W, Yue Y, et al. Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning <sup>1</sup>H NMR spectroscopy in conjunction with multivariate data analysis. *J Proteome Res* 2007;6:2605–14.
- Tan Y, Yin P, Tang L, Xing W, Huang Q, Cao D, et al. Metabonomics study of stepwise hepatocarcinogenesis from the model rats to patients: potential biomarkers effective for small hepatocellular carcinoma diagnosis. *Mol Cell Proteomics* 2012;11 [M111.010694].
- Patterson AD, Maurhofer O, Beyoglu D, Lanz C, Krausz KW, Pabst T, et al. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. *Cancer Res* 2011;71:6590–600.
- Wang X, Wang X, Xie G, Zhou M, Yu H, Lin Y, et al. Urinary metabolite variation is associated with pathological progression of the post-hepatitis B cirrhosis patients. *J Proteome Res* 2012;11:3838–47.
- Hao S, Xin J, Lian J, Xie Q, Chen D, Guo Y, et al. Establishing a metabolomic model for the prognosis of hepatitis B virus-induced acute-on-chronic liver failure treated with different liver support systems. *Metabolomics* 2011;7:400–12.
- Lian JS, Liu W, Hao SR, Guo YZ, Huang HJ, Chen DY, et al. A serum metabolomic study on the difference between alcohol- and HBV-induced liver cirrhosis by ultra performance liquid chromatography coupled to mass spectrometry plus quadrupole time-of-flight mass spectrometry. *Chin Med J (Engl)* 2011;124:1367–73.
- Zhang A, Sun H, Han Y, Yan G, Wang X. Urinary metabolic biomarker and pathway study of hepatitis B virus infected patients based on UPLC-MS system. *PLoS One* 2013;8:e64381.

- [46] Pares A, Perez-Cormenzana M, Mayo R, Bannasar A, Mas A, Castro A. Lipidic and bile acid metabolomic profile in patients with primary biliary cirrhosis and pruritus. *Hepatology* 2012;56:1129A.
- [47] Legido-Quigley C, McDermott L, Vilca-Melendez H, Murphy GM, Heaton N, Lindon JC, et al. Bile UPLC–MS fingerprinting and bile acid fluxes during human liver transplantation. *Electrophoresis* 2011;32:2063–70.
- [48] Yang J, Zhao X, Liu X, Wang C, Gao P, Wang J, et al. High performance liquid chromatography–mass spectrometry for metabolomics: potential biomarkers for acute deterioration of liver function in chronic hepatitis B. *J Proteome Res* 2006;5:554–61.
- [49] Goto T, Myint KT, Sato K, Wada O, Kakiyama G, Iida T, et al. LC/ESI–tandem mass spectrometric determination of bile acid 3-sulfates in human urine 3 $\beta$ -Sulfoxy-12 $\alpha$ -hydroxy-5 $\beta$ -cholanoic acid is an abundant nonamidated-sulfate. *J Chromatogr B* 2007;846:69–77.
- [50] Park JY, Park BK, Ko JS, Bang S, Song SY, Chung JB. Bile acid analysis in biliary tract cancer. *Yonsei Med J* 2006;47:817–25.
- [51] Sokol RJ, Dahl R, Devereaux MW, Yerushalmi B, Kobak GE, Gumprich E. Human hepatic mitochondria generate reactive oxygen species and undergo the permeability transition in response to hydrophobic bile acids. *J Pediatr Gastroenterol Nutr* 2005;41(2):235–43.
- [52] Gowda GA. Human bile as a rich source of biomarkers for hepatopancreatobiliary cancers. *Biomark Med* 2010;4:299–314.
- [53] Dong J, Cai X, Zou L, Chen C, Xue X, Zhang X, et al. Lysophosphatidylcholine biomarkers of lung cancer detected by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Chem Res Chin U* 2011;27:750–5.
- [54] Dong J, Cai X, Zhao L, Xue X, Zou L, Zhang X, et al. Lysophosphatidylcholine profiling of plasma: discrimination of isomers and discovery of lung cancer biomarkers. *Metabolomics* 2010;6:478–88.
- [55] An Z, Chen Y, Zhang R, Song Y, Sun J, He J, et al. Integrated ionization approach for RRLC–MS/MS-based metabolomics: finding potential biomarkers for lung cancer. *J Proteome Res* 2010;9:4071–81.
- [56] Laviano A, Cascino A, Muscaritoli M, Fanfarillo F, Rossi Fanelli F. Tumor-induced changes in host metabolism: a possible role for free tryptophan as a marker of neoplastic disease. *Adv Exp Med Biol* 2003;527:363–6.
- [57] Frickenschmidt A, Frohlich H, Bullinger D, Zell A, Laufer S, Gleiter CH, et al. Metabolomics in cancer diagnosis: mass spectrometry-based profiling of urinary nucleosides from breast cancer patients. *Biomarkers* 2008;13:435–49.
- [58] Cai X, Dong J, Zou L, Xue X, Zhang X, Liang X. Metabonomic study of lung cancer and the effects of radiotherapy on lung cancer patients: analysis of highly polar metabolites by ultra performance HILIC coupled with Q-TOF MS. *Chromatographia* 2011;74:391–8.
- [59] Wedge DC, Allwood JW, Dunn W, Vaughan AA, Simpson K, Brown M, et al. Is serum or plasma more appropriate for intersubject comparisons in metabolomic studies? An assessment in patients with small-cell lung cancer. *Anal Chem* 2011;83:6689–97.
- [60] Laiakis EC, Morris GA, Fornace AJ, Howie SR. Metabolomic analysis in severe childhood pneumonia in the Gambia, West Africa: findings from a pilot study. *PLoS One* 2010;5 [pii: e12655].
- [61] Mene P, Punzo G. Uric acid: bystander or culprit in hypertension and progressive renal disease? *J Hypertens* 2008;26:2085–92.
- [62] Rodriguez-Nunez A, Camina F, Lojo S, Rodriguez-Segade S, Castro-Gago M. Concentrations of nucleotides, nucleosides, purine bases and urate in cerebrospinal fluid of children with meningitis. *Acta Paediatr* 1993;82:849–52.
- [63] Fabre JE, Nguyen M, Latour A, Keifer JA, Audoly LP, Coffman TM, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat Med* 1999;5:1199–202.
- [64] Ma YL, Qin HL, Liu WJ, Peng JY, Huang L, Zhao XP, et al. Ultra-high performance liquid chromatography–mass spectrometry for the metabolomic analysis of urine in colorectal cancer. *Dig Dis Sci* 2009;54:2655–62.
- [65] Qiu Y, Cai G, Su M, Chen T, Zheng X, Xu Y, et al. Serum metabolite profiling of human colorectal cancer using GC–TOFMS and UPLC–QTOFMS. *J Proteome Res* 2009;8:4844–50.
- [66] Tan B, Qiu Y, Zou X, Chen T, Xie G, Cheng Y, et al. Metabonomics identifies serum metabolite markers of colorectal cancer. *J Proteome Res* 2013;12:3000–9.
- [67] Qiu Y, Cai G, Su M, Chen T, Liu Y, Xu Y, et al. Urinary metabolomic study on colorectal cancer. *J Proteome Res* 2010;9:1627–34.
- [68] Wang W, Feng B, Li X, Yin P, Gao P, Zhao X, et al. Urinary metabolic profiling of colorectal carcinoma based on online affinity solid phase extraction–high performance liquid chromatography and ultra performance liquid chromatography–mass spectrometry. *Mol Biosyst* 2010;6:1947–55.
- [69] Mal M, Koh PK, Cheah PY, Chan EC. Ultra-pressure liquid chromatography/tandem mass spectrometry targeted profiling of arachidonic acid and eicosanoids in human colorectal cancer. *Rapid Commun Mass Spectrom* 2011;25:755–64.
- [70] Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, et al. Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 2009;457:910–4.
- [71] Saylor PJ, Karoly ED, Smith MR. Prospective study of changes in the metabolomic profiles of men during their first three months of androgen deprivation therapy for prostate cancer. *Clin Cancer Res* 2012;18:3677–85.
- [72] Chen J, Zhang X, Cao R, Lu X, Zhao S, Fekete A, et al. Serum 27-nor-5 $\beta$ -cholestane-3,7,12,24,25 pentol glucuronide discovered by metabolomics as potential diagnostic biomarker for epithelium ovarian cancer. *J Proteome Res* 2011;10:2625–32.
- [73] Zhang T, Wu X, Yin M, Fan L, Zhang H, Zhao F, et al. Discrimination between malignant and benign ovarian tumors by plasma metabolomic profiling using ultra performance liquid chromatography/mass spectrometry. *Clin Chim Acta* 2012;413:861–8.
- [74] Sperner-Unterwieser B, Neurauder G, Klieber M, Kurz K, Meraner V, Zeimet A, et al. Enhanced tryptophan degradation in patients with ovarian carcinoma correlates with several serum soluble immune activation markers. *Immunobiology* 2011;216:296–301.
- [75] Murph M, Tanaka T, Pang J, Felix E, Liu S, Trost R, et al. Liquid chromatography mass spectrometry for quantifying plasma lysophospholipids: potential biomarkers for cancer diagnosis. *Methods Enzymol* 2007;433:1–25.
- [76] Jia L, Chen J, Yin P, Lu X, Xu G. Serum metabolomics study of chronic renal failure by ultra performance liquid chromatography coupled with Q-TOF mass spectrometry. *Metabolomics* 2008;4:183–9.
- [77] Sato E, Kohno M, Yamamoto M, Fujisawa T, Fujiwara K, Tanaka N. Metabolomic analysis of human plasma from haemodialysis patients. *Eur J Clin Invest* 2011;41:241–55.
- [78] Duan H, Guan N, Wu Y, Zhang J, Ding J, Shao B. Identification of biomarkers for melamine-induced nephrolithiasis in young children based on ultra high performance liquid chromatography coupled to time-of-flight mass spectrometry (U-HPLC–Q-TOF/MS). *J Chromatogr B* 2011;879:3544–50.
- [79] Sysi-Aho M, Ermolov A, Gopalacharyulu PV, Tripathi A, Seppänen-Laakso T, Maukonen J, et al. Metabolic regulation in progression to autoimmune diabetes. *PLoS Comput Biol* 2011;7:e1002257.
- [80] Posada-Ayala M, Zubiri I, Martin-Lorenzo M, Sanz-Maroto A, Molero D, Gonzalez-Calero L, et al. Identification of a urine metabolomic signature in patients with advanced-stage chronic kidney disease. *Kidney Int* 2014;85:103–11.
- [81] Lanza IR, Zhang S, Ward LE, Karakelide H, Raftery D, Nair KS. Quantitative metabolomics by <sup>1</sup>H NMR and LC–MS/MS confirms altered metabolic pathways in diabetes. *PLoS One* 2010;5:e10538.
- [82] Felig P, Marliss E, Ohman JL, Cahill Jr CF. Plasma amino acid levels in diabetic ketoacidosis. *Diabetes* 1970;19:727–8.
- [83] Pacy PJ, Nair KS, Ford C, Halliday D. Failure of insulin infusion to stimulate fractional muscle protein synthesis in type I diabetic patients. Anabolic effect of insulin and decreased proteolysis. *Diabetes* 1989;38:618–62.
- [84] Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J. Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. *J Clin Invest* 1995;95:2926–37.
- [85] Gu Y, Zhang Y, Shi X, Li X, Hong J, Chen J. Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabolomics. *Talanta* 2010;81:766–72.
- [86] Huo T, Cai S, Lu X, Sha Y, Yu M, Li F. Metabonomic study of biochemical changes in the serum of type 2 diabetes mellitus patients after the treatment of metformin hydrochloride. *J Pharm Biomed* 2009;49:976–82.
- [87] Suhre K, Meisinger C, Döring A, Altmaier E, Belcredi P, Gieger C, et al. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. *PLoS One* 2010;5:e13953.
- [88] Boden G, Chen X, Iqbal N. Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. *Diabetes* 1998;47:1609–12.
- [89] Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 2002;32:14–23.
- [90] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* Apr 13 2006;440:944–8.
- [91] Schinner S, Scherbaum WA, Bornstein SR, Barthel A. Molecular mechanisms of insulin resistance. *Diabet Med* 2005;22:674–82.
- [92] Jové M, Portero-Otín M, Naudí A, Ferrer I, Pamplona R. Metabolomics of human brain aging and age-related neurodegenerative diseases. *J Neuropathol Exp Neurol* 2014;73:640–57.
- [93] Trushina E, Mielke MM. Recent advances in the application of metabolomics to Alzheimer's disease. *Biochim Biophys Acta-Mol Basis Dis* 2014;1842:1232–9.
- [94] Ibáñez C, Simó C, Barupal DK, Fiehn O, Kivipelto M, Cedazo-Minguez A, et al. A new metabolomic workflow for early detection of Alzheimer's disease. *J Chromatogr A* 2013;1302:65–71.
- [95] Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. *PLoS One* 2013;8:e63644.
- [96] Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science* 2002;298:789–91.
- [97] Rodriguez JJ, Noristani HN, Verkhatsky A. The serotonergic system in ageing and Alzheimer's disease. *Prog Neurobiol* 2012;99:15–41.
- [98] Ibáñez C, Simó C, Martín-Álvarez PJ, Kivipelto M, Winblad B, Cedazo-Minguez A, et al. Toward a predictive model of Alzheimer's disease progression using capillary electrophoresis–mass spectrometry metabolomics. *Anal Chem* 2012;84(20):8532–40.
- [99] Jiang Z, Sun J, Liang Q, Cai Y, Li S, Huang Y, et al. A metabolomic approach applied to predict patients with cerebral infarction. *Talanta* 2011;84:298–304.
- [100] Rafii M, Elango R, House JD, Courtney-Martin G, Darling P, Fisher L, et al. Measurement of homocysteine and related metabolites in human plasma and urine by liquid chromatography electrospray tandem mass spectrometry. *J Chromatogr B* 2009;877:3282–91.
- [101] Cha MH, Jones AD, Ko MM, Zhang C, Lee MS. Metabolic profiles distinguish non-dampness-phlegm and dampness-phlegm patterns among Korean patients with acute cerebral infarction. *Evid Based Complement Altern Med* 2013;2013:517018.
- [102] Orešič M, Tang J, Seppänen-Laakso T, Mattila I, Saarni SE, Saarni SI, et al. Metabolome in schizophrenia and other psychotic disorders: a general population-based study. *Genome Med* 2011;3:19.



- [103] Ko MM, Kang BK, Lim JH, Lee MS, Cha MH. Genetic association of NPY gene polymorphisms with dampness-phlegm pattern in Korean stroke patients. *Evid Based Complement Altern Med* 2012;2012:109796.
- [104] Kim HJ, Bae HS, Park SU, Moon SK, Park JM, Jung WS. Clinical approach to the standardization of oriental medical diagnostic pattern identification in stroke patients. *Evid Based Complement Altern Med* 2011;2011:768492.
- [105] Pietiläinen KH, Sysi-Aho M, Rissanen A, Seppänen-Laakso T, Yki-Järvinen H, Kaprio J, et al. Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects—a monozygotic twin study. *PLoS One* 2007;2:e218.
- [106] Barber MN, Risis S, Yang C, Meikle PJ, Staples M, Febbraio MA, et al. Plasma lysophosphatidylcholine levels are reduced in obesity and type 2 diabetes. *PLoS One* 2012;7:e41456.
- [107] Orešič M, Tang J, Seppänen-Laakso T, Mattila I, Saarni SE, Saarni SI, et al. Metabolome in schizophrenia and other psychotic disorders: a general population-based study. *Genome Med* 2011;3:19.
- [108] Cheryl SY, Woon PS, Liu JJ, Ong WY, Tsai GC, Sim K. Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: a decade of advance. *Neurosci Biobehav Rev* 2010;34:958–77.
- [109] Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML, et al. Genetic variation at the 22q11 PRODH/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci U S A* 2002;99:3717–22.
- [110] Kempf L, Nicodemus KK, Kolachana B, Vakkalanka R, Verchinski BA, Egan MF, et al. Functional polymorphisms in PRODH are associated with risk and protection for schizophrenia and fronto-striatal structure and function. *PLoS Genet* 2008;4:e1000252.
- [111] Xuan J, Pan G, Qiu Y, Yang L, Su M, Liu Y, et al. Metabolomic profiling to identify potential serum biomarkers for schizophrenia and risperidone action. *J Proteome Res* 2011;10:5433–43.
- [112] Zhao YY, Lin RC. Metabolomics in nephrotoxicity. *Adv Clin Chem* 2014;65:69–89.
- [113] Chen H, Miao H, Feng YL, Zhao YY, Lin RC. Metabolomics in dyslipidemia. *Adv Clin Chem* 2014;66:101–19.
- [114] Zhao YY, Wu SP, Liu S, Zhang Y, Lin RC. Ultra-performance liquid chromatography–mass spectrometry as a sensitive and powerful technology in lipidomic applications. *Chem Biol Interact* 2014. <http://dx.doi.org/10.1016/j.cbi.2014.06.029> [in press].
- [115] Zhao YY, Shen X, Cheng XL, Wei F, Bai X, Lin RC. Urinary metabolomics study on the protective effects of ergosta-4,6,8(14),22-tetraen-3-one on chronic renal failure in rats using UPLC Q-TOF/MS and a novel MS<sup>E</sup> data collection technique. *Process Biochem* 2012;47:1980–7.
- [116] Zhang ZH, Zhao YY, Cheng XL, Dai Z, Zhou C, Bai X, et al. General toxicity of *Pinellia ternate* (Thunb.) Berit. in rat: a metabolomic method for profiling of serum metabolic changes. *J Ethnopharmacol* 2013;149:303–10.
- [117] Zhao YY, Zhang L, Long FY, Cheng XL, Bai X, Wei F, et al. UPLC-Q-TOF/HSMS/MS<sup>E</sup>-based metabolomics for adenine-induced changes in metabolic profiles of rat faeces and intervention effects of ergosta-4,6,8(14),22-tetraen-3-one. *Chem Biol Interact* 2013;301:31–8.
- [118] Zhang ZH, Zhao YY, Cheng XL, Lin RC, Dai Z, Zhou C. Metabolomic study of biochemical changes in the rat urine induced by *Pinellia ternata* (Thunb.) Berit. *J Pharm Biomed Anal* 2013;85:186–93.
- [119] Zhao YY, Cheng XL, Wei F, Bai X, Tan XJ, Lin RC, et al. Intrarenal metabolomic investigation of chronic kidney disease and its TGF-β1 mechanism in induced-adenine rats using UPLC Q-TOF/HSMS/MS<sup>E</sup>. *J Proteome Res* 2013;12:692–703.
- [120] Zhao YY, Lei P, Chen DQ, Feng YL, Bai X. Renal metabolic profiling of early renal injury and renoprotective effects of *Poria cocos* epidermis using UPLC Q-TOF/HSMS/MS<sup>E</sup>. *J Pharm Biomed Anal* 2013;81–82:202–9.