

Metabolomics Deciphering Therapeutic Effects and Mechanism of Traditional Chinese Medicines

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ABSTRACT

Traditional Chinese medicine (TCM), an ancient medical system, has the advantages of multi-targeting and multi-ingredient preparations, and is different from modern pharmacology. However, because of the complexity of TCM and the limitation of present investigation method, the research for deciphering the scientific basis and systematic features of TCM is difficult to go further. Metabolomics has a significant increased study in recent years and enables mapping of early biochemical changes in disease and hence provides an opportunity to develop predictive biomarkers. Moreover, its method and design resemble those of traditional Chinese medicine. Therefore, it can provide useful tools for exploring essence of TCM, and will help to in-depth understand Chinese medicine syndromes. In this mini-paper, particular attention will be paid to the past successes in applications of robust metabolomics to contribute to small metabolites discovery in TCM development.

Keywords: Metabolomics, Biomarkers, Metabolites, Traditional Chinese medicine, Syndromes

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INTRODUCTION

Traditional Chinese medicine (TCM) is embodiment with holistic, self-contained and personal developed, entrenched from Yin-Yang and Wuxing theory^[1]. It is a complex comprehensive for medical practice, which is a way for people to diagnose, treatment, and prevention the disease's happening for more than 3000 years. Observation and listening, questioning, and pulse analysis are the main steps for comprehensive analysis of clinical information in TCM^[2]. Detecting balance statue between body and mind, which is reflected at all levels. Traditional Chinese culture and philosophical ideologies, expresses gorgeous dialectical thought, maintain human body, spirit and mind obsessed by harmony status, which were all reflected in TCM. Internal imbalances can stem from a variety of factors and lead to a plethora of conditions ranging from short distresses to chronic disease processes^[3]. It not only focs on the disease defined by detailed pathological variations, but also concentrates on the whole functional state of the people. On our modern society, TCM is getting increasingly popular to enhance the health conditions of human beings and prevent diseases, especially reveal great advantages in early combination intervention.

However, many challenges and insufficient scientific study both exist in the system of TCM. Lacking of modern and technologic approaches is the main aspect; which limits the development of TCM in the world. Systems biology, defined by National Institute of General Medical Sciences, is a

discipline of biology, mathematics, engineering, and physical sciences that participates experimental and computational way to researching biological developments in cells, tissues, and organisms^[4]. The systems biology is the computational integration of data created the series of genetics, transcriptomics, proteomic and metabolomics platforms to study the way by different levels of biomolecular organization, which offers new prospects to study the causes of human disease and discovery possible treatments^[5]. Metabolomics can offer an effective way to explain complex sample analysis. It enables people to comprehensive the complexion of TCM, such as Chinese medicines, herbal remedies, and origins. Because of the idea of the metabolomics is to deal with the whole body of detection, identifying, quantitating and cataloguing the markers changing, it is familiar with the conception of the TCM^[6]. Metabolomics is a study of global metabolite profiles among cells, tissues or organisms under diseases processes. Metabolites are the result of the interface of the genomics with its environment. But it's not only the terminal product of gene expression but also a part of the regulatory system in an integrated manner. Metabolomics joining genomics, transcriptomics, and proteomics constitutes the "omics" to understand the global systems biology^[7]. It adopts the "top-down" strategy to research the function of organisms from terminal symptoms of metabolic network and study these metabolites changes of the whole system affected by interventions in holistic context. Metabolomics of medicinal herbal medicine are particularly cherished natural resource for TCM research.

METABOLOMICS TECHNOLOGIES

Metabolomics was developed in the 1970s, it was also called metabolic profile at that time. Nuclear Magnetic Resonance (NMR) spectroscopy, and gas chromatography–mass spectrometry (GC–MS), has been extensively used as methods to detect these low- molecules in biological samples. GC–MS is a typical example to measure the qualitative and quantitative metabolites of biological samples. Meanwhile, rapid improvement technologies was achieved in the field of NMR spectroscopy, which made NMR as a sensitivity analytical tool to detect metabolites in samples of biofluids in non-targeted and no invasion way^[8]. Striking results happened in 1983, Van der Greef et al. used the technology for adopting soft-ionization MS with pattern recognition to identify gender differences in cases of urine metabolic profiling^[9]. From that period, the prominence of suitable data preprocessing was enhanced in the domain of metabolic profile. In the late of 1980s, the NMR-based metabolomics approach was introduced by Nicholson to study urine and blood in biofluid^[10–11].

Because metabolomics is a course to understand all endogenous metabolites present in a given biological system and the comprehensive qualitative and quantitative analysis of a biological sample. These metabolites including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins and carbohydrates, can present clues for people to better understand disease phenotypes^[12]. Analytical techniques are required to advance with super sensitivity, selectivity, accuracy and higher resolution^[13]. This revolution in ion source technologies for MS included ion sources including atmospheric pressure chemical ionization, electrospray ionization, fast atom bombardment, and atmospheric pressure photo-ionization. Combining with these modern instrument analytical technologies, metabolomics will continue to be earlier, less invasive, much more beneficial for increasing the coverage of detected endogenous metabolites. MS instruments are experiencing rapid growth in the field of sensitivity, resolution, accuracy and so on.

Metabolomics offers a method to research metabolomics that reflects the whole processes underlying biological systems. Before mass detection, MS applications in metabolomics use a separation method, such as (liquid chromatography) LC, with high-performance liquid chromatography (HPLC), ultra Performance Liquid Chromatography (UPLC), GC and CE. Mass analyzers were also usually used in metabolomics, including time-of-flight (TOF), quadrupole, ion trap and so on. Furthermore, NMR used in the field of metabolomics is undergoing an extremely increasing after Nicholson and his team expands the conception about metabolomics in 1980s. Many studies have been applied this application in different areas, such as plants, disease diagnostics, drug discovery and environmental science.

APPLICATION OF METABOLOMICS IN TCM

Under the TCM theories, formula consist of various kinds of crude drugs, they interaction to achieve synergistic efficacy.

TCM uses the principle of multiple components, which multiple to get multiple targets, and it has been developed into a whole healthcare system^[14]. Besides, we still lack a comprehensive understanding for the interaction among constituents in the crude drugs, which is the foundational of the complex TCM formulae. Researching pharmacodynamic material basis effect on system biology, and disease diagnosis in TCM is also another important part in holistic body. Metabolomics offers a valuable approach to research TCM in the characterization of the chemical components, the global efficacy for TCM, studying the metabolites behind the Chinese medicine, which is the potential to impact the origin of the disease. It offers a way to understand diversification of TCM, such as Chinese ZHENG, multi-component and multi-targets. Metabolomics is seen as a potential way to revolution TCM research and to in-depth study scientific based herbal medicine. To study the based herbal medicine of TCM is the value of metabolomics, would be explored in the field of TCM in the world.

METABOLOMICS IN QUALITY CONTROL OF TCM

WHO and FDA are widely range of promotion for chromatographic fingerprint technique, which has been used as an accuracy and systemic characterization of compositions, to control quality of herbal medicine or drug. Due to the quality control for TCM is becoming a challenge, which is a limit criterion for TCM development in concentrations of active ingredients in formula and crude drugs^[15]. Because crude drugs with different growing conditions, such as different primary plants, different cultivation environment, various harvest or altitudes, which are important factor for quality control of this ancient medicine^[16]. It is necessary for us to develop an integrated profiling approach to capture the whole spectrum for ingredients and quantitatively determines of key ingredients in TCM in formula and crude drugs. Li and his colleagues to research *Shengmai* injection to develop an integrated profiling method to captures the entire spectrum for ingredients and quantitatively seven key ingredients, which are determines the quality of *Shengmai* injection. Using metabolomics platform to establish a basis on “seven-marker-” quality standard and evaluate different manufactures as well as different batches of this product generated by one manufacturer^[17]. ‘Zangyinchen’ is a TCM, that is a drug for action of hepatic and gallbladder diseases. Fan et al to use a rapid and comprehensive ¹H NMR-based metabolomics way to characterization and comparison of complete metabolites of two species of ‘Zangyinchen’, named *Swertia mussotii* Franch and *Swertia chirayita* Buch. Ham, objective to establish a method for identification and quality control. There are many metabolites identified, including iridoid glycosides, xanthenes, triterpenoids. After statistical analysis and quantitative method, the results show that *S. mussotii* had higher contents of gentiopicrin, isoorientin, glucose, loganic acid, and choline, besides *S. chirayita* exhibited

higher levels of swertiamarin, oleanolic acid, valine, and fatty acids^[18]. Ginseng, including white ginseng and red ginseng, are widely used in the ancient Chinese medicine practice with different purpose. Zhang and her workmates to use ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry-based metabolomics tactic was recognized to assessment the typical chemical components for white ginseng and red ginseng. Through PCA and OPLS-DA methods, they identify these two types of species. Finally, they found ginsenoside Rg3, a nitrogen-containing component and ginsenoside 20(R)-Rh1 were typical components of red ginseng, malonyl ginsenoside Rb1/isomer and malonyl ginsenoside Rg1/isomer were found as characteristic components for white ginseng. It was believed that using the identified characteristic components is a way to identify the consistent quality. It is an effective way to distinguish these two species^[19]. Sukanda Tianniam used metabolite fingerprinting to evaluate the different regions of quality of *Angelica acutiloba* dried roots. Through PCA and PLS-DA, various toki samples were successful discriminated. The metabolomics method would enhance our understanding of the dominant relationship among different cultivation areas^[20]. Wang et al. had used HPLC-DAD fingerprint chromatograph to evaluate the quality of *Phellodendri amurensis* cortex of different 11 batches. The samples were clustered into 4 regions in PCA, which is similar with the result of Hierarchical clustering analysis (HCA)^[21]. Mao and his co-workers to apply metabolomics technology to probe the metabolic profile and representative compounds for distinguishing between leaves of *Panax ginseng* and *Panax quinquefolius*. They rapidly find the potential biomarkers of them. Nine ginsenosides of 86 components identified are regarded as the typical chemical markers between *Panax ginseng* and *Panax quinquefolius*^[22]. *Fang feng*, which is *Radix Saposhnikoviae*, has been widely used to treat pyrexia, rheumatism, headache and convulsion for thousands of years in China. Those researchers used high-performance liquid chromatography-electrospray ionization time-of-flight mass spectrometry and statistical analysis technique to discriminate different geographical locations of *Fang feng*. They identified 10 potential markers as the main difference among diverse sources in TCM. Besides, these markers were quantified could provide a method to optimize the quality control of Chinese medicine^[23]. Using metabolomics technologies, Sorbitol and a glucose/4-aminobutyric acid were chosen as important quantitative biomarkers to discriminate *Angelica* roots from different species and cultivation area^[24]. *Curcuma phaeocaulis*, *C. kwangsiensis* and *C. Wenyujin* were three ecotypes of curcuma species. Xiang and his team used metabolomics technology platform to identity curzerenone, germacrone, curdione and epicurzerenone as chemical marker to discrimination different species of curcuma^[25]. *Flos Lonicerae Japonicae* (FLJ) is a famous ancient drug to remedy fever and inflammation in China for many centuries. A comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF/MS) method was built to quality evaluation

about Non-fumigated processing of FLJ and sulfur-fumigation^[26]. All the studies show that the metabolomics is very powerful tools to establish the quality control of TCM and provides insights into the potential biomarker of typical Chinese medicine.

METABOLOMICS IN TOXICITY OF TCM

TCM also owned adverse drug reactions in clinical, which has been more commonly recognized in recent years. The problems about toxicity of TCM are attained more attention by international community. Through a lot of experimental research and clinical experience, lacking of subtle and trustworthy biomarkers of toxicity about TCM is main barrier^[27]. But metabolomics provide a approach to study *in vivo* metabolic profile, which can offer information about drug toxicity and toxicity attenuation mechanism^[28]. Metabolomics analyses of samples can characterize toxicities in different changing of organism. Detecting compound-specific signal pathway, and discovering the changes in these pathways, we will find the biomarkers of toxicity^[29]. Here we supposed to introduce system-based metabolomics into TCM research, expecting to establish the toxicity of TCM in a better way.

Lu and her workers used urinary metabolomics method to discover the characteristic potential metabolites endogenous biomarkers in rats treated with *Xanthii Fructus*. The drug is usually called “Cang-Erzi” in traditional Chinese Medicine as a treatment for headache, skin itching. But the toxicity of this drug is considered as necessary aspect to be considered. Using Ultra-performance liquid chromatography coupled with quadrupole time-off light mass spectrometry (UPLC-QTOF-MS), these researchers discover the potential biomarker in rats treated with *Xanthii Fructus* by urinary metabolomics. Between control group and high dosage group, there were ten biomarkers as toxicity metabolites by OPLS-DA^[30]. Dong et al. studied the toxicity of Chuanwu, the mother root of *Aconitum carmichaelii* Debx. They revealed toxicity biomarker by rats was administered by CW in different dose for 6 months, at the same time through pattern recognition methods, 17 metabolites with significant changes and 9 pathways were identified and validated^[31]. Furthermore, Wang et al. used metabolomics technologies method to discovery the toxicological risk of mother and lateral root of *Aconitum carmichaelii* Debx, respectively named “Chuanwu” and “Fuzi”. In traditional Chinese medicine theory, Paozhi is a approach to detoxifying measure, which can decompose poisonous Aconitum alkaloids into less or nontoxic condition. At the same time, they studied the metabolomics characters of the crud and its processed by UPLC-HDMS with PCA and OPLS-DA. At last, biomarkers were identified between the crude and processed group^[32]. In the TCM, Rhizoma Glycyrrhizae (Gancao) was used to reduce the toxicity of Fuzi. Sun and his team study the Gancao the constituent of Glycyrrhetic acid (GA) to reduce the toxicity of the Fuzi’s mesaconitine. Fifteen male Wistar were administered mesaconitine, and

administered mesaconitine with one pretreatment with GA, and a control group. Urinary biomarkers were identified between control group and mesaconitine-induced rats, and the metabolic profiles of GA-pretreated rats apparently closer to the control group than mesaconitine group^[33]. *Ban Xia* is from the plant *Pinelliaternata* (Thunb.) Berit, which has therapeutic effects in clinic practice. But its side effects and toxicity cannot be ignored. Zhi-Hao Zhang and his colleagues used metabolomics method to discovery the toxicity of BX and combination with blood biochemistry, histopathological examinations to evaluate the toxicity of BX for rats. There were 9 biomarkers was proved as the potential toxicity biomarkers of BX^[34]. Combined the urinary metabolomics and traditional assays of Kansui, Researchers discovered the toxicity of *Kansui* would perturbation the TCA cycle, increased anaerobic glycolysis, and disturbed amino acids metabolism compared with normal rats^[35]. By RPLC-Q-TOF/MS, researching the biomarkers of CW and prepared CW, there were 22 key biomarkers have been seen as responsible for the reason of mechanism of decreasing toxicity during paozhi process^[36]. Overall, in the aspect of detecting of toxicity and biomarker discovery, these metabolomics researching in the field of TCM and its reduction of toxicity enhance our ability for predictive about drug toxicity.

METABOLOMICS IN EFFECTIVENESS OF TCM

Formula of TCM and single medicine of TCM are designed to improve clinical efficacy, these Chinese medicine are used for different signs and characteristics for patients follow the concepts of TCM. The characteristic of Chinese medicine has multiple components and multiple targets. This characteristic would become a spirit of motivation for drug development. Many researches have been done for investigating the mechanism of the clinical efficacy of TCM. Furthermore, the development of metabolomics may provide a better understanding platform for the mechanism, theory and action of the efficacy TCM^[37-42]. Using the metabolomics combining with pharmacology is a forceful technique to discovery the unique biomarkers, active compounds. Also, provide evidence of disease development, at the same time, to study the mechanism of drug treatment of diseases. Overall, metabolomics with its characteristic of holistic provides the opportunity to detect the essence of TCM.

Shuang-huang-lian injection (SHLI) is an effective drug for the treatment of acute respiratory tract infection, pneumonia, influenza in clinical practice, which is a famous Chinese patent medicine. Gao *et al* adopted UPLC-Q/TOF-MS to discovery the metabolomics profiling of pyrexia rats induced by yeast. At the same time, it discovered that SHLI affected a systemic recovery from the rats with pyrexia-induced metabolic perturbation. In a word, they used metabolomics method to explain the antipyretic mechanisms of this Chinese patent medicine at first, which demonstrated the

necessity of using metabolomics as a powerful way for Chinese patent drug^[43]. Liver diseases are very common in the world. Yinchenhao(YCH, *Artemisia annua*L) is a famous crude Chinese medicine, which has been used in clinical practice for treating liver disease for thousands years in Asia. Sun *et al* used α -naphthylisothiocyanate (ANIT) to induce liver injury of rats, and combined with metabolomics techniques and pattern recognition approaches to identify the liver injury's biomarker and the effective of YCH. Five potential biomarkers has been seen as the effective action by the treatment of YCH. It told us that changed biomarkers and pathways provide significant evidence to understand the Chinese medicine mechanisms and holistic theory^[44]. Systemic lupus erythematosus (SLE) is a type of complex autoimmune disease with many clinical practices. Currently, we lack of an effective method for the treatment of this disease, and the current method frequently have adverse effects^[45]. Chinese medicine, *Jieduquyuzyin* prescription (JP), with *Radix Paeoniae Rubra*, *Radix Rehmanniae*, *Carapax Trionycis*, *etc*, has a great therapeutic effect on SLE, which has fewer side effect than glucocorticoids and immunosuppresses. Rapid resolution liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (RRLC-Q-TOF/MS) was used to test the biomarker of SLE model in serum metabolomics. 13 metabolites were identified and characterized as differential metabolites between SLE group and control group. After treatment with JP, 7 metabolites were effective treatment, which involved the metabolism of unsaturated fatty acid, phospholipid and tryptophan pathway^[46]. Induced by the H1N1 pneumonia caused the wide attention of scientists around the world, Lijuan Chen and his partners provide another thought to treat this disease. A traditional Chinese medicine modified Jiu Wei Qiang Huo decoction (MJWQH) has been proved for good therapeutic effect. These researchers determined determine metabolomics biomarkers in mouse serum with a reverse-phase liquid chromatography with quadrupole time-of-flight mass spectrometry. In mice serum, thirteen biomarkers of H1N1-induced pneumonia were identified. Physiological and biochemical indexes showed that the effectiveness of the MJWQH to this disease. Importantly, this drug reversed the unusual biomarkers to control group from the infection group^[47]. In the traditional Chinese medicine, Huang-Lian-Jie-Du-Decoction (HLJDD) is a famous recipe to treat antipyretic and detoxifying. It shows much therapeutic effect about neuroprotective activities, and can treat about nervous system disease including stroke. However, its mechanism of neuroprotective activities is unclear. Due to this characteristic of comprehensively and holistically increase difficult of understanding the recipe, metabolomics technique and statistical analyses provided a possible way to disclose the mechanism of this medicine. This method explained, in the aspect of membrane and mitochondrial metabolism, HLJDD could remission stroke rats. Furthermore, this decoction shows a effective way to treat ischemic stroke^[48]. Kai-Xin-San (KXS) is an herbal formulae that has been widely used to treat Alzheimer's disease (AD) in China.

A report from Chu *et al*^[49] found that KXS significantly improved the cognitive impairment and reduced A β 1-40 levels, and 36 metabolites could correlate with the regulation effects of KXS treatment, and metabolic profiling of AD were close to normal states via regulating the perturbed pathways after KXS treatment.

METABOLOMIC EVALUATION OF ACUPUNCTURE EFFICACY

Acupuncture is a classical therapy in theory system of TCM over 3000 years. This method used hair-thin metal needles to break the specific acupoint, the skin with specific points, to ameliorate or treat diseases^[50]. Acupuncture is a comprehensive and systemically way to cure disease. But its molecular mechanism is difficult to explain. Metabolomics can offer a global assessment of endogenous metabolites, and close to the happening of physiological of disease state or wellbeing state.

Menopause is a common unavoidable ageing consequence for women. However, both nature and surgical menopauses often bring harmful for women. They often experience loss of bone, depression feeling and more serious involvement some chronic diseases. In clinical practice, hormone replacement therapy is the priority index to treat this type of disease. But its side-effect covers many aspects and increase dangers for breast and ovarian cancer. In TCM, acupuncture is a effective method for women to relieve the painful. Zhang and his team systematically analyzed the two type of method, including acupoint laser-irradiation and catgut-embedding, to ovariectomy-induced rat. In the meanwhile, using NMR and GC-FID/MS methods to discuss the disease causes disorder about lipid peroxidation, glycolysis, TCA cycle, and so on. The experience showed that the acupoint in the method of metabolomic disorder has more effective than HRT with nilestriol, especially on the aspect of alleviating lipid peroxidation, restoring glucose homeostasis^[51]. Zunsanli is a point of "The Stomach Meridian of Foot-Yangming". People often use this acupoint to cure a serious of disease, including stomach ache, abdominal pain and so on. Up to now, we have little understanding about its biological and mechanism. Yan *et al* invited 20 males with average age of 25.4 ± 4.2 years, who experienced acupuncture stimulation for Zunsanli for 30 min, during two weeks every day. The urine and saliva samples were collected at day points. Acupuncture-treated ST-36 will brings perturbation low-weight metabolite. Metabolomics analyze the mechanism of metabolite profile changing and infer the pathways and the biological processes. Urinary metabolomics explained alpha-linolenic acid metabolism, citrate cycle, alanine, aspartate, and glutamate metabolism pathways et al. were severely disconcerted, and there are 53 different biomarkers were recognized^[52]. Saliva metabolomics clarified the four main pathways were main affected, and 26 metabolites were identified. These results may be helpful us to clarify the mechanism of ST-36 in the aspects of urine metabolomics

and saliva metabolomics^[53]. These works shows that the potential of metabolomics in the research of acupuncture.

METABOLOMIC EVALUATION OF ZHENG RESEARCH

TCM ZHENG is a basic theory of TCM. The accuracy of ZHENG differentiation is the basic for TCM treatment. Four diagnostic method of ZHENG is a common practice for doctors in clinical practice. It needs to develop a novel process to study the mechanism of ZHENG, and study the physical function of the whole body. Metabolomics provide an enable way to differential the TCM ZHENG and understand the biological mechanism^[54]. "Neijing" is the first literature to mention the ZHENG of 'Kidney-Yang Deficiency syndrome', which is characteristic as dysfunction of warm and the disorder of modern Nerve Endocrine Immune System. This type of ZHENG causes people to feel aversion to cold, cold limbs, reproductive hypofunction and so on. Rats are injected exogenous glucocorticoid to copy the syndrome have been seen as classical way. And reflect the hypothalamus-hypothalamus -target spool disturbance in the physiological and biochemical aspects. This paper explained the characteristic of the rats' model of 'Kidney-Yang Deficiency syndrome'. The urinary metabolomics, which was detected by UPLC/MS, can explain to the significant difference between control group and model group. 13 metabolites have been identified as significantly changed biomarkers. These biomarkers affected the pathway of energy metabolism, gut microflora. The results are useful us to realize the 'Kidney-Yang Deficiency syndrome', which shows that metabolomics is a valuable method to study the mechanism of TCM's ZHENG theory^[55]. Myocardial ischemia syndrome (heart-qi deficiency, HQD) is a common disease in the world. Deficiency of heart qi flustered, shortness of breath, for the performance of fast or slow, weak pulse. As with deficiency of heart Yang is hand foot cold, chills, pale complexion. In clinical practice, ECG ST-segment and T-wave changing is golden index for myocardial ischemia. Because heart qi deficiency, sometimes there will be a transient features. The existing approach is unsatisfactory for the diagnosis of patients with HQD. Metabolomics techniques will explain the underlying mechanism of HQD. The three essential elements, including UPLC/MS, multivariate analysis and database searching, were used to analyses the global metabolic, seventeen biomarkers were thought as significant biomarkers between control group and HQD group. This research explained HQD perturbed the pathway of glycolysis or gluconeogenesis metabolism, biosynthesis of unsaturated fatty acids metabolism and so on,. These results demonstrated that the underlying of the mechanism of HQD in the aspects of molecule^[56]. In the traditional Chinese medicine, presbycusis has been considered associated with kidney deficiency. Tao Wu and his teams to research the relationship between them based on metabolomics technology and data analysis. By means of pure-tone audiometry and kidney deficiency

symptom scoring table to evaluate auditory function and kidney deficiency. The results tell us the more hearing loss in the elderly, the more kidney deficiency score. The biomarkers were identified between presbycusis patients and the controls. This research explained glutathione metabolism, amino acid metabolism, glucose metabolism, NMDA receptors, and GABA receptors may be the potential mechanism of presbycusis, which also explain the mechanism of presbycusis and kidney deficiency^[57].

CONCLUSION

In conclusion, metabolomics is a significant method to study TCM theory and application. Hence, it provides an opportunity to develop predictive biomarkers. Moreover, its method and design resemble those of traditional Chinese medicine. We believe the comprehensive metabolomics method is a powerful tool to understand the therapeutic basis and to explain the action mechanism of Chinese medicine. Overall, the initiatives of metabolomics may provide a new way to explore the compatibility of the formula and establishment a bridge for evaluating between new techniques platform and TCM. The applications of metabolomics in the ancient medical system will strengthen the thoughtful of the holistic and reconciliation of TCM. It has a global and key role in moving the TCM sciences forward.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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