



Review

Potential role of metabolomics approaches in the area of traditional Chinese medicine: As pillars of the bridge between Chinese and Western medicine

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ABSTRACT

Traditional Chinese medicine (TCM) is a holistic approach to health that attempts to bring the body, mind and spirit into harmony. Entering 21st century, TCM is getting more and more popular in the whole world for improving health condition of human beings and preventing or healing diseases, especially shows great advantages in early intervention, combination therapies and personalized medicine, etc. However, like almost all other ethnopharmacology, TCM also faces severe challenges and suffers from insufficient modern research owing to lack of scientific and technologic approaches, restricts the development of TCM in the world. Fortunately, a novel analytical technique, metabolomics (or metabonomics), adopts a 'top-down' strategy to reflect the function of organisms from terminal symptoms of metabolic network and understand metabolic changes of a complete system caused by interventions in holistic context. Its property consists with the holistic thinking of TCM, may beneficially provide an opportunity to scientifically express the meaning of evidence-based Chinese medicine, such as Chinese medicine syndromes (CMS), preventive treatment, action of Chinese medicine, Chinese medical formulae (CMF) and acupuncture efficacy. This review summarizes potential applications of robust metabolomics approaches in the area of traditional oriental medicine, and highlights the key role of metabolomics to resolve special TCM issues.

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Contents

1. Introduction	859
2. Metabolomic evaluation of CMS	860
3. Preventive treatment of TCM	863
4. Metabolomic analysis of Chinese medicine	863
5. Metabolomic dissection of CMF	864
6. Metabolomic evaluation of acupuncture efficacy	865
7. Conclusion and future perspective	865
Acknowledgments	866
References	866

Abbreviations: TCM, traditional Chinese medicine; WM, Western medicine; CMS, Chinese medicine syndromes; CMF, Chinese medical formulae; NMR, proton nuclear magnetic resonance; MS, mass spectroscopy; PCA, principal component analysis; PLS, partial least squares; MGS, Morning Glory Seed; YCHT, Yin Chen Hao Tang; LW, Liu Wei Di Huang Wan; UPLC-MS, ultra-performance liquid chromatography-mass spectrometry; RA, rheumatoid arthritis; CHD, coronary heart disease; XBSS, Xin-blood stasis syndrome; ALD, alcohol-induced liver disease.

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1. Introduction

TCM, a complex medical science, reflects traditional Chinese culture and philosophical principles, embodies rich dialectical thought, places the human body into a large system for observation and adjusts humans to remain in a healthy status [1]. Admittedly, it shows great advantages in early intervention, combination therapies, personalized medicine, etc. There are abundant systematic concepts of health and the wisdom of life-cultivation in TCM, not only including the connotation of modern health, but also with many of its own characteristics. The health concept of TCM includes the holistic view of unison between man and universe, the harmo-

nious unity of fusion of shape and soul, the people-oriented view of values and the balance of qi-blood-yin-yang in the human body. The unique features of TCM for maintaining health consist of prevention, emotion regulation, obeying nature. Taking full advantage of the health concept and regulation methods of TCM will promote and make great contributions to human health. Focusing on the weak links and shortages of modern medicine, the valuable experiences and theoretical advantages of TCM in clinical trials and mechanism studies should be brought to light so as to bring Chinese medicine into the frame of modern medicine and to contribute to the world medicine.

TCM has a long history and been accepted by the academic community and patients as superior and a unique valuable property in China. However, like almost all other traditional medicines, TCM is also facing severe challenges or problems. The largest obstacle suffers from insufficient modern scientific research, that not only lowering the position of TCM also restricting the development of TCM in the abroad. One of the reasons for such failure is that the methodology used in the TCM research follows basically the path of partitioned reductive analysis, which is unable to capture practically the characteristics of TCM scientific system, such as the holistic and dynamic nature of diseases, and the interaction among various biological components. Further, the features and advantages of traditional oriental medicine are difficult to understand owing to lack of modern scientific and technologic approaches, restricts the actual value of TCM bring into full play effectiveness [1]. For example, many aspects of TCM are still practicing in its original form and although it has been effective in treating many conditions especially chronic ones, it lacks necessary well-defined molecular mechanism and sometimes even molecular basis. Prescribed medicines in TCM are normally mixtures from a number of plants and their composition is not well defined. To conduct a systemic strategic reductive analysis on human body and diseases under the guidance of holistic view will be an utmost important way for developing TCM towards the future of healthcare. The modernization of TCM does not have to follow the partitioned reductive approach, which has been practiced in the development of modern science in recent centuries. Therefore, it is indispensable to strengthen TCM research, which undoubtedly demands significant amount of analytical power and efforts. There have been many recent attempts to address these issues but most of them were still based on the “reductionism” philosophy, whereas TCM is based on “holism” philosophy instead of “reductionism” as in the case of “omics” theories. Fortunately, as a systemic approach, metabolomics adopts a ‘top-down’ strategy to reflect the function of organisms from terminal symptoms of metabolic network and understand metabolic changes of a complete system caused by interventions in holistic context [2,3]. This property is in concert with the holistic efficacy of TCM, suggesting that metabolomics has the potential to impact our understanding of the theory behind the evidence-based Chinese medicine [1]. Marker metabolites can be therapeutic targets as well [4]. The systemic thinking and strategy of the metabolomics and its aim at grasping integral function have provided unprecedented enlightenment for the modern TCM research. Additionally, metabolomics represents a global understanding of metabolite complement of integrated living systems and dynamic responses to the changes of both endogenous and exogenous factors and has many potential applications and advantages for the research of complex systems. It opens up the possibility of studying the effect of TCM, in complex biological systems, abridging it with Western medicine (WM) [5].

Metabolomics was originally proposed as a method of functional genomics, an emerging subject of the post-genome era, which, together with genomics, transcriptomics and proteomics, jointly constitutes the ‘Systems Biology’ [6,7]. The primary definition of metabolomics, as “the quantitative measurement of the

dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification” [7], has recently demonstrated significant potential in many fields such as responses to environmental stress [8,9], toxicology [10–12], nutrition [13–15], studying global effects of genetic manipulation [16–18], cancer [19–24], comparing different growth stages [25–27], diabetes [28,29], disease diagnosis [30] and natural product discovery [31,32]. One of the major benefits of metabolomics in the study of disease and drug therapy is that metabolic profiling can usually be achieved using urine or plasma samples. The accessibility of urine and plasma clearly makes these samples ideal for large-scale research. Integrity of metabolomic processes includes sample collection, pretreatment, data analysis, and metabolic variation interpretation. Flowchart of metabolomic analysis was shown in Fig. 1. Generally, two techniques are mainly available for the metabolic analysis of biological samples: proton nuclear magnetic resonance (NMR) spectroscopy [33–36] and mass spectroscopy (MS) [37,38]. Depending on a series analysis of different sample spectra and combination with chemical pattern recognition methods, metabolomics can be used to identify organisms in pathophysiological state, gene function, drug toxicity and efficacy, and associated biomarkers [39]. One area of considerable interest in the field of metabolomics is that of personalised healthcare, whereby an individual’s drug treatment is tailored so as to achieve maximal efficacy while avoiding adverse drug reactions. Metabolomics holds the promise of a comprehensive, non-invasive analysis of metabolic biomarkers that could detect early-stage disease, identify residual disease post-surgery, and help to monitor treatment response and detect early treatment toxicity. Surprisingly, the global metabolite profile involves measuring low molecular-weight metabolites (<1 kDa) in complex biofluids/tissues to study perturbations in response to physiological challenges, toxic insults or disease processes [40]. Metabolomics approaches as pillars of the potential bridge between Chinese and WM, may beneficially influence and provide an opportunity to explain the theoretical meaning of evidence-based Chinese medicine.

Nowadays, some animal or disease model are not even clearly characterized or validated and metabolomics are still confounding and controversial concepts. Trying to connect western medicine and traditional oriental medicine using metabolomics approach is probably premature at this stage since there is no definitive clinical characterization of traditional oriental medicine. Hence, to address this challenge, explanation of the relationship between TCM and WM is essential. In this review, we present and discuss applicable approaches of using the robust metabolomic techniques to resolve the key issues and challenges as well as particular focus will be paid to the potential role and significant achievement of metabolomics to contribute to biomarker discovery in TCM research.

2. Metabolomic evaluation of CMS

The two medical systems are highly complementary as the reductionist aspects. WM are favourable in acute disease situations and the holistic aspects of Chinese medicine offer more opportunities in chronic conditions and for prevention [41]. Metabolomics may play a key role in building the bridge between TCM and WM. Especially, metabolomics-based diagnosis can be used to capture phenotype information, leading towards a scientific basis for a more refined patient characterization, new diagnostic tools and personalized health strategies. Key insights from this process of integrating Western and Chinese medicine will pave the way for a patient-centred health care ecosystem.

Facing the complicated life phenomenon, metabolomics take an organic conception of the human body, which conforms to the way of thinking of TCM. Kidney yin deficiency syndrome is

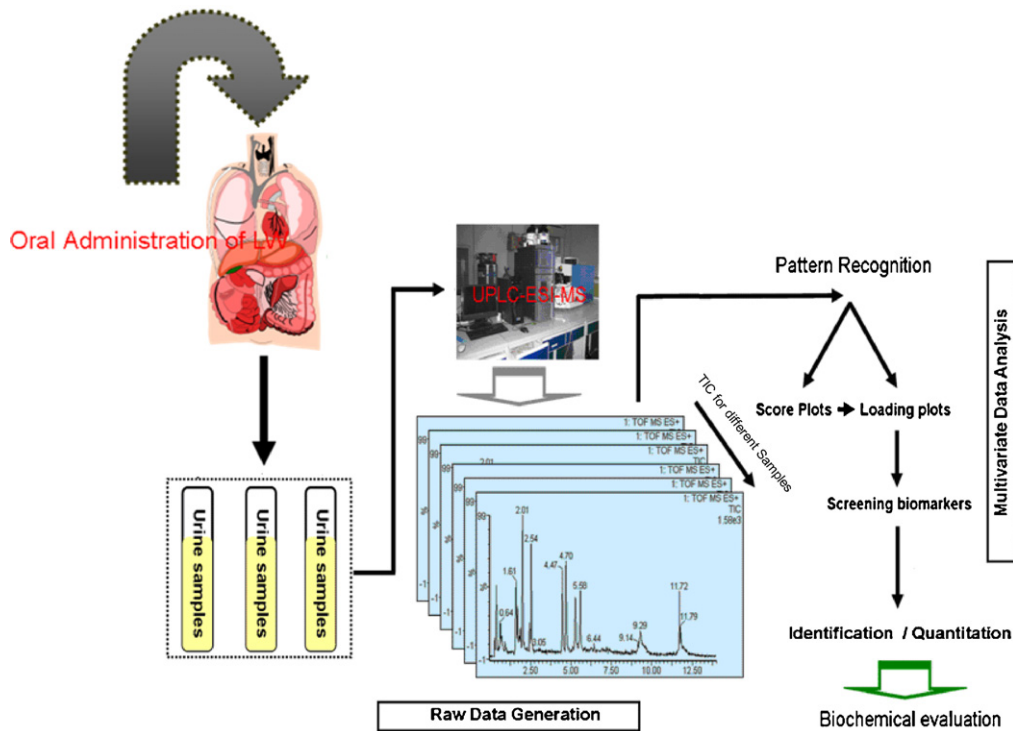


Fig. 1. The overall flowchart of the metabolomic investigation in TCM.

a common disease in China, especially for old people. However, with the increasing pace of life, many young people begin to suffer from this disease. Liuwei Dihuang Wan (LW), one of the most important Chinese patent medicines consists of six herbs including *Radix Rehmanniae Preparata*, *Fructus Macrocarpii*, *Rhizoma Dioscoreae Oppositae*, *Poria*, *Rhizoma Alismatis* and *Cortex Moutan Radicis*, is widely used in eastern Asia for treating kidney yin deficiency. It has long been used clinically in treatment of many kinds of diseases with the sign of kidney Yin insufficiency, such as dizziness, tinnitus, weakness and soreness of the loins and the limbs, emission and sweating, the effectiveness has been well recorded during long-term clinical practice [42]. The first three crude drugs are known as “Sanbu”, which invigorate yin of the kidney, and the rest as “Sanxie”, which attenuate the effects of invigoration. In a word, “SanBu” coordinates with “SanXie” to replenish kidney essence and LW is applied to treat kidney yin deficiency in clinic. Wang et al. [43] adopted ultra-performance liquid chromatography-mass spectrometry (UPLC–MS) to investigate the metabolic profiling of rats with kidney yin deficiency induced by thyroxine and reserpine. It could be shown that the changes in metabolic profiling were restored to their baseline values after treatment with LW according to the PCA (Principal component analysis) score plots (Fig. 2), indicating 20 ions (8 in the positive mode, 14 in the negative mode and 2 in both) as “differentiating metabolites” (Fig. 3). PCA score plots separated urine samples into different blocks, and samples subjected to the same treatment were located on the same trajectory, indicating that treatments have greatly disturbed the normal urine metabolic profiles of rats. Adoption of metabolomic approach would do much help for exploring the scientific connotation and intrinsic quality of CMS as well as differentiation of syndrome, especially deepen the evaluation of the therapeutic effects of TCM. There is good reason to think that metabolomics will find particular utility in CMS investigation, which may inform us new information on pathways and processes involved in these responses. Investigations into the characters of the ‘Kidney-Yang Deficiency syndrome’ induced by

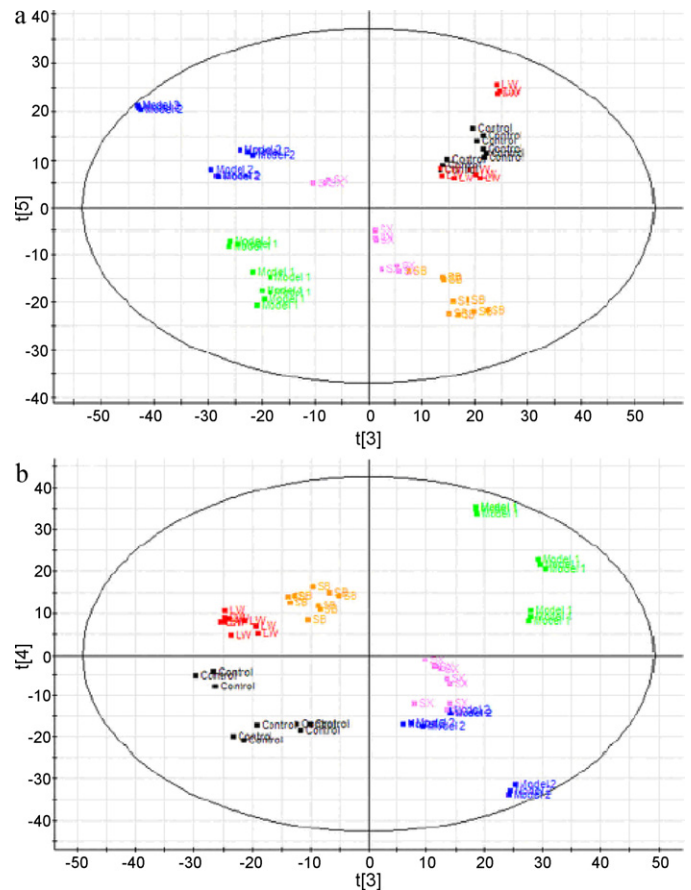


Fig. 2. PCA score plots of urine samples collected from different treatment groups of rats (a) in negative ESI mode and (b) in positive ESI mode. Key: (■) Control; (■) LW; (■) Model 1; (■) Model 2; (■) Sanbu; (■) Sanxie. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

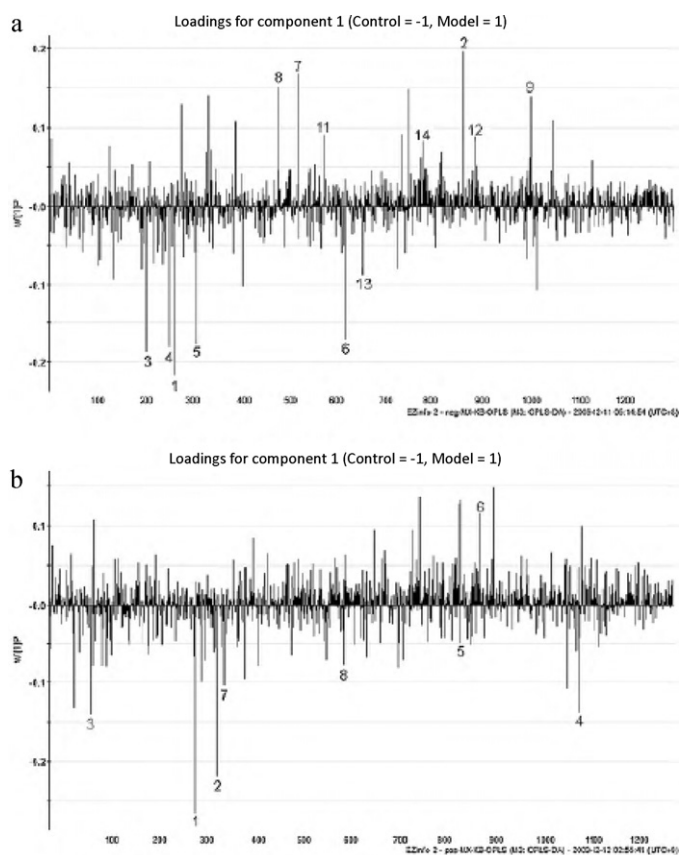


Fig. 3. Loadings for component in (a) negative ESI mode and (b) positive ESI mode.

high dose of hydrocortisone and the therapeutic effects of *Rhizoma Drynariae*, classic TCM in treating the syndrome, have been performed by robust metabolomics method based on UPLC/MS [44]. It was found that significant difference in metabolic profiling was observed from hydrocortisone-induced group compared with the pre-dose group by using PCA. The time-dependent regression tendency in *Rhizoma Drynariae* treatment group was obtained, and the major metabolic alterations responsible for group separation were linked to some significantly changed metabolites like phenylalanine, N(2)-succinyl-L-ornithine, phenylacetyl glycine, creatinine, hippurate, L-proline, and citrate. Biochemical changes were related to the disturbance of amino acid metabolism, energy metabolism and gut microflora, which are helpful to further understand the 'Kidney-Yang Deficiency syndrome' and the therapeutic mechanism of *Rhizoma Drynariae*. Therefore, metabolomics not only opens out mechanism of classical TCM theory on syndrome but also enriches current research on complex diseases [45].

Siwutang, a novel prescription consisting of *Paeonia lactiflora* Pall, *Angelica sinensis*, *Rehmannia glutinosa* and *Rhizoma Chuanxiong*, treated cyclophosphamide induced "blood deficiency" in mice [46]. PCA result showed conspicuous difference in the metabolic profiles between groups. Compared with the control group, the model group contained lower concentration of lactate, 3-hydroxybutyrate, choline, glucose, and higher concentration leucine/isoleucine in serum. When they were dosed with Siwutang for 7 days, the effects above-mentioned were reversed. The injury established by injecting cyclophosphamide is a kind of proper model to develop further metabolomics researches. Damage of mitochondria, disorder of energy metabolism and osmoregulation

were observed in cyclophosphamide caused blood deficiency model by NMR-based metabolomics method. Siwutang could improve these effects. TCM differs from its Western counterpart in a number of ways. One of the more striking ways involves a conceptually oriented diagnostic system that relies more on the clinician's reading of the patient's symptoms and signs than on laboratory findings [47]. A study was conducted by Qiu et al. [48] to investigate the metabolic responses to *Herba Cistanches* intervention in a rat model of the hydrocortisone-induced "kidney-deficiency syndrome". It revealed that *Herba Cistanches* caused a systemic recovery from the hydrocortisone-induced metabolic perturbation in rats. Interestingly, the TCM concepts of "Xinxueyuzheng (heart blood stasis obstruction pattern)" and "Qiyinliangxuzheng (qi and yin deficiency pattern)" for myocardial ischemia rat models were constructed by Yan et al. [49]. Endogenous metabolites in rat plasma, such as valine, serine, threonine, ornithine, hydroxyproline, lysine, 2-hydroxybutanoic acid, 3-hydroxybutanoic acid, galactofuranose and inositol were identified and indicated as the potential biomarkers, suggesting that the two "patterns" are involved in dysfunction in oxidative stress, energy metabolism and amino acid metabolism. A metabolomic approach has been explored to find subtypes of rheumatoid arthritis (RA) patients enabling a development towards more personalized medicine [50]. The RA patients were diagnosed according to Chinese medicine theory and divided into 2 groups, the RA Heat and RA Cold group. The result showed that RA patients could be divided in 2 groups according to Chinese medicine theory. Molecular differences between the RA Cold and RA Heat groups were found which suggest differences in apoptotic activity. Subgrouping of patients according to Chinese medicine diagnosis has the potential to provide opportunities for better treatment outcomes by targeting Western or Chinese medicine treatment to specific groups of patients. Additional metabolomic study of a biochemical modification *in vivo* induced by high dose of hydrocortisone, which led to a unique pathologic condition similar to the "kidney deficiency syndromes", an early stage of obesity and diabetes in TCM, could be used as a potentially powerful tool to investigate the biochemical changes of CMS, as an early diagnostic means [51]. Plasma metabolic products in patients of coronary heart disease (CHD) with Xin-blood stasis syndrome (XBSS) or non-XBSS was identified by GC-MS, the changes of their main elements in different groups were analyzed by PCA and partial least squares (PLS) analysis [30]. Occurrence of XBSS was positively correlated with octadecanoic acid, arachidonic acid, urea, lactic acid and beta-hydroxy, butanoic acid contents, and negatively correlated with oleic acid, L-proline, glycine, and citric acid contents. Discrepancy analysis on changeful metabolites showed that the contents of arachidonic acid, octadecanoic acid, lactic acid, urea, beta-hydroxy butanoic acid and oleic acid increased in CHD patients, especially in those with XBSS ($P < 0.01$). Analyzed from plasmic metabolite spectrum view, CHD-XBSS is related with glyco-metabolism and lipid metabolism, also with the stress induced by hypoxia and agonism. Luo et al. [52] had determined the changes of the plasma metabolic phenotype in rats with chronic restraint stress (rats with syndrome of liver qi stagnation and spleen deficiency). NMR spectra of metabolites in the rat blood were differentially changed after chronic stress. Specific, characteristic metabolic products can be identified by metabolomics analyses, which lead to interpretation of biological feature of CMS. Discovery of biomarkers from the metabolome of a specific CMS will facilitate the modernized study of TCM system, promote the quantitative and scientific elucidation of TCM, help predict the disease on-set, and along with a better understanding action mechanism of the TCM. Therefore, metabolomic analysis is an approach with good development prospects to studies of CMS.

3. Preventive treatment of TCM

Personalized medicine – defined as customized medical care for each patient's unique condition – in the broader context of personalized health, will make significant strides forward when a systems approach is implemented to achieve the ultimate in disease phenotyping and to create novel therapeutics that address system-wide molecular perturbations caused by disease processes [53]. Metabolomics incorporates the most advanced approaches to molecular phenotype system readout and provides the ideal therapeutic technology platform for the discovery of biomarker patterns associated with healthy and diseased states, for use in personalized health monitoring programs and for the design of individualized interventions. Combination preventive treatment with individualized optimization are likely to become a major focus. TCM is a scientific discipline, which develops the related theories from the long-term clinical practices [54]. Preventive treatment of TCM first recorded in “the yellow emperor's classic of internal medicine” which is a classical Chinese medicine book, a fundamental method of treating disease to take preventive or treatment measures to prevent the development of disease, consists of three meanings: firstly, disease control, stressing regimen and prevention of diseases; secondly, emphasis on early diagnosis and treatment and control the evolution of the disease; thirdly, the prognosis and prevention of disease recurrence. Premedical intervention before diseases is one of the principal practices in TCM for tackling health problems and diseases, especially the subhealth condition following stress-induced pathophysiological situations. Scientific interpretations for various preventive treatments in TCM present the idea that disease prevention should surpass medical intervention in humans [55]. Metabolomics has the potential to enable mapping of early biochemical changes in disease and hence provides an opportunity to develop predictive biomarkers that can trigger earlier interventions. Several studies have shown that early-intervention metabolomics approach might provide deeper insight into the disease-preventive mechanisms in clinical trials [15,56,57]. These proof-of-metabolomics studies provided opportunity for preventive treatment of TCM. Elimination of cancer in the 21st century is likely to depend not only on more effective individualized treatment, but also upon earlier detection and prevention of different malignancies [58]. A metabolomics approach was used to identify early intervention, noninvasive biomarkers of alcohol-induced liver disease (ALD) which is a leading cause of nonaccident-related deaths in the world [59]. Although liver damage caused by ALD is reversible when discovered at the earlier stages, current risk assessment tools are relatively nonspecific. Identification of an early specific signature of ALD would aid in therapeutic intervention and recovery. In this study, the metabolic changes associated with ALD were examined using alcohol-fed male Ppara-null mouse as a model of ALD. PCA of MS-based urinary metabolic profile showed that indole-3-lactic acid was exclusively elevated by alcohol exposure in Ppara-null mice. Elevation of indole-3-lactic acid was mechanistically related to the molecular events associated with development of ALD in alcohol-treated Ppara-null mice.

As an early diagnostic means, metabolomic investigation of chronic unpredictable mild stress rats was carried out [60]. Clear separation between the model and control group was achieved, and the level of twelve metabolites, including amino acids, sugar, organic acids and fatty acids, were significantly different between the controls and chronic unpredictable mild stress group. These observations suggested that the depressed state may be associated with perturbation of amino acid metabolism, energy metabolism and glycometabolism. Thus, metabolomics approach could be used as a potential powerful tool to investigate the biochemical change in certain physiopathological conditions, such as depression, as an early diagnostic means. The future of metabolomics rests with its

ability to monitor subtle changes in the metabolome that occur prior to the detection of a gross phenotypic change reflecting disease. The integrated analysis of metabolomics and other “omics” may provide more sensitive ways to detect changes related to disease and discover novel biomarkers. Knowledge regarding these multivariant characteristics is critical for establishing validated and predictive metabolomic models for disease prevention. Understanding the metabolome will not only provide insights into the critical sites of regulation in health promotion, but will also assist in identifying biomarkers for establishing preventative or therapeutic approaches for health. While unraveling the metabolome will not be simple, the societal implications are enormous [61].

4. Metabolomic analysis of Chinese medicine

Chinese medicine could serve as a source of inspiration for drug development. In the recent years, the international community has attached increasing importance to Chinese medicine. Many studies have been implemented to investigate the action mechanisms of Chinese medicine. Among these studies, many prevailing metabolomic-based methods have been implemented to facilitate Chinese medicine investigation. Some favourable outcomes have been gained in the action studies of Chinese medicine, such as the action target organs assay, the establishment of action pattern, the elucidation of action mechanism and the exploration of action material foundation. Using metabolomics in combination with pharmacology is a robust way for the discovery of novel biological active compounds and targets as well as for proving the occurrence of synergy and prodrugs [62]. It is believed that metabolomics will greatly promote Chinese medicine research and be beneficial to the modernization of TCM in terms of extending the application of modern means.

HPLC-MS/MS-based metabolomics method was used to find the possible biomarker of *Rhizoma Coptidis* in rat urine [63]. It demonstrated that the metabolome between treated group and control group had difference in rat urine sample. A large number of metabolites were found including malic acid, oxalacetic acid, 2-ketoglutaric acid, arachidonic acid and other compounds. It was consistent with pharmacological effects of *Rhizoma Coptidis*, such as antiinflammatory, anticephalic nerve and energy metabolism inhibition. Metabolomics NMR-based was an attractive method for non-selective and comprehensive analysis of *Ginkgo* extracts, which were very complex mixtures prepared from raw leaf extracts by a series of extraction and prepurification steps [64]. *Ginkgo biloba* leaves exerted multi-directional lipid-lowering effects on the rat metabolome, including limitation of the absorption of cholesterol, inactivation of HMGCoA and favourable regulation of profiles of essential polyunsaturated fatty acid [65]. A LC/MS based metabolomics approach was also applied to characterize the anti-aging effect of total flavones of *Epimedium*, a traditional Chinese medicine. Result indicated that anti-aging effects of total flavones of *Epimedium* might due to the intervention on lipid metabolism and its property of anti-oxidation. Especially, *Epimedium* flavanoids could reverse this age-dependent change at different levels in a synchronous manner [66,67]. This work suggests metabolomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of TCM.

The molecular compositions of rosemary (*Rosmarinus officinalis* L.) extracts and their dependence on extraction solvents, seasons, and drying processes were systematically characterized using NMR spectroscopy and multivariate data analysis. Results showed that the rosemary metabolome was dominated by 33 metabolites including sugars, amino acids, organic acids, polyphenolic acids, and diterpenes, among which quinate, cis-4-glucosyloxycinnamic acid, and 3,4,5-trimethoxyphenylmethanol were found in rose-

mary for the first time [68]. Metabolite profiling of five medicinal Panax herbs including *Panax ginseng* (Chinese ginseng), *Panax notoginseng* (Sanchi), *Panax japonicus* (Rhizoma Panacis Majoris), *Panax quinquefolium* L. (American ginseng), and *P. ginseng* (Korean ginseng) were performed using UPLC–MS and multivariate statistical analysis technique. PCA of the analytical data showed that the five Panax herbs could be separated into five different groups of phytochemicals. The chemical markers were identified through the loadings plot of PCA, and were identified tentatively by the accurate mass of TOF/MS and partially verified by the available reference standards [69]. Pharmacodynamic effects of the ginsenoside Rg3 on the metabolome in urine of healthy and liver-tumor-bearing rats have been investigated [70]. Seventeen biomarker candidates including three apolar metabolites were detected for global analysis of highly complex biosamples. It may not only increase the number of discovered biomarkers but consequently improve the comprehensive information on metabolic changes. Dried Citrus peels, known as Chenpi in Chinese medicine, are a traditional medicine for the treatment of indigestion and inflammatory syndromes. A targeted metabolomic approach was applied to discriminate Chenpi with different storage periods [71]. It was found that the long-term stored Chenpi had a higher total phenolic content and superior 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity levels compared to the regular stored Chenpi, and that heat treatment increased both total phenolic content and bioactivity. Metabolomics combined with chromatographic fingerprinting seem possible for one to reveal the working mechanism of TCM and to further control their intrinsic quality [72]. For the development of a quality control strategy in TCM, metabolite profiling in combination with multivariate statistics is a sophisticated method for quality assessment of natural products, and provides a base for the establishment of a quality control strategy [73,74].

Concurrently, a number of metabolites involved in glucose metabolism, citric acid cycle and amino acid metabolism were affected immediately after the intake of green tea and the proposed approach provided a more comprehensive picture of the metabolic changes after intake of green tea in human urine [75]. Interestingly, green and black tea intake also had a different impact on endogenous metabolites in urine and plasma. Green tea intake caused a stronger increase in urinary excretion of several citric acid cycle intermediates, which suggests an effect of green tea flavanols on human oxidative energy metabolism and/or biosynthetic pathways [76]. Metabolic changes in Wistar rats caused by Aconitum alkaloids aconitine, mesaconitine, and hypaconitine which are the main components in a commonly used traditional Chinese herbal medicine *Fu Zi* were investigated by means of metabolomic approaches and metabolites with significant changes or with a tendency to change, in the aconitine and mesaconitine groups were dissimilar, suggesting a possible difference in the mechanisms of these alkaloids [77]. The corresponding mechanism of hypaconitine was different from those of aconitine and mesaconitine, based on the differences of perturbed metabolic patterns between groups [78]. Potential biomarkers of the effects of Hei-Shun-Pian, the processed lateral root of *Aconitum carmichaelii* Debx. (*Ranunculaceae*), on the metabolic profile of rats, suggests the effect of Hei-Shun-Pian on rat heart in a dose dependent manner [79]. Metabolic perturbations associated with liver toxicity induced by Huang-yao-zi (*Dioscorea bulbifera* L.) were evaluated using NMR to determine the correlations between metabolomic profiling and histopathologic/biochemical observations and to discover biomarkers for liver toxicity [80]. It revealed that the levels of taurine, creatine, betaine, dimethylglycine, acetate, glycine were elevated, whereas, the levels of succinate, 2-oxoglutarate, citrate, hippurate and urea were reduced. PLS-DA of NMR spectra revealed two apparent clusters between control groups and treatment groups,

indicating metabolic changes observed in response to Huang-yao-zi treatment. Together, these findings clearly demonstrate that metabolomics analysis may be useful for predicting hepatotoxicity induced by Huang-yao-zi. Morning Glory Seed (MGS), a well-known TCM, which was used for the treatment of edema, simple obesity and lung fever. Metabolomic characters of the nephrotoxicity induced-MGS was evaluated [81,82]. It revealed that the levels of eight endogenous metabolites as biomarkers were significantly changed in urine from MGS treated rats. The underlying regulations of MGS-perturbed metabolic pathways, such as lysophosphatidylcholines formation and sphingolipids cycle, were closely associated with the identified metabolites.

Cinnabar, an important traditional Chinese mineral medicine, has been widely used as a Chinese patent medicine ingredient for sedative therapy. However, the pharmaceutical effects of cinnabar, especially in the whole organism, were subjected to few investigations. In Wei's study [83], an NMR-based metabolomics approach has been applied to investigate the pharmaceutical effects of cinnabar after intragastrical administration on male Wistar rats. NMR spectra were analyzed using multivariate pattern recognition techniques to show the time- and dose-dependent biochemical variations induced by cinnabar. The metabolic signature of urinalysis from cinnabar-treated animals exhibited an increase in the levels of creatinine, acetate, acetoacetate, taurine, hippurate and phenylacetyl glycine, together with a decrease in the levels of trimethyl-N-oxide, dimethylglycine and Krebs's cycle intermediates (citrate, 2-oxoglutarate and succinate). The metabolomics analyses of serum showed elevated concentrations of ketone bodies (3-d-hydroxybutyrate and acetoacetate), branched-chain amino acids (valine, leucine and isoleucine), choline and creatine as well as decreased glucose, lipids and lipoproteins from cinnabar-treated animals. It can be concluded that the metabolomics is a potentially powerful tool to explore the therapeutic basis of TCM.

5. Metabolomic dissection of CMF

The complexity of medicine suggests that treatment protocols should be carefully designed, and the construction of a prescription is an art in fighting disease. Increasing evidence demonstrates that, in treating illnesses, treatment regimens containing multiple herbs with distinct but related mechanisms can usually amplify the therapeutic efficacies of each agent, leading to maximal therapeutic efficacy with minimal adverse effects [84,85]. In the past few years, the pharmaceutical industry has seen a shift from the search for "magic bullets" that specifically target a single disease-causing molecule to the pursuit of combination therapies that comprise more than one active ingredient. Interestingly, combinatory therapeutic strategies has been advocated over thousands of years by prescriptions called formula in TCM. Typically, formula consist of several types of medicinal herbs or minerals, in which one represents the principal component, and others serve as adjuvant ones to assist the effects or facilitate the delivery of the principal component. It is believed that multiple components could hit multiple targets and exert synergistic therapeutic efficacies. The therapeutical effect of TCM usually attributed to the synergism mechanism among multiple herbs and constituents, which was named as 'formula compatibility' of TCM [86]. However, the precise mechanisms of formula remain to be addressed by using molecular approaches, thus hampering the modernization of TCM. Studies on formula compatibility/synergism principles are essential, and metabolomics is considered as one of the available and ideal means in this process. The rapid development of metabolomics, especially the advances in the high-throughput and comprehensive research technologies provide new strategies in the analysis of active components of the formula.

The integrative approach of metabolomics is in line with the holistic concept and practices of TCM. The promise of metabolomics, a new “omics” technique, to validate effect of Chinese medicines and the compatibility of Chinese formulas has been appreciated and performed. Wang et al. [87] evaluated metabolomic characters of the hepatotoxicity induced by alcohol and the intervention effects of Yin Chen Hao Tang (YCHT), a classic traditional Chinese medicine formula composed of *Flos Artemisiae*, *Gardeniae Jasminoidis*, *Fructus* and *Radix et Rhizoma Rhei* for treatment of jaundice and liver disorders in China. The greatest difference in metabolic profiling was observed from alcohol-treated rats compared with the control and YCHT-treated rats. The positive ions m/z 664.3126 was elevated in urine of alcohol-treated rats, whereas, ions m/z 155.3547 and 708.2932 were at a lower concentration compared with that in urine of control rats, however, these ions did not indicate a statistical difference between control rats and YCHT-treated rats. The ion m/z 664.3126 was found to correspond to ceramide, providing further support for an involvement of the sphingomyelin signaling pathway in alcohol hepatotoxicity and the intervention effects of YCHT (Fig. 4). Recently, the therapeutic mechanism of LW was undertaken to explore by UPLC/ESI-HDMS and the changes in metabolic profiling were restored to their baseline values after treatment with LW according to the PCA score plots, indicating that treatments have greatly disturbed the normal urine metabolic profiles of rats [43]. There were different phenotypes of metabolites based on HPLC-UV urinary profiling after administration of LW, and those could be conveniently discriminated by PCA. In addition, the results also indicated that LW could restore the metabolite network that disturbed by inflammation, which would be a proof of therapeutic efficacy of LW to inflammation by metabolomics study [88]. Urinary metabolomics Based UPLC-MS was used to evaluate the efficacy and mechanism of Xindi soft capsule, consisting of sea buckthorn flavonoids (e.g. quercetin, kaempferol and isorhamnetin), which is a TCM preparation to blood stasis [89]. With pattern recognition analysis (PCA and PLS-DA) of urinary metabolites, a clear separation of acute blood stasis model group and healthy control group was achieved, the dose groups were located between acute blood stasis model group and healthy control group showing a tendency of recovering to healthy control group, high dose and middle dose were more effective than low dose. Some significantly changed metabolites like cholic acid, phenylalanine and kynurenic acid have been found and identified and used to explain the mechanism. It revealed that the metabolomics method is a valuable tool in the action mechanism of

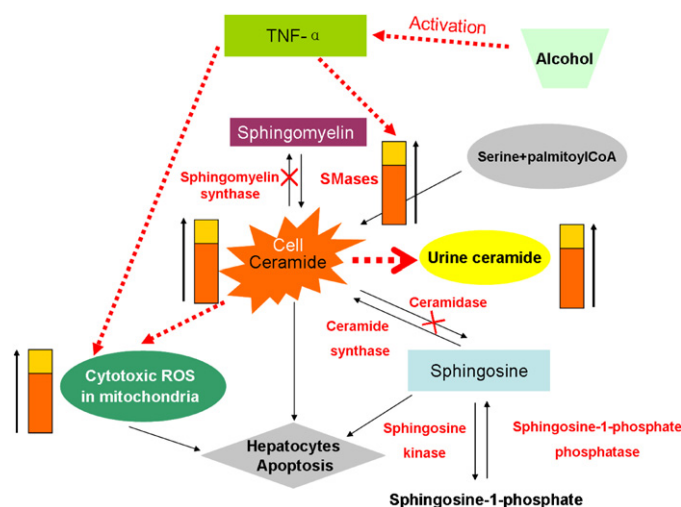


Fig. 4. The proposed metabolic pathway for explanation relationship between alcohol hepatotoxicity and the increased content of ceramide in urine.

Chinese TCM preparation. Compound Danshen Tablets, an herbal (*Salvia miltiorrhiza* Bge.) compound preparation, was presented protective effects on myocardial ischemia by reversing potential biomarkers to sham levels, especially for the four metabolites in the pathway of purine metabolism (hypoxanthine, xanthine, inosine and allantoin) [90]. Based on the symptoms and characteristics of patients and guided by the theories of TCM, formula are designed to contain a combination of different kinds of plants or minerals to improve clinical efficacy. Siwutang, a classic TCM formula, treated cyclophosphamide induced “blood deficiency” model by NMR-based-metabolomics method [46]. When mice were dosed with Siwutang for 7 days, cyclophosphamide caused “blood deficiency” model were reversed in PCA. It could be shown that Siwutang can improve therapeutical and pharmacological effects. Furthermore, the initiatives of metabolomics may pave a new way to explain the formula compatibility and contribute to the establishment of a new technique platform for evaluating the efficacy of TCM formula.

6. Metabolomic evaluation of acupuncture efficacy

Chinese acupuncture handed down from the ancient times, still survives in clinical practice nowadays, and its therapeutic principles innovated in modern clinical practice. At present, acupuncture has attracted extensive attention in the domestic and overseas circles in TCM field. Comprehensive analysis reveals that acupuncture therapy for migraine [91], apoplexy [92], tinnitus [93], stroke [94], etc. has been widely approved in China, and are still having rather strong advantages and characteristics. A report indicated that acupuncture and moxibustion can increase human immunity, reduce urinary protein, improve renal function, antagonize the side-effects of glucocorticoid hormones, etc. Medication combined with acup-moxibustion has the advantages of convenience, lower cost, safety, no adverse effects, etc. [95]. However, some important traditional therapeutic principles have been lost due to technology limitation, hence it is difficult to reflect the features and advantages of acupuncture. Generally, a systemic and multidisciplinary study on this topic is rather deficient up to now, and should be definitely necessary for further improving clinical therapeutic effect in the light of the requirements of evidence-based medicine in further researches. Numerous experimental studies have demonstrated that acupuncture could correct various metabolic disorders such as hyperglycemia, overweight, hyperphagia, hyperlipidemia, inflammation, altered activity of the sympathetic nervous system and insulin signal defect, all of which contribute to the development of insulin resistance [96]. Acupuncture has the potential to improve insulin sensitivity. It revealed the mechanisms responsible for the beneficial effects of acupuncture, though further investigations were warranted. NMR-based metabolomic approach in the study of biological effects of acupuncture, functional dyspepsia patients acupuncture-treated significantly changed the levels of leucine/isoleucine, lactate and glucose, and slightly changed lipids level towards those of the healthy controls, demonstrating its therapeutic effects on the relief of functional dyspepsia symptoms [97].

7. Conclusion and future perspective

In this review, we delineate and discuss metabolomic approaches to resolve special TCM issues. According to the status and problems, it is concluded that TCM modernization has become necessary and urgent. Modernization of TCM means the combination of TCM with modern technology, academic thought and scientific culture, in which the most important point is to elucidate the active component of TCM, especially the material foundation of compound prescriptions and their pharmacodynamic mechanisms. Metabolomics/metabonomics is a post genomic technology which

seeks to provide a comprehensive profile of all the metabolites present in a biological sample. This property agrees with the holistic thinking of TCM, a unique medical system assisting the ancient Chinese in dealing with disease, plays an increasingly important role in the study of TCM and explains the scientific meaning of evidence-based Chinese medicine. Furthermore, the initiatives of metabolomics may pave a new way to elucidate the action of TCM in the light of modern sciences and evaluate the efficacy of TCM and its modernization. Application of combined metabolomics and TCM methodologies will facilitate and deepen the evaluation of the therapeutic effects of formula, intrinsic quality of CMS, possible action mechanism of TCM and Chinese herbal and mineral medicine as well as acupuncture, so as to stride over the gap between the traditional medicine and the future medicine. Much progress is expected in the coming few years.

We urgently desiderate strengthening modernization studies of TCM and believe that the comprehensive metabolomic approach is a potentially powerful tool to explore the therapeutic basis and to clarify the possible action mechanism of TCM. Overall, with the further development of metabolomic analytical techniques, especially multi-analyzed techniques, we expect that metabolomics, as pillars of the bridge between western and Chinese medicine, will greatly promote TCM research and be beneficial to the modernization of TCM and establish the international standards.

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