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Review Article

Metabolomics in the Diagnosis, Pathogenesis, and Treatment of Chronic Liver Diseases Using Traditional Chinese Medicine

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Abstract

Chronic liver disease (CLD) is a major global health challenge, characterized by chronic inflammation that can progress to liver fibrosis, cirrhosis, and ultimately hepatocellular carcinoma. Early identification of biomarkers is crucial for effective intervention. Traditional Chinese medicine (TCM) has shown potential in improving CLD symptoms and protecting the liver, although its mechanisms remain unclear. Metabolomics, the comprehensive study of metabolites, offers a promising approach to understanding CLD pathogenesis and identifying biomarkers. Notably, metabolomics aligns with TCM's holistic approach and may help reveal its therapeutic mechanisms. This review summarizes key metabolites associated with CLD diagnosis and progression and discusses how TCM may modulate metabolic pathways to alleviate CLD symptoms. These insights could lead to improved diagnostic and therapeutic strategies for CLD.

Introduction

Chronic liver disease (CLD) is a leading global health issue, with an annual mortality rate of approximately two million people.[1] It encompasses a variety of liver disorders, including viral hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), autoimmune liver diseases (AILDs), and drug-induced liver injury (DILI).[2] The progressive nature of CLD can lead to liver fibrosis, cirrhosis, and an increased risk of hepatocellular carcinoma (HCC).[3] Early detection of CLD and its progression is critical for improving patient outcomes, with the identification of reliable biomarkers being key to this goal.

Keywords: Metabolomics; Chronic liver disease; Traditional Chinese medicine; Treatment; Diagnostic; Pathogenesis; Viral hepatitis; Alcoholic liver disease; Non-alcoholic fatty liver disease; Autoimmune liver disease; Drug-induced liver injury; Hepatocellular carcinoma.

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Metabolomics, the systematic study of metabolites, has emerged as a powerful tool for understanding the metabolic changes underlying CLD and for discovering potential biomarkers.[4,5] By analyzing alterations in metabolic pathways, metabolomics provides insights into disease mechanisms that are difficult to capture using traditional approaches.[6,7] Additionally, traditional Chinese medicine (TCM) offers a promising therapeutic approach for CLD, particularly in the context of personalized and holistic treatment.[8,9] However, the mechanisms by which TCM exerts its effects remain poorly understood. Integrating metabolomics with TCM may provide a unique perspective on these mechanisms and help guide future therapeutic strategies.

This review will explore the role of metabolomics in CLD, summarize key metabolic alterations associated with the disease, and discuss how TCM may influence metabolic pathways to mitigate liver damage. By integrating these perspectives, we aimed to contribute to a more comprehensive understanding of CLD pathogenesis and inform the development of novel diagnostic and therapeutic approaches.

Overview of metabolomics

Metabolomics is an established discipline in the post-genomic era that integrates genomics, transcriptomics, and proteomics, forming the core of systems biology. It is considered one of the most dynamic fields in life sciences research.[10] The term "metabolomics"

was coined by Nicholson at the University of London in 1999.[11] This field involves the study of metabolic networks within biological systems, focusing on dynamic changes in metabolites in response to stimuli or perturbations, such as specific gene mutations or environmental factors.[12] Metabolomics targets endogenous small molecules with a relative molecular mass smaller than 1,000.[10] Compared to genomics, transcriptomics, and proteomics, the number of metabolites is significantly lower, eliminating the need for whole-genome sequencing or extensive expressed sequence tag analysis.[13] Minor variations in gene and protein expression can be reflected and amplified through metabolites.[13] Analyzing metabolites in biological samples can provide insights into physiological or pathological conditions in the human body.[14] The identification of common metabolites across diverse biological samples enhances the applicability of metabolomics, enabling its use in a wide range of clinical and research contexts.[14]

However, metabolomics research faces several challenges and limitations. The complexity of the metabolome, which involves a vast array of metabolites with different chemical properties, presents significant difficulties in comprehensive analysis. Unlike genomics or proteomics, metabolites are highly dynamic and influenced by factors such as environmental conditions, diet, and disease states.[15] This dynamic nature complicates the establishment of standard profiles or biomarkers across individuals or populations. Moreover, the sensitivity and resolution of analytical instruments are crucial; detecting low-abundance metabolites can be challenging. Issues such as metabolite overlap, isomerism, and ion suppression in mass spectrometry further complicate accurate quantification and identification.[16] Additionally, the complexity of metabolic networks and the lack of comprehensive databases for all potential metabolites in diverse biological contexts complicate data interpretation.[17]

To address these challenges, recent advancements have focused on improving the sensitivity and accuracy of analytical techniques. Enhanced sample preparation protocols, such as solid-phase microextraction and derivatization techniques, improve extraction efficiency and reduce matrix interference.[18] Furthermore, combining analytical techniques, such as gas chromatography-mass spectrometry (GC-MS) with liquid chromatography-mass spectrometry (LC-MS), provides complementary information, enabling more comprehensive profiling of metabolites.[19] Advanced data analysis algorithms, including machine learning and artificial intelligence, have also shown promise in enhancing metabolite identification, quantification, and interpretation of complex datasets.[20]

Common analytical techniques in metabolomics include nuclear magnetic resonance (NMR), GC-MS, and LC-MS.[21] Among these, NMR is one of the earliest and most widely used techniques. It relies on nuclear spin properties, which absorb radiation to generate nuclear energy conversion within an external magnetic field.[22] NMR offers advantages such as minimal sample volume requirements and the ability to analyze samples with little or no complex preprocessing, in contrast to techniques like GC-MS or LC-MS that often require extensive sample preparation such as derivatization or extraction.[23] Additionally, NMR enables the non-invasive acquisition of spectra from biofluids (e.g., blood, urine, or serum), reflecting the metabolic state of the body and making it suitable for *in vivo* analysis without requiring tissue biopsy.[24] Currently, GC-MS and LC-MS are among the most widely used and effective metabolomics techniques.[25] GC-MS is ideal for analyzing small, thermally stable, volatile, and easily vaporized compounds, while LC-MS is suitable for polar compounds with larger molecular masses and lower thermal stability.[26] In clinical research, blood, urine, and tissue samples are commonly used. These samples contain various polar, non-volatile, and non-gaseous substances, making LC-MS a more versatile tool compared to GC-MS.[27] In recent years, as metabolomics research has advanced, its role in diagnosing CLDs and exploring their mechanisms has become increasingly significant.[28,29]

Applications of metabolomics in the diagnosis and mechanism exploration of CLDs

Viral hepatitis

Viral hepatitis, particularly hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, is a leading cause of liver disease in China.[30] With the widespread adoption of metabolomics technologies, the metabolic profiling of viral hepatitis (including HBV and HCV infections) has been extensively studied, uncovering numerous biomarkers associated with disease progression and severity.[31] For example, in a study comparing serum metabolites from patients at different stages of chronic hepatitis B (CHB) and healthy individuals, researchers identified several metabolic disruptions linked to disease progression. Disturbances in ammonia metabolism, glutamine and glutamate metabolism, alanine metabolism, branched-chain amino acid imbalance, and tricarboxylic acid cycle disruption were found to be major contributors. Aspartate, glutamate, glutamine, and alanine fluctuations were particularly characteristic of disease progression.[32] Similarly, a study on serum metabolites in CHB revealed that alanine, malic acid, and 5-methoxytryptamine could effectively differentiate CHB patients from healthy controls. These metabolites are involved in energy metabolism, macromolecule synthesis, and redox maintenance pathways, suggesting their role in the disease.[33] In another investigation, metabolomic analysis of urine samples from CHB patients uncovered significant disruptions in three key metabolic pathways: cytochrome P450 metabolism of xenobiotics, phenylalanine metabolism, and amino sugar and nucleotide sugar metabolism. Key metabolites, including biotin sulfoxide, 5-oxo-eicosanoic acid, D-glucosaminyl glucoside, and 2-methylhypoxanthine, were significantly altered in CHB patients compared to healthy controls.[34] Further supporting these findings, a study of saliva metabolites confirmed differences in various metabolites such as succinic acid, putrescine, acetic acid, tyrosine, and lactic acid in CHB patients compared to healthy individuals. These changes affected nine metabolic pathways, including glycolysis, gluconeogenesis, pyruvate metabolism, and the citric acid cycle, providing a broader understanding of metabolic alterations in CHB.[35]

Metabolomics has also been instrumental in diagnosing and predicting the progression of hepatitis B cirrhosis (HBC). Researchers found that seven omega-6-derived oxidized lipids, such as 9,10-dihydroxyoctadecenoic acid and thromboxane B2, were altered in patients with HBC. These lipids, combined with traditional markers like alpha-fetoprotein, age, and gender, significantly enhanced the prediction accuracy of HBC.[36] Another study showed that six metabolites (α-hydroxyhippuric acid, tyrosyl-β-glucuronide, 3-hydroxyisovaleric acid, uracil riboside, estrone, and glycochenodeoxycholate) were linked to different stages of HBC as classified by the Child-Pugh score, reflecting abnormalities in amino acid, bile acid, and hormone metabolism.[37]

In the case of HCV infection, metabolomics has also provided valuable insights into disease progression and treatment outcomes. For example, choline and histidine levels were found to be higher in patients with late-stage (F2-F4) fibrosis due to chronic HCV

infection, with the choline-to-uric acid ratio being a particularly effective marker for distinguishing disease severity.[38] Metabolic profiling of patients with decompensated cirrhosis from HCV infection revealed increased levels of plasma fatty acids, bile acids, and aromatic amino acids, as well as decreased levels of branched-chain amino acids and tricarboxylic acid cycle metabolites. These findings highlight important metabolic shifts associated with disease progression. Lysophosphatidylcholine, taurocholic acid, and ethyl succinate were identified as key metabolites that accurately differentiate compensated and decompensated stages of HCV-related liver cirrhosis.[39] Additionally, studies on HCV patients undergoing liver transplantation have shown that certain metabolites, such as sphingomyelins and phosphatidylcholines, can predict the progression of fibrosis post-transplant.[40] Furthermore, metabolomics has been shown to predict the virological response to antiviral therapy in HCV-infected patients. High serum tryptophan levels prior to treatment were associated with a better virological response, indicating that metabolomic profiling could help guide treatment decisions.[41]

ALD

ALD is characterized by impaired metabolic processes, and metabolomics has become an invaluable tool for uncovering the metabolic disruptions linked to ethanol consumption.[42] Studies employing this approach have provided deep insights into the molecular changes underlying ALD, particularly in distinguishing the stages of the disease. For example, capillary electrophoresis-mass spectrometry-based metabolomics revealed 19 metabolites associated with alcohol intake, with serine, guanidinosuccinic acid, and glutamine identified as promising biomarkers for early-stage ALD and potential predictors of progression to more severe liver injury, such as alcoholic hepatitis and alcoholic cirrhosis.[43]

Metabolic dysregulation is particularly evident in the caffeine and tryptophan pathways in ALD. Untargeted and targeted metabolomics approaches have demonstrated that specific caffeine metabolites—such as 1-methylxanthine, paraxanthine, and 5-acetylamino-6-amino-3-methyluracil—can distinguish the severity of ALD, particularly in differentiating alcoholic hepatitis from milder liver conditions.[44] In addition, the abundance of 10 tryptophan metabolites was strongly correlated with Model for End-Stage Liver Disease scores, commonly used for assessing liver dysfunction severity in patients with alcoholic cirrhosis.[45] Notably, nine of these metabolites showed significant differences between healthy individuals and ALD patients, with two—quinaldic acid and indole sulfate—specifically linked to advanced stages of ALD, such as cirrhosis and liver failure.[45]

Comprehensive serum metabolite profiling has further illuminated key alterations in ALD. Elevated levels of methylated nucleotides, γ-glutamyl amino acids, bile acids, uracil, and campesterol were observed in patients with alcoholic cirrhosis, while branched-chain amino acids, serotonin, and urate salts were found to be reduced in both alcoholic hepatitis and cirrhosis. These findings suggest that a broad range of metabolites could serve as biomarkers for assessing ALD progression, with 3-ureidopropionic acid, cis-3,3-dimethylglutaryl glycine, retinol, and valine highlighted as particularly promising biomarkers for distinguishing early-stage alcohol-related liver injury from more advanced conditions such as alcoholic cirrhosis.[46]

Another critical metabolic pathway in ALD is bile acid metabolism. Alterations in bile acid profiles have been identified as a key metabolic signature for the onset and progression of ALD. The serum ratio of glycoursodeoxycholic acid to glycochenodeoxycholic acid has been suggested as a non-invasive biomarker for predicting ALD severity, particularly for differentiating alcoholic hepatitis from cirrhosis and potentially predicting the risk of liver failure in patients with advanced disease.[47]

NAFLD

The spectrum of NAFLD ranges from simple non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH) and NAFLD-related cirrhosis.[48] While non-alcoholic fatty liver is generally considered a benign condition that can often be reversed through diet and exercise, it carries the risk of progressing to NASH and eventually cirrhosis, which may lead to HCC.[49] One of the greatest challenges in managing NAFLD is the non-invasive diagnosis of NASH, as no reliable biomarkers currently exist to detect or predict the inflammation characteristic of the disease.[50]

Recent advancements in metabolomics have provided significant insights into NAFLD, offering powerful tools for early detection and risk prediction. For instance, a predictive model called the NASH ClinLipMet score has been developed. This model incorporates a combination of biomarkers, such as aspartate aminotransferase, fasting insulin, the patatin-like phospholipase domain-containing protein-3 genotype, and specific amino acids (e.g., glutamate, glycine, isoleucine), to assess the likelihood of progression to NASH.[51] Another innovative approach, proposed by Mayo *et al*.,[52] uses a diagnostic algorithm based on 20 serum triglycerides to differentiate NAFLD histological phenotypes, from simple hepatic steatosis to advanced NASH.

Alterations in circulating amino acids, such as branched-chain amino acids (leucine, isoleucine, valine) and aromatic amino acids (tryptophan, tyrosine, phenylalanine), are commonly observed in NAFLD patients.[53–55] Research has shown that increased plasma levels of branched-chain amino acids correlate with hepatocyte ballooning and inflammation, while other amino acids like glutamate, serine, and glycine are linked to insulin resistance and can differentiate fibrosis stages in NAFLD.[54]

Metabolomic profiling has also proven useful in distinguishing NAFLD subtypes. For example, Masarone *et al*.[56] employed untargeted plasma metabolomics combined with machine learning to classify biopsy-confirmed NAFLD patients into distinct groups: pure steatosis, NASH, and NAFLD-related cirrhosis. Their model, which included biomarkers like glycine, taurine-conjugated bile acids, phenylalanine, and branched-chain amino acids, demonstrated high accuracy (94.0%), sensitivity (94.1%), and specificity (93.8%) in classifying these subtypes.[56]

Additionally, bile acid profiles are closely associated with the severity of NASH. Significant changes in bile acid composition have been observed, with NASH patients showing elevated levels of primary bile acids and reduced levels of secondary bile acids.[57] These bile acid alterations are not only indicative of disease severity but also linked to specific pathological features. For instance, steatosis is primarily associated with taurine-conjugated bile acids, while lobular inflammation and hepatocyte ballooning are more closely tied to glycine-conjugated bile acids.[57]

AILDs

AILDs, including autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC), are chronic inflammatory conditions that significantly impact liver function.[58] While these diseases are increasingly recognized, their complex etiology remains poorly understood, making diagnosis and treatment particularly challenging. Liver biopsy, although the gold standard for diagnosing AILDs, is invasive, painful, costly,

and prone to sampling errors and interpretational variability.[59]

Metabolomics has gained considerable attention in recent years as a promising tool for identifying non-invasive biomarkers to more accurately diagnose and assess the severity of AILDs. For instance, a study using NMR spectroscopy analyzed plasma samples from patients with AIH, PBC, metabolic dysfunction-associated liver disease, chronic viral hepatitis, and healthy controls.[60] This research identified 15 metabolites that could distinguish AIH from other liver diseases, with AIH patients showing elevated levels of alanine, aspartate, glutamate, choline, betaine, dimethylamine, and several branched-chain amino acids (valine, leucine, isoleucine).[60]

Additionally, a shift in energy metabolism has been suggested in AIH patients. Elevated plasma levels of acetate, lactate, acetone, acetoacetate, and glucose point to the activation of aerobic glycolysis—a metabolic change typically associated with immune activation.[61]

For PBC, a serum metabolomics study identified significant alterations in glucose, fatty acid, and amino acid metabolism.[62] A diagnostic model was developed using metabolites such as 4-hydroxyproline, 3-hydroxyisovalerate, citrate, and ketone esters, achieving excellent diagnostic performance with a 95% confidence interval (0.868–0.976).[62]

Bile acid metabolism has also proven to be a crucial factor in distinguishing different AILDs. Targeted serum bile acid analysis has shown that PBC patients have significantly higher levels of chenodeoxycholic acid, lithocholic acid, and tauroursodeoxycholic acid compared to those with AIH.[63] These bile acids not only help differentiate the two conditions but also correlate with disease severity, as reflected in the Child-Pugh classification.[63]

Furthermore, unique metabolic changes in PSC were observed during liver transplantation studies. In bile from PSC patients, a notable enrichment of dipeptides and polyamines was detected.[64] This finding suggests that such metabolic alterations may play a role in disrupting liver homeostasis and influencing gut microbiota composition, both of which are critical in the pathogenesis of PSC.[64]

DILI

In China, DILI accounts for approximately 10% of hospitalized patients with liver diseases.[65] While mild DILI is primarily characterized by elevated transaminase levels, severe cases can progress to liver failure and even death.[66] The absence of specific and sensitive biomarkers for DILI complicates early diagnosis and intervention, often resulting in poor patient prognosis.[67] Recent studies employing metabolomics have identified serum biomarkers associated with DILI and explored potential indicators for predicting its severity and progression.[68] Notable findings include significantly elevated levels of palmitic acid, taurochenodeoxycholic acid, glycochenodeoxycholate, and taurochenodeoxycholate in the serum of DILI patients compared to healthy controls, alongside a marked reduction in serum lysophosphatidylethanolamine levels.[68] These metabolites, involved in bile acid biosynthesis, alpha-linolenic acid metabolism, and glycerophospholipid metabolism, have emerged as promising clinical biomarkers for DILI.[69] Furthermore, metabolites such as deoxycholic acid, glycochenodeoxycholate, taurochenodeoxycholate, chenodeoxycholate, deoxycholate, and lithocholic acid have been identified as independent risk factors for distinguishing severe DILI from milder forms, suggesting their potential as predictive biomarkers for liver injury severity.[70] Plasma metabolomic and lipidomic profiling in anti-tuberculosis DILI has revealed potential early predictive markers and highlighted the involvement of iron deposition and unsaturated fatty acid biosynthesis pathways in liver injury pathogenesis.[71] In addition, urinary metabolomic analysis of 14 patients with anti-tuberculosis DILI and 30 age- and gender-matched non-DILI controls identified 28 key differential metabolites associated with bile secretion, niacin and niacinamide metabolism, tryptophan metabolism, and other pathways. These metabolites demonstrate potential for predicting DILI risk in patients undergoing anti-tuberculosis treatment.[72]

HCC

HCC often progresses silently, without noticeable symptoms, until it reaches an advanced stage.[73] This delayed onset significantly contributes to its high mortality rate, making early detection, diagnosis, and treatment crucial for improving patient outcomes. Metabolomics has emerged as a powerful tool in oncology, offering the potential to identify metabolic disruptions associated with cancer onset and progression. By detecting these changes early, metabolomics can play a pivotal role in diagnosing and assessing the prognosis of HCC.[74]

In a study of Bangladeshi patients with HBV-related HCC, urine metabolites were analyzed using NMR spectroscopy. Higher levels of carnitine were observed in HCC patients compared to those with chronic HBV infection, cirrhosis, or healthy controls, while metabolites such as creatinine, hippurate, and trimethylamine-N-oxide were significantly lower in the HCC group.[75] These findings suggest that HCC impacts specific metabolic pathways involved in energy production and detoxification. Another study conducted in Egypt explored urine metabolomics in HCC patients, identifying several metabolites that distinguished tumor patients from both healthy controls and cirrhosis patients. Increased concentrations of glycine, trimethylamine-N-oxide, hippurate, citrate, creatinine, and carnitine were observed in the urine of HCC patients.[76] These changes indicate alterations in energy metabolism and may be linked to abnormal chromosomal methylation pathways. A more in-depth analysis of portal vein blood from HCC patients revealed significant alterations in key metabolic processes, including primary bile acid biosynthesis, taurine metabolism, and phenylalanine and tryptophan metabolism. Elevated levels of metabolites such as DL-3-phenyllactic acid, L-tryptophan, glycodeoxycholic acid, and 1-methylnicotinamide were found, correlating with liver dysfunction and poorer survival outcomes.[77] Conversely, lower levels of arachidonic acid and phenol were detected, suggesting a potential protective role against tumor development.[77] A large-scale prospective study analyzed serum samples from over 520,000 participants before HCC diagnosis. The study identified several metabolites positively correlated with HCC risk, including N1-acetylspermidine, indole red, hydroxyphenyllactic acid, and sphingosine. In contrast, metabolites such as retinol, dehydroepiandrosterone sulfate, glycerophosphocholine, and creatine were negatively correlated with HCC risk.[78] These findings highlight the involvement of metabolic pathways related to bile acids, amino acids, phospholipids, and steroids, as well as the influence of environmental and dietary factors in HCC development.

In summary, metabolomics provides a comprehensive understanding of the biochemical alterations in CLDs, enabling the identification of biomarkers with diagnostic and prognostic value. Metabolites directly reflect disruptions in critical biological pathways associated with liver disease. For instance, amino acids such as alanine, glutamine, and branched-chain amino acids indicate hepatocellular function and nitrogen metabolism—processes central to the liver's metabolic roles.[79] Similarly, bile acids provide insights into hepatic synthesis, transport, and detoxification pathways, often impaired during disease progression.[80] Lipid-derived metabo-

lites and fatty acids highlight disruptions in energy storage and membrane dynamics, particularly in cirrhosis and fibrosis.[81] These metabolites serve as indicators of systemic metabolic changes caused by liver dysfunction, making them valuable for non-invasive diagnosis, disease staging, and prognostic assessment. However, inconsistencies in sample collection protocols (e.g., fasting status, storage conditions) and the use of different analytical platforms (e.g., NMR, GC-MS, LC-MS) can lead to variations in metabolite detection. These challenges emphasize the need for standardized methodologies and multi-center collaborations to enhance the reproducibility and comparability of findings. Future research should focus on standardizing methodologies and validating findings across diverse populations to improve clinical applicability. A summary of metabolomics applications in diagnosing CLD and HCC is provided in Table 1.[32-41,43–47,51,52,54,56,57,60–64,68,70–72,75–78]

The application of metabolomics in studying microbial-derived metabolites in CLDs

The human gut microbiota comprises a number of microbial cells approximately equal to the number of human cells in the body. However, the collective genome of the gut microbiota contains 450 times more genes than the human genome.[82] The gut microbiota plays a central role in regulating host metabolism and immune responses by producing metabolites such as short-chain fatty acids, bile acids, and amino acid derivatives.[83–85] These microbial metabolites influence liver function through the gut-liver axis, with metabolomics providing critical insights into their roles in the pathogenesis of CLDs.[86]

Research has demonstrated a clear association between gut dysbiosis and various liver diseases, underscoring the importance of the gut-liver axis.[87] Dysbiosis, characterized by reduced beneficial bacterial populations and altered metabolite profiles, impacts liver health and disease through multiple mechanisms. For instance, secondary bile acids and lipopolysaccharides (pathogen-associated molecular patterns) can activate Toll-like receptors in the liver, triggering pro-inflammatory cytokine release and exacerbating liver damage.[88] Studies on NAFLD have revealed that elevated levels of deoxycholic acid-derived bile acids and diminished *Lactobacilli* populations are linked to advanced disease stages.[89] In experimental models, a high-cholesterol diet induced gut microbiota changes, such as an increase in Proteobacteria and Desulfobulbus, which were associated with elevated taurine-conjugated bile acids and reduced indole derivatives. These changes drove the progression of steatosis, steatohepatitis, fibrosis, and hepatocarcinogenesis.[90]

Specific microbial metabolites have been identified as both biomarkers and therapeutic targets in various liver diseases. For example, reduced *Blautia* abundance and elevated succinate levels are associated with immune globulin G4-related sclerosing cholangitis. Decreased *Ruminococcus* abundance and reduced microbial-derived secondary bile acids are linked to PSC.[91] Alterations in tryptophan and glutamate metabolism pathways are connected to the progression of ALD.[92] In CHB, gut microbiota-derived metabolites such as phenylalanine and tyrosine strongly correlate with liver function markers, providing valuable insights into disease mechanisms.[93] For HCC, metabolites such as acetate, glutamate, and arachidonic acid are associated with early disease recurrence.[94] Microbial species like *Veillonella* and *Gemella*, along with metabolites like indoleacetic acid and taurochenodeoxycholic acid, show promise as non-invasive diagnostic biomarkers for HCC.[95] Additionally, microbial metabolites have been implicated in liver toxicity. For instance, 1-phenyl-1,2-propanedione, a gut-derived metabolite, exacerbates acetaminophen-induced liver injury by depleting hepatic glutathione levels.[96]

In summary, the gut microbiota produces a diverse array of metabolites that play significant roles in liver health and disease. These metabolites—including secondary bile acids, short-chain fatty acids, and indole derivatives—not only influence the gut but also affect liver function via the portal circulation, contributing to CLD progression. The integration of metabolomics and microbiome research has provided valuable insights into these mechanisms, uncovering novel diagnostic and therapeutic opportunities for CLDs. The application of metabolomics in studying microbial-derived metabolites in CLDs is summarized in Table 2.[89–96]

Application of metabolomics in the treatment of CLDs with TCM

TCM is increasingly recognized worldwide for its multi-component formulations targeting various biological pathways, offering novel avenues for drug discovery and supplemental therapies.[97] Metabolomics, a powerful approach analyzing extensive metabolic responses of biological systems to pathological stimuli and therapeutic interventions, provides crucial insights into disease mechanisms by mapping systemic metabolic changes in complex biological matrices.[98] This approach aligns well with the holistic philosophy of TCM, enhancing its potential to assess biological activities, elucidate mechanisms of action, and drive drug development.[99,100]

Recent studies have applied metabolomics to evaluate the efficacy and underlying biochemical mechanisms of TCM in treating CLDs. For instance, plasma metabolomics revealed that *Tripterygium wilfordii* could reverse metabolic disruptions in bile acid and fatty acid metabolism caused by concanavalin A, demonstrating its protective effect against AIH, primarily attributed to its active compound, *Triptolide*.[101] Likewise, *Schisandra chinensis* has shown potential in modulating the arachidonic acid metabolism pathway, offering protective effects against acetaminophen-induced DILI, as revealed by plasma metabolomics studies.[102]

Further research has highlighted the therapeutic potential of other herbs. For example, *Gardenia jasminoides* modulates amino acid metabolism and exerts beneficial effects on ALD through ultra-performance liquid chromatography-mass spectrometry-based metabolomics.[103] Likewise, *Artemisia capillaris* has been shown to influence the tricarboxylic acid cycle, sphingolipid metabolism, and taurine-hypotaurine pathways, contributing to its protective role in alcoholic liver injury.[104] Additionally, studies on *inulin polysaccharides* have demonstrated their capacity to improve dysregulated blood lipid and amino acid metabolism in NAFLD rats, further emphasizing the broad therapeutic potential of TCM for liver diseases.[105] Additionally, metabolomics analyses reveal that *curcumin* extract may exert therapeutic effects on NAFLD by modulating lipid metabolism, particularly pathways involving steroid hormone biosynthesis, glycerophospholipid metabolism, and glycerolipid metabolism.[106] Untargeted metabolomics has also identified the regulation of amino acid metabolism as a potential therapeutic mechanism of *verbascoside* in treating CHB.[107]

In addition to single-ingredient herbs, Chinese herbal compound formulas have proven effective for treating CLDs, especially for complex diseases with multiple symptoms. The increasing application of metabolomics to study these formulas has revealed their multifaceted mechanisms of action. For instance, *Sancao Granule*, composed of *Herba qiancao*, *Herba xianxiancao*, *Radix scutellariae*, *Rhizoma coptidis*, *Forsythiae Fructus*, and *Radix glycyrrhizae*, regulates multiple metabolic pathways, including fatty acid

Table 1. Application of metabolomics in the diagnosis of chronic liver diseases

Disease	Sample	Metabolites	Metabolic pathways	Applications	References
CHB	Serum	Aspartate, glutamate, glutamine, and methionine	Amino acid metabolism	Prognostic assessment	32
CHB	Serum	Alanine, malic acid, and 5-methoxytryptamine	Energy metabolism and redox reactions	Diagnosis	33
CHB	Urine	Biotin sulfoxide, 5-oxodocosanoic acid, D-glucosamine, and 2-methylhippuric acid	Phenylalanine metabolism, amino acid and nucleotide metabolism	Diagnosis	34
CHB	Saliva	Acetic acid, putrescine, acetic acid, succinic acid, tyrosine, lactic acid, butyric acid, pyruvic acid, pyridoxal, and p-hydroxybenzoic acid	Glycolysis and amino acid metabolism	Diagnosis	35
HBC	Serum	9,10-dihydroxyoctadecenoic acid, 14,15-dihydroxyeicosatrienoic acid, 13-hydroxyoctadecadienoic acid, 12-hydroxyeicosatetraenoic acid, 11-HETE, and thromboxane B2	Lipid metabolism	Prognostic assessment	36
HBC	Urine	Alpha-hydroxyhippurate, tyrosine-beta-glucuronide, 3-hydroxyisovalerate, indoleacetic acid succinate, estrone, and glycochenodeoxycholate	Amino acid metabolism, bile acid metabolism, hormone, and intestinal microbiota metabolism	Diagnosis	37
HCV infection	Serum	Choline and histidine	Glutathione metabolism and amino acid metabolism	Diagnosis	38
HCV infection	Plasma	Lysophosphatidylcholine, bovine bile acid, and acetic acid	Fatty acid metabolism, bile acid metabolism, and amino acid metabolism	Diagnosis	39
HCV infection	Serum	Metabolites of two phosphatidylcholines and two phospholipids	Metabolism of sphingomyelin and phosphatidylcholine	Prognostic assessment	40
HCV infection	Serum	Tryptophan	Tryptophan metabolism	Guide treatment decisions	41
ALD	Plasma	Succinic acid, guanidinosuccinic acid, glutamine	Glutamate and glutamine metabolism	Prognostic assessment	43
ALD	Urine	1-methylxanthine, hypoxanthine, and 5-acetamido-6-amino-3-methyluracil	Caffeine metabolism	Diagnosis	44
ALD	Urine	Quinaldic acid, indole-3-carboxylic acid	Tryptophan metabolism	Diagnosis	45
ALD	Serum	3-Ureidopropionate, (3R)-3,3-dimethylglutaryl-glycine, retinol, and methionine	Amino acid metabolism	Diagnosis	46
ALD	Serum	Taurine, glycine-conjugated bile acids	Bile acid metabolism	Prognostic assessment	47
NAFLD	Serum	Glutamate, glycine, lsoleucine, phosphatidylcholine (16:0), and phosphatidylethanolamine (40:6)	Lipid metabolism	Prognostic assessment	51
NAFLD	Serum	Triglycerides	Lipid metabolism	Diagnosis	52
NAFLD	Serum	Glutamate, serine, and glycine	Amino acid metabolism	Diagnosis	54
NAFLD	Plasma	Glycocholic acid, taurocholic acid, phenylalanine, and branched-chain amino acids	Amino acid metabolism and bile acid metabolism	Diagnosis	56
NAFLD	Plasma	Taurocholate and glycocholate	Bile acid metabolism	Prognostic assessment	57
AIH	Plasma	Alanine, aspartate, glutamate, choline, betaine, dimethylamine, valine, leucine, and isoleucine	Amino acid metabolism	Diagnosis	60

(continued)

Table 1. *(continued)*

Disease	Sample	Metabolites	Metabolic pathways	Applications	References
AIH	Plasma	Pyruvate, lactic acid, acetic acid, acetoacetate, and glucose	Aerobic glycolysis	Diagnosis	61
PBC	Serum	4-Hydroxyproline, 3-Hydroxyisobutyrate, citrate, and acetoacetate	Glucose metabolism, fatty acid metabolism, and amino acid metabolism	Diagnosis	62
PBC	Serum	Goose deoxycholic acid, lithocholic acid, and taurine-conjugated bile acid	Bile acid metabolism	Prognostic assessment	63
PSC	Bile	Dipeptides and polyamine metabolites	Bile acid metabolism and lipid metabolism	Diagnosis	64
DILI	Serum	Cholic acid, deoxycholic acid, and lithocholic acid	Bile acid metabolism	Diagnosis	68
DILI	Serum	Deoxycholic acid, glycoursodeoxycholic acid, and taurodeoxycholic acid	Bile acid metabolism	Prognostic assessment	70
DILI	Plasma	Niacin, glycyl-L-leucine, kudzu alkaloid, and 18-β-glycyrrhetinic acid, among 22 metabolites	Iron deficiency, and unsaturated fatty acid biosynthesis	Predict disease onset	71
DILI	Urine	Choline, kudzu alkaloid, N-acetylputrescine, uric acid, among 28 metabolites	Bile secretion, niacin and niacinamide metabolism, tryptophan metabolism	Predict disease onset	72
HCC	Urine	Carnitine, creatinine, urate salts, and trimethylamine-N-oxide	Choline metabolism and intestinal microbiota metabolism	Diagnosis	75
HCC	Urine	Glycine, trimethylamine-N-oxide, urate salts, citrate salts, creatinine, creatine, and carnitine	Energy metabolism and aberrant chromosomal methylation metabolism	Diagnosis	76
HCC	Serum	DL-3-phenyllactic acid, L-tryptophan, glycoursodeoxycholic acid and 1-methylnicotinamide, linoleic acid, and phenol	Amino acid metabolism and bile acid metabolism	Prognostic assessment	77
HCC	Serum	N1-acetylputrescine, indigo, hydroxyphenyllactic acid, tyrosine, sphingosine, leucylprolyl, glycoursodeoxycholate, glycodeoxycholate, 7-methylxanthine, retinol, dehydroepiandrosterone sulfate, glycerophosphocholine, gamma-carboxyethyl hydroxybenzopyran, and creatine	Bile acid metabolism, amino acid metabolism, phospholipid metabolism, and steroid metabolism	Prognostic assessment	78

AIH, autoimmune hepatitis; ALD, Alcoholic liver disease; CHB, chronic hepatitis B; DILI, drug-induced liver injury; HBC, hepatitis B cirrhosis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.

biosynthesis and arachidonic acid metabolism. This comprehensive metabolic modulation significantly improved concanavalin A-induced AIH in mice by reducing serum biochemical markers, alleviating histological damage, and inhibiting neutrophil infiltration in liver tissue.[108]

Similarly, *Dachaihu Formula* has shown promise in improving metabolic disturbances in NAFLD by regulating pathways related to glycine/serine/threonine metabolism, glucose-6-phosphate metabolism, and arachidonic acid metabolism.[109] Another study demonstrated that *Shenling Baizhu Formula*, a combination of herbs including *Lotus plumule* and *Coix seed*, modulates sphingolipid metabolism, providing a potential therapeutic strategy for improving liver lipid profiles in NAFLD rats.[110]

Other compound formulas, such as *Longchai Formula*, regulate phospholipid metabolism, contributing to liver cell repair and energy metabolism, offering potential treatments for CHB.[111] *Jiawei Xiaoyao Formula* has also been linked to intervention in HCC in rats by modulating key metabolic pathways such as bile acid biosynthesis and cholesterol metabolism, providing a promising avenue for addressing liver-qi stagnation and spleen deficiency.[112]

The cumulative application of metabolomics techniques in TCM treatments for CLDs underscores the valuable role of integrative, multi-target therapeutic strategies in modern medicine. The applications of metabolomics in TCM treatments for CLDs are summarized in Table 3.[101–112]

Limitations

Despite its significant promise, the application of metabolomics in the study of CLDs faces several limitations. A primary challenge lies in the variability of sample populations across different geographic regions, introducing confounding factors such as genetic, environmental, and lifestyle differences that may compromise the generalizability of results.[113] Additionally, discrepancies in the

Table 2. The application of metabolomics in microbial-derived metabolites of chronic liver disease

Disease	Microbiota	Metabolites	Metabolic pathways	Applications	References
NAFLD	*Lachnospiraceae and lactobacillaceae*	Deoxycholic acid	Bile acid metabolism	Diagnosis	89
NAFLD	*Genus Helicobacter, genus desulfurobacterium, genus anaerobiospirillum, family desulfurobacteriaceae*	Tauroursodeoxycholic acid and 3-indolepropionic acid	Cholesterol metabolism and bile acid metabolism	Prognostic assessment	90
IgG4-related sclerosing cholangitis	*Brucella bacterium*	Succinic acid	Succinic acid metabolism	Diagnosis	91
PSC	*Lactobacillus genus*	Secondary bile acids	Bile acid metabolism	Diagnosis	91
ALD	*Pseudomonas and agrobacterium*	Short-chain fatty acids, bile acids, indole compounds	Linoleic acid metabolism, histidine metabolism, fatty acid degradation, and glutamate metabolism	Prognostic assessment	92
CHB	*Streptococcus, vibrio, and haemophilus*	Phenylalanine and tyrosine	Amino acid metabolism	Diagnosis	93
HCC	*Vibrio, streptococcus pneumoniae, and bifidobacterium species*	Twenty-three metabolites including acetic acid, glutamate, and arachidonic acid	Lipid metabolism, glucose metabolism, and amino acid metabolism	Prognostic assessment	94
HCC	*Enterobacter cloacae and Lactococcus lactis*	Waardenburg, taurocholic deoxycholic acid, glycocholic deoxycholic acid, theophylline, and xanthine	Bile acid metabolism and purine metabolism	Diagnosis	95
DILI	*Brewer's yeast*	1-Phenyl-1,2-propanedione	Glutathione metabolism	Diagnosis	96

ALD, alcoholic liver disease; CHB, chronic hepatitis B; DILI, drug-induced liver injury; HCC, hepatocellular carcinoma; IgG4, immune globulin G4; NAFLD, non-alcoholic fatty liver disease; PSC, primary sclerosing cholangitis.

Table 3. Application of metabolomics in traditional Chinese medicine treatment of chronic liver diseases

Drug	Disease	Sample	Metabolomics platform	Metabolic pathways	References
Chinese herbal monomers					
Tripterygium wilfordii and triptolide	AIH	Plasma	UPLC-Q-TOF-MS	Bile acid metabolism and fatty acid metabolism	101
Schisandra	DILI	Plasma	UPLC-Q-TOF-MS	Arachidonic acid metabolism	102
Gardenoside	ALD	Serum	UPLC-Q-TOF-MS	Amino acid metabolism	103
Wormwood	ALD	Serum	UPLC-Q-TOF-MS	Tricarboxylic acid cycle, sphingolipid metabolism, and bile acid metabolism	104
Inulin	NAFLD	Serum	GC-MS	Lipid metabolism and amino acid metabolism	105
Turmeric extract	NAFLD	Serum	UPLC-Q-TOF-MS	Fatty acid metabolism	106
Syringin	CHB	Serum		Amino acid metabolism	107
Traditional Chinese Medicine formula			UPLC-MS		
Sancao Granules	AIH	Serum	UPLC-Q-TOF-MS	Fatty acid metabolism	108
Dachaihu Formula	NAFLD	Serum	UPLC-Q-TOF-MS	Fatty acid metabolism and amino acid metabolism	109
Shenling Bai zhu formula	NAFLD	Serum	UPLC-MS	Sphingolipid metabolism	110
Longchai formula	CHB	Serum	UPLC-Q-TOF-MS	Phospholipid metabolism	111
Jiawei Xiaoyao formula	HCC	Plasma	ESI-QTRAP-MS/MS	Bile acid metabolism, phenylalanine metabolism, and cholesterol metabolism	112

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; CHB, chronic hepatitis B; DILI, drug-induced liver injury; ESI-QTRAP-MS/MS, electrospray Ionization quadrupole linear ion trap tandem mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; UPLC-MS, ultra-performance liquid chromatography-mass spectrometry; UPLC-Q-TOF-MS, ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry.

types of biological samples used, alongside variations in detection platforms and analytical methodologies, often lead to inconsistencies and hinder the reproducibility of findings across studies.[114] Furthermore, most current metabolomics research on CLDs remains non-targeted, focusing predominantly on identifying differential metabolites rather than deeply exploring their biological implications. This approach limits our understanding of the underlying pathophysiological mechanisms of these diseases.[115]

Challenges also arise in applying metabolomics to TCM research. Variations in animal models, the composition of herbal formulas, and differences in processing methods can all influence metabolite profiles and their regulation, complicating the interpretation of experimental results. Quality control of Chinese herbal medicines is critical to ensuring treatment consistency and efficacy, and metabolomics holds promise in identifying factors affecting medicinal quality. However, the inherent chemical complexity of herbal formulas, which often contain numerous bioactive compounds, further complicates their analysis and standardization.

To address these limitations and enhance the utility of metabolomics, future research should focus on several critical areas. First, there is an urgent need to standardize metabolomics platforms, quantification techniques, and sample collection procedures to improve the reproducibility and comparability of results across studies. Expanding sample sizes and ensuring geographic diversity will help mitigate population variability and enhance the generalizability of findings. Second, research should prioritize targeted metabolomics, focusing on specific metabolites and their biological roles, to gain deeper insights into metabolic dysregulation in CLDs and TCM. This approach will help elucidate the mechanisms of action of bioactive compounds in both individual herbs and complex formulations. Third, integration of multi-omics approaches, such as genomics, transcriptomics, proteomics, and network pharmacology, will provide a more comprehensive understanding of the molecular mechanisms underlying CLDs and the pharmacological effects of TCM. This integration will bridge traditional practices with modern molecular biology, strengthening the scientific foundation for TCM research. Finally, metabolomics holds significant potential for quality control in TCM, as it can identify biomarkers for assessing the quality and consistency of medicinal materials, supporting the development of standardized quality assurance systems to ensure the safety and efficacy of herbal treatments.

Conclusions

Metabolomics has proven invaluable in advancing our understanding of CLDs and TCM by providing insights into metabolic changes, disease mechanisms, and therapeutic effects. While challenges such as population variability, methodological inconsistencies, and the complexity of TCM research persist, the potential of metabolomics to revolutionize both clinical and basic research remains substantial. Future investigations should prioritize methodological standardization, expand targeted studies, and integrate multi-omics approaches to further enhance its application and impact in these fields.

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Conflict of interest

The authors declare no conflicts of interest in this work.

Author contributions

Conception and preparation of the manuscript (SMY, YLL, CZ), table making (HCZ), drafting the manuscript via an intense literature survey (HCZ, SCW, JHL, TTH, YQS, LPW), review and editing of the manuscript (JJ), and supervision of the manuscript (XD, RLW). All authors have approved the final version and publication of the manuscript.

References

[1] Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol 2019;70(1):151–171. doi:10.1016/j.jhep.2018.09.014, PMID:30266282.
[2] Kardashian A, Serper M, Terrault N, Nephew LD. Health disparities in chronic liver disease. Hepatology 2023;77(4):1382–1403. doi:10.1002/hep.32743, PMID:35993341.
[3] Ginès P, Castera L, Lammert F, Graupera I, Serra-Burriel M, Allen AM, *et al*. Population screening for liver fibrosis: Toward early diagnosis and intervention for chronic liver diseases. Hepatology 2022;75(1):219–228. doi:10.1002/hep.32163, PMID:34537988.
[4] Sillé F, Hartung T. Metabolomics in Preclinical Drug Safety Assessment: Current Status and Future Trends. Metabolites 2024;14(2):98. doi:10.3390/metabo14020098, PMID:38392990.
[5] Wang R, Li B, Lam SM, Shui G. Integration of lipidomics and metabolomics for in-depth understanding of cellular mechanism and disease progression. J Genet Genomics 2020;47(2):69–83. doi:10.1016/j.jgg.2019.11.009, PMID:32178981.
[6] Alseekh S, Aharoni A, Brotman Y, Contrepois K, D'Auria J, Ewald J, *et al*. Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices. Nat Methods 2021;18(7):747–756. doi:10.1038/s41592-021-01197-1, PMID:34239102.
[7] Jacob M, Lopata AL, Dasouki M, Abdel Rahman AM. Metabolomics toward personalized medicine. Mass Spectrom Rev 2019;38(3):221–238. doi:10.1002/mas.21548, PMID:29073341.
[8] Tang J, Xiong K, Zhang T, Han Han. Application of Metabolomics in Diagnosis and Treatment of Chronic Liver Diseases. Crit Rev Anal Chem 2022;52(5):906–916. doi:10.1080/10408347.2020.1842172, PMID:33146026.
[9] Amathieu R, Triba MN, Goossens C, Bouchemal N, Nahon P, Savarin P, *et al*. Nuclear magnetic resonance based metabolomics and liver diseases: Recent advances and future clinical applications. World J Gastroenterol 2016;22(1):417–426. doi:10.3748/wjg.v22.i1.417, PMID:26755887.
[10] Nicholson JK, Lindon JC. Systems biology: Metabonomics. Nature 2008;455(7216):1054–1056. doi:10.1038/4551054a, PMID:18948945.
[11] Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 1999;29(11):1181–1189. doi:10.1080/004982599238047, PMID:10598751.
[12] Guijas C, Montenegro-Burke JR, Warth B, Spilker ME, Siuzdak G. Metabolomics activity screening for identifying metabolites that modulate phenotype. Nat Biotechnol 2018;36(4):316–320. doi:10.1038/nbt.4101, PMID:29621222.
[13] Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomark-

ers and towards mechanisms. Nat Rev Mol Cell Biol 2016;17(7):451–459. doi:10.1038/nrm.2016.25, PMID:26979502.
[14] Liu R, Bao ZX, Zhao PJ, Li GH. Advances in the Study of Metabolomics and Metabolites in Some Species Interactions. Molecules 2021;26(11):3311. doi:10.3390/molecules26113311, PMID:34072976.
[15] Gomez-Casati DF, Zanor MI, Busi MV. Metabolomics in plants and humans: applications in the prevention and diagnosis of diseases. Biomed Res Int 2013;2013:792527. doi:10.1155/2013/792527, PMID:23986911.
[16] Wang S, Blair IA, Mesaros C. Analytical Methods for Mass Spectrometry-Based Metabolomics Studies. Adv Exp Med Biol 2019;1140:635–647. doi:10.1007/978-3-030-15950-4_38, PMID:31347076.
[17] Kusonmano K, Vongsangnak W, Chumnanpuen P. Informatics for Metabolomics. Adv Exp Med Biol 2016;939:91–115. doi:10.1007/978-981-10-1503-8_5, PMID:27807745.
[18] Bojko B. Solid-phase microextraction: a fit-for-purpose technique in biomedical analysis. Anal Bioanal Chem 2022;414(24):7005–7013. doi:10.1007/s00216-022-04138-9, PMID:35606454.
[19] Fiehn O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. Curr Protoc Mol Biol 2016;114:30.4.1–30.4.32. doi:10.1002/0471142727.mb3004s114, PMID:27038389.
[20] Mittal A, Mohanty SK, Gautam V, Arora S, Saproo S, Gupta R, *et al*. Artificial intelligence uncovers carcinogenic human metabolites. Nat Chem Biol 2022;18(11):1204–1213. doi:10.1038/s41589-022-01110-7, PMID:35953549.
[21] Jeppesen MJ, Powers R. Multiplatform untargeted metabolomics. Magn Reson Chem 2023;61(12):628–653. doi:10.1002/mrc.5350, PMID:37005774.
[22] Peng Y, Zhang Z, He L, Li C, Liu M. NMR spectroscopy for metabolomics in the living system: recent progress and future challenges. Anal Bioanal Chem 2024;416(9):2319–2334. doi:10.1007/s00216-024-05137-8, PMID:38240793.
[23] Markley JL, Brüschweiler R, Edison AS, Eghbalnia HR, Powers R, Raftery D, *et al*. The future of NMR-based metabolomics. Curr Opin Biotechnol 2017;43:34–40. doi:10.1016/j.copbio.2016.08.001, PMID:27580257.
[24] Crook AA, Powers R. Quantitative NMR-Based Biomedical Metabolomics: Current Status and Applications. Molecules 2020;25(21):5128. doi:10.3390/molecules25215128, PMID:33158172.
[25] Segers K, Declerck S, Mangelings D, Heyden YV, Eeckhaut AV. Analytical techniques for metabolomic studies: a review. Bioanalysis 2019;11(24):2297–2318. doi:10.4155/bio-2019-0014, PMID:31845604.
[26] Stettin D, Poulin RX, Pohnert G. Metabolomics Benefits from Orbitrap GC-MS-Comparison of Low- and High-Resolution GC-MS. Metabolites 2020;10(4):143. doi:10.3390/metabo10040143, PMID:32260407.
[27] Rontani JF. Use of Gas Chromatography-Mass Spectrometry Techniques (GC-MS, GC-MS/MS and GC-QTOF) for the Characterization of Photooxidation and Autoxidation Products of Lipids of Autotrophic Organisms in Environmental Samples. Molecules 2022;27(5):1629. doi:10.3390/molecules27051629, PMID:35268730.
[28] Lin X, Gao J, Zhou L, Yin P, Xu G. A modified k-TSP algorithm and its application in LC-MS-based metabolomics study of hepatocellular carcinoma and chronic liver diseases. J Chromatogr B Analyt Technol Biomed Life Sci 2014;966:100–108. doi:10.1016/j.jchromb.2014.05.044, PMID:24939728.
[29] Fang ZZ, Gonzalez FJ. LC-MS-based metabolomics: an update. Arch Toxicol 2014;88(8):1491–1502. doi:10.1007/s00204-014-1234-6, PMID:24710571.
[30] Yue T, Zhang Q, Cai T, Xu M, Zhu H, Pourkarim MR, *et al*. Trends in the disease burden of HBV and HCV infection in China from 1990-2019. Int J Infect Dis 2022;122:476–485. doi:10.1016/j.ijid.2022.06.017, PMID:35724827.
[31] Yang J, Wang D, Li Y, Wang H, Hu Q, Wang Y. Metabolomics in viral hepatitis: advances and review. Front Cell Infect Microbiol 2023;13:1189417. doi:10.3389/fcimb.2023.1189417, PMID:37265499.
[32] Nguyen HTT, Wimmer R, Le VQ, Krarup HB. Metabolic fingerprint of progression of chronic hepatitis B: changes in the metabolome and novel diagnostic possibilities. Metabolomics 2021;17(2):16. doi:10.1007/s11306-020-01767-y, PMID:33495863.
[33] Gao R, Cheng J, Fan C, Shi X, Cao Y, Sun B, *et al*. Serum Metabolomics to Identify the Liver Disease-Specific Biomarkers for the Progression of Hepatitis to Hepatocellular Carcinoma. Sci Rep 2015;5:18175. doi:10.1038/srep18175, PMID:26658617.
[34] 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(5):e64381. doi:10.1371/journal.pone.0064381, PMID:23696887.
[35] Gilany K, Mohamadkhani A, Chashmniam S, Shahnazari P, Amini M, Arjmand B, *et al*. Metabolomics analysis of the saliva in patients with chronic hepatitis B using nuclear magnetic resonance: a pilot study. Iran J Basic Med Sci 2019;22(9):1044–1049. doi:10.22038/ijbms.2019.36669.8733, PMID:31807248.
[36] Lu Y, Fang J, Zou L, Cui L, Liang X, Lim SG, *et al*. Omega-6-derived oxylipin changes in serum of patients with hepatitis B virus-related liver diseases. Metabolomics 2018;14(3):26. doi:10.1007/s11306-018-1326-z, PMID:30830341.
[37] 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(7):3838–3847. doi:10.1021/pr300337s, PMID:22624806.
[38] Shanmuganathan M, Sarfaraz MO, Kroezen Z, Philbrick H, Poon R, Don-Wauchope A, *et al*. A Cross-Platform Metabolomics Comparison Identifies Serum Metabolite Signatures of Liver Fibrosis Progression in Chronic Hepatitis C Patients. Front Mol Biosci 2021;8:676349. doi:10.3389/fmolb.2021.676349, PMID:34414211.
[39] Salgüero S, Rojo D, Berenguer J, González-García J, Fernández-Rodríguez A, Brochado-Kith O, *et al*. Plasma metabolomic fingerprint of advanced cirrhosis stages among HIV/HCV-coinfected and HCV-monoinfected patients. Liver Int 2020;40(9):2215–2227. doi:10.1111/liv.14580, PMID:32593189.
[40] Cano A, Mariño Z, Millet O, Martínez-Arranz I, Navasa M, Falcón-Pérez JM, *et al*. A Metabolomics Signature Linked To Liver Fibrosis In The Serum Of Transplanted Hepatitis C Patients. Sci Rep 2017;7(1):10497. doi:10.1038/s41598-017-10807-y, PMID:28874799.
[41] Saito T, Sugimoto M, Igarashi K, Saito K, Shao L, Katsumi T, *et al*. Dynamics of serum metabolites in patients with chronic hepatitis C receiving pegylated interferon plus ribavirin: a metabolomics analysis. Metabolism 2013;62(11):1577–1586. doi:10.1016/j.metabol.2013.07.002, PMID:23953890.
[42] Fahoum K, Ying X, Magahis PT, Ross J, Basu E, Shen NT, *et al*. Non-invasive markers of inflammation in alcohol-associated liver disease: A scoping review. J Gastroenterol Hepatol 2024;39(2):245–255. doi:10.1111/jgh.16432, PMID:38054575.
[43] Harada S, Takebayashi T, Kurihara A, Akiyama M, Suzuki A, Hatakeyama Y, *et al*. Metabolomic profiling reveals novel biomarkers of alcohol intake and alcohol-induced liver injury in community-dwelling men. Environ Health Prev Med 2016;21(1):18–26. doi:10.1007/s12199-015-0494-y, PMID:26459263.
[44] Xu R, He L, Vatsalya V, Ma X, Kim S, Mueller EG, *et al*. Metabolomics analysis of urine from patients with alcohol-associated liver disease reveals dysregulated caffeine metabolism. Am J Physiol Gastrointest Liver Physiol 2023;324(2):G142–G154. doi:10.1152/ajpgi.00228.2022, PMID:36513601.
[45] Xu R, Vatsalya V, He L, Ma X, Feng W, McClain CJ, *et al*. Altered urinary tryptophan metabolites in alcohol-associated liver disease. Alcohol Clin Exp Res (Hoboken) 2023;47(9):1665–1676. doi:10.1111/acer.15148, PMID:37431708.
[46] Calzadilla N, Zilberstein N, Hanscom M, Al Rashdan HT, Chacra W, Gill RK, *et al*. Serum metabolomic analysis in cirrhotic alcohol-associated liver disease patients identified differentially altered microbial metabolites and novel potential biomarkers for disease severity. Dig Liver Dis 2024;56(6):923–931. doi:10.1016/j.dld.2023.10.006, PMID:37923598.
[47] Yang Z, Kusumanchi P, Ross RA, Heathers L, Chandler K, Oshodi A, *et al*. Serum Metabolomic Profiling Identifies Key Metabolic Signatures Associated With Pathogenesis of Alcoholic Liver Disease in Humans. Hepatol Commun 2019;3(4):542–557. doi:10.1002/hep4.1322, PMID:30976744.
[48] Clayton-Chubb D, Kemp W, Majeed A, Lubel JS, Hodge A, Roberts SK. Understanding NAFLD: From Case Identification to Interventions, Outcomes, and Future Perspectives. Nutrients 2023;15(3):687.

doi:10.3390/nu15030687, PMID:36771394.
[49] Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021;18(4):223–238. doi:10.1038/s41575-020-00381-6, PMID:33349658.
[50] Papatheodoridi M, Cholongitas E. Diagnosis of Non-alcoholic Fatty Liver Disease (NAFLD): Current Concepts. Curr Pharm Des 2018;24(38):4574–4586. doi:10.2174/1381612825666190117102111, PMID:30652642.
[51] Zhou Y, Orešič M, Leivonen M, Gopalacharyulu P, Hyysalo J, Arola J, *et al*. Noninvasive Detection of Nonalcoholic Steatohepatitis Using Clinical Markers and Circulating Levels of Lipids and Metabolites. Clin Gastroenterol Hepatol 2016;14(10):1463–1472.e6. doi:10.1016/j.cgh.2016.05.046, PMID:27317851.
[52] Mayo R, Crespo J, Martínez-Arranz I, Banales JM, Arias M, Mincholé I, *et al*. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. Hepatol Commun 2018;2(7):807–820. doi:10.1002/hep4.1188, PMID:30027139.
[53] Yamakado M, Tanaka T, Nagao K, Imaizumi A, Komatsu M, Daimon T, *et al*. Plasma amino acid profile associated with fatty liver disease and co-occurrence of metabolic risk factors. Sci Rep 2017;7(1):14485. doi:10.1038/s41598-017-14974-w, PMID:29101348.
[54] Gaggini M, Carli F, Rosso C, Buzzigoli E, Marietti M, Della Latta V, *et al*. Altered amino acid concentrations in NAFLD: Impact of obesity and insulin resistance. Hepatology 2018;67(1):145–158. doi:10.1002/hep.29465, PMID:28802074.
[55] Hasegawa T, Iino C, Endo T, Mikami K, Kimura M, Sawada N, *et al*. Changed Amino Acids in NAFLD and Liver Fibrosis: A Large Cross-Sectional Study without Influence of Insulin Resistance. Nutrients 2020;12(5):1450. doi:10.3390/nu12051450, PMID:32429590.
[56] Masarone M, Troisi J, Aglitti A, Torre P, Colucci A, Dallio M, *et al*. Untargeted metabolomics as a diagnostic tool in NAFLD: discrimination of steatosis, steatohepatitis and cirrhosis. Metabolomics 2021;17(2):12. doi:10.1007/s11306-020-01756-1, PMID:33458794.
[57] Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, *et al*. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. Hepatology 2018;67(2):534–548. doi:10.1002/hep.29359, PMID:28696585.
[58] Arndtz K, Hirschfield GM. The Pathogenesis of Autoimmune Liver Disease. Dig Dis 2016;34(4):327–333. doi:10.1159/000444471, PMID:27170385.
[59] Carbone M, Neuberger JM. Autoimmune liver disease, autoimmunity and liver transplantation. J Hepatol 2014;60(1):210–223. doi:10.1016/j.jhep.2013.09.020, PMID:24084655.
[60] Dimou A, Zachou K, Kostara C, Azariadis K, Giannoulis G, Lyberopoulou A, *et al*. NMR-based metabolomic signature: An important tool for the diagnosis and study of pathogenesis of autoimmune hepatitis. Hepatology 2024;80(2):266–277. doi:10.1097/HEP.0000000000000767, PMID:38305739.
[61] Wang JB, Pu SB, Sun Y, Li ZF, Niu M, Yan XZ, *et al*. Metabolomic Profiling of Autoimmune Hepatitis: The Diagnostic Utility of Nuclear Magnetic Resonance Spectroscopy. J Proteome Res 2014;13(8):3792–3801. doi:10.1021/pr500462f, PMID:24940827.
[62] Hao J, Yang T, Zhou Y, Gao GY, Xing F, Peng Y, *et al*. Serum Metabolomics Analysis Reveals a Distinct Metabolic Profile of Patients with Primary Biliary Cholangitis. Sci Rep 2017;7(1):784. doi:10.1038/s41598-017-00944-9, PMID:28400566.
[63] Ma ZH, Wang XM, Wu RH, Hao DL, Sun LC, Li P, *et al*. Serum metabolic profiling of targeted bile acids reveals potentially novel biomarkers for primary biliary cholangitis and autoimmune hepatitis. World J Gastroenterol 2022;28(39):5764–5783. doi:10.3748/wjg.v28.i39.5764, PMID:36338890.
[64] Tietz-Bogert PS, Kim M, Cheung A, Tabibian JH, Heimbach JK, Rosen CB, *et al*. Metabolomic Profiling of Portal Blood and Bile Reveals Metabolic Signatures of Primary Sclerosing Cholangitis. Int J Mol Sci 2018;19(10):3188. doi:10.3390/ijms19103188, PMID:30332763.
[65] Li X, Tang J, Mao Y. Incidence and risk factors of drug-induced liver injury. Liver Int 2022;42(9):1999–2014. doi:10.1111/liv.15262, PMID:35353431.
[66] Leise MD, Poterucha JJ, Talwalkar JA. Drug-induced liver injury. Mayo Clin Proc 2014;89(1):95–106. doi:10.1016/j.mayocp.2013.09.016, PMID:24388027.
[67] Fontana RJ. Pathogenesis of idiosyncratic drug-induced liver injury and clinical perspectives. Gastroenterology 2014;146(4):914–928. doi:10.1053/j.gastro.2013.12.032, PMID:24389305.
[68] Ma Z, Wang X, Yin P, Wu R, Zhou L, Xu G, *et al*. Serum metabolome and targeted bile acid profiling reveals potential novel biomarkers for drug-induced liver injury. Medicine (Baltimore) 2019;98(31):e16717. doi:10.1097/MD.0000000000016717, PMID:31374067.
[69] Xie Z, Chen E, Ouyang X, Xu X, Ma S, Ji F, *et al*. Metabolomics and Cytokine Analysis for Identification of Severe Drug-Induced Liver Injury. J Proteome Res 2019;18(6):2514–2524. doi:10.1021/acs.jproteome.9b00047, PMID:31002254.
[70] Xie Z, Zhang L, Chen E, Lu J, Xiao L, Liu Q, *et al*. Targeted Metabolomics Analysis of Bile Acids in Patients with Idiosyncratic Drug-Induced Liver Injury. Metabolites 2021;11(12):852. doi:10.3390/metabo11120852, PMID:34940610.
[71] Wang MG, Wu SQ, Zhang MM, He JQ. Plasma metabolomic and lipidomic alterations associated with anti-tuberculosis drug-induced liver injury. Front Pharmacol 2022;13:1044808. doi:10.3389/fphar.2022.1044808, PMID:36386176.
[72] Wang MG, Wu SQ, Zhang MM, He JQ. Urine metabolomics and microbiome analyses reveal the mechanism of anti-tuberculosis drug-induced liver injury, as assessed for causality using the updated RUCAM: A prospective study. Front Immunol 2022;13:1002126. doi:10.3389/fimmu.2022.1002126, PMID:36483548.
[73] Chan YT, Zhang C, Wu J, Lu P, Xu L, Yuan H, *et al*. Biomarkers for diagnosis and therapeutic options in hepatocellular carcinoma. Mol Cancer 2024;23(1):189. doi:10.1186/s12943-024-02101-z, PMID:39242496.
[74] Lee CW, Yu MC, Lin G, Chiu JC, Chiang MH, Sung CM, *et al*. Serum metabolites may be useful markers to assess vascular invasion and identify normal alpha-fetoprotein in hepatocellular carcinoma undergoing liver resection: a pilot study. World J Surg Oncol 2020;18(1):121. doi:10.1186/s12957-020-01885-w, PMID:32493393.
[75] Cox IJ, Aliev AE, Crossey MM, Dawood M, Al-Mahtab M, Akbar SM, *et al*. Urinary nuclear magnetic resonance spectroscopy of a Bangladeshi cohort with hepatitis-B hepatocellular carcinoma: A biomarker corroboration study. World J Gastroenterol 2016;22(16):4191–4200. doi:10.3748/wjg.v22.i16.4191, PMID:27122669.
[76] Shariff MI, Gomaa AI, Cox IJ, Patel M, Williams HR, Crossey MM, *et al*. Urinary metabolic biomarkers of hepatocellular carcinoma in an Egyptian population: a validation study. J Proteome Res 2011;10(4):1828–1836. doi:10.1021/pr101096f, PMID:21275434.
[77] Liu J, Geng W, Sun H, Liu C, Huang F, Cao J, *et al*. Integrative metabolomic characterisation identifies altered portal vein serum metabolome contributing to human hepatocellular carcinoma. Gut 2022;71(6):1203–1213. doi:10.1136/gutjnl-2021-325189, PMID:34344785.
[78] Stepien M, Keski-Rahkonen P, Kiss A, Robinot N, Duarte-Salles T, Murphy N, *et al*. Metabolic perturbations prior to hepatocellular carcinoma diagnosis: Findings from a prospective observational cohort study. Int J Cancer 2021;148(3):609–625. doi:10.1002/ijc.33236, PMID:32734650.
[79] Paulusma CC, Lamers WH, Broer S, van de Graaf SFJ. Amino acid metabolism, transport and signalling in the liver revisited. Biochem Pharmacol 2022;201:115074. doi:10.1016/j.bcp.2022.115074, PMID:35568239.
[80] Jia W, Li Y, Cheung KCP, Zheng X. Bile acid signaling in the regulation of whole body metabolic and immunological homeostasis. Sci China Life Sci 2024;67(5):865–878. doi:10.1007/s11427-023-2353-0, PMID:37515688.
[81] Badmus OO, Hillhouse SA, Anderson CD, Hinds TD, Stec DE. Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): functional analysis of lipid metabolism pathways. Clin Sci (Lond) 2022;136(18):1347–1366. doi:10.1042/CS20220572, PMID:36148775.
[82] Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, *et al*. An integrated catalog of reference genes in the human gut microbiome. Nat Biotechnol 2014;32(8):834–841. doi:10.1038/nbt.2942, PMID:24997786.
[83] Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet 2012;13(4):260–270. doi:10.1038/

nrg3182, PMID:22411464.
[84] Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148(6):1258–1270. doi:10.1016/j.cell.2012.01.035, PMID:22424233.
[85] Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI. Human nutrition, the gut microbiome and the immune system. Nature 2011;474(7351):327–336. doi:10.1038/nature10213, PMID:21677749.
[86] Abu-Ali GS, Mehta RS, Lloyd-Price J, Mallick H, Branck T, Ivey KL, *et al*. Metatranscriptome of human faecal microbial communities in a cohort of adult men. Nat Microbiol 2018;3(3):356–366. doi:10.1038/s41564-017-0084-4, PMID:29335555.
[87] Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, *et al*. The gut-liver axis and the intersection with the microbiome. Nat Rev Gastroenterol Hepatol 2018;15(7):397–411. doi:10.1038/s41575-018-0011-z, PMID:29748586.
[88] Ohtani N, Hara E. Gut-liver axis-mediated mechanism of liver cancer: A special focus on the role of gut microbiota. Cancer Sci 2021;112(11):4433–4443. doi:10.1111/cas.15142, PMID:34533882.
[89] Smirnova E, Muthiah MD, Narayan N, Siddiqui MS, Puri P, Luketic VA, *et al*. Metabolic reprogramming of the intestinal microbiome with functional bile acid changes underlie the development of NAFLD. Hepatology 2022;76(6):1811–1824. doi:10.1002/hep.32568, PMID:35561146.
[90] Zhang X, Coker OO, Chu ES, Fu K, Lau HCH, Wang YX, *et al*. Dietary cholesterol drives fatty liver-associated liver cancer by modulating gut microbiota and metabolites. Gut 2021;70(4):761–774. doi:10.1136/gutjnl-2019-319664, PMID:32694178.
[91] Liu Q, Li B, Li Y, Wei Y, Huang B, Liang J, *et al*. Altered faecal microbiome and metabolome in IgG4-related sclerosing cholangitis and primary sclerosing cholangitis. Gut 2022;71(5):899–909. doi:10.1136/gutjnl-2020-323565, PMID:34035120.
[92] Ganesan R, Gupta H, Jeong JJ, Sharma SP, Won SM, Oh KK, *et al*. Characteristics of microbiome-derived metabolomics according to the progression of alcoholic liver disease. Hepatol Int 2024;18(2):486–499. doi:10.1007/s12072-023-10518-9, PMID:37000389.
[93] Wang J, Wang Y, Zhang X, Liu J, Zhang Q, Zhao Y, *et al*. Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients. Front Microbiol 2017;8:2222. doi:10.3389/fmicb.2017.02222, PMID:29180991.
[94] Zheng C, Lu F, Chen B, Yang J, Yu H, Wang D, *et al*. Gut microbiome as a biomarker for predicting early recurrence of HBV-related hepatocellular carcinoma. Cancer Sci 2023;114(12):4717–4731. doi:10.1111/cas.15983, PMID:37778742.
[95] Li X, Yi Y, Wu T, Chen N, Gu X, Xiang L, *et al*. Integrated microbiome and metabolome analysis reveals the interaction between intestinal flora and serum metabolites as potential biomarkers in hepatocellular carcinoma patients. Front Cell Infect Microbiol 2023;13:1170748. doi:10.3389/fcimb.2023.1170748, PMID:37260707.
[96] Gong S, Lan T, Zeng L, Luo H, Yang X, Li N, *et al*. Gut microbiota mediates diurnal variation of acetaminophen induced acute liver injury in mice. J Hepatol 2018;69(1):51–59. doi:10.1016/j.jhep.2018.02.024, PMID:29524531.
[97] Zhao CQ, Zhou Y, Ping J, Xu LM. Traditional Chinese medicine for treatment of liver diseases: progress, challenges and opportunities. J Integr Med 2014;12(5):401–408. doi:10.1016/S2095-4964(14)60039-X, PMID:25292339.
[98] Wishart DS. Metabolomics for Investigating Physiological and Pathophysiological Processes. Physiol Rev 2019;99(4):1819–1875. doi:10.1152/physrev.00035.2018, PMID:31434538.
[99] Wang M, Chen L, Liu D, Chen H, Tang DD, Zhao YY. Metabolomics highlights pharmacological bioactivity and biochemical mechanism of traditional Chinese medicine. Chem Biol Interact 2017;273:133–141. doi:10.1016/j.cbi.2017.06.011, PMID:28619388.
[100] Wang P, Wang Q, Yang B, Zhao S, Kuang H. The Progress of Metabolomics Study in Traditional Chinese Medicine Research. Am J Chin Med 2015;43(7):1281–1310. doi:10.1142/S0192415X1550 0731, PMID:26477800.
[101] Zhang T, Rao Q, Dai M, Wu ZE, Zhao Q, Li F. Tripterygium wilfordii protects against an animal model of autoimmune hepatitis. J Ethnopharmacol 2023;309:116365. doi:10.1016/j.jep.2023.116365, PMID:36907478.
[102] Li X, Li M, Deng S, Yu T, Ma Y, Yang H, *et al*. A network pharmacology-integrated metabolomics strategy for clarifying the action mechanisms of Schisandrae Chinensis Fructus for treating drug-induced liver injury by acetaminophen. Bioorg Med Chem 2021;31:115992. doi:10.1016/j.bmc.2020.115992, PMID:33421914.
[103] Qiu S, Zhang AH, Guan Y, Sun H, Zhang TL, Han Y, *et al*. Functional metabolomics using UPLC-Q/TOF-MS combined with ingenuity pathway analysis as a promising strategy for evaluating the efficacy and discovering amino acid metabolism as a potential therapeutic mechanism-related target for geniposide against alcoholic liver disease. RSC Adv 2020;10(5):2677–2690. doi:10.1039/c9ra09305b, PMID:35496090.
[104] Zhang A, Sun H, Wang X. Urinary metabolic profiling of rat models revealed protective function of scoparone against alcohol induced hepatotoxicity. Sci Rep 2014;4:6768. doi:10.1038/srep06768, PMID:25341677.
[105] Zhu H, Wang Z, Wu Y, Jiang H, Zhou F, Xie X, *et al*. Untargeted metabonomics reveals intervention effects of chicory polysaccharide in a rat model of non-alcoholic fatty liver disease. Int J Biol Macromol 2019;128:363–375. doi:10.1016/j.ijbiomac.2019.01.141, PMID:30690116.
[106] Wang Y, Niu M, Jia GL, Li RS, Zhang YM, Zhang CE, *et al*. Untargeted Metabolomics Reveals Intervention Effects of Total Turmeric Extract in a Rat Model of Nonalcoholic Fatty Liver Disease. Evid Based Complement Alternat Med 2016;2016:8495953. doi:10.1155/2016/8495953, PMID:27366193.
[107] Jiang YC, Li YF, Zhou L, Zhang DP. Comparative metabolomics unveils molecular changes and metabolic networks of syringin against hepatitis B mice by untargeted mass spectrometry. RSC Adv 2019;10(1):461–473. doi:10.1039/c9ra06332c, PMID:35492557.
[108] Yang Y, Li F, Wei S, Liu X, Wang Y, Liu H, *et al*. Metabolomics profiling in a mouse model reveals protective effect of Sancao granule on Con A-Induced liver injury. J Ethnopharmacol 2019;238:111838. doi:10.1016/j.jep.2019.111838, PMID:30930257.
[109] Cui H, Li Y, Wang Y, Jin L, Yang L, Wang L, *et al*. Da-Chai-Hu Decoction Ameliorates High Fat Diet-Induced Nonalcoholic Fatty Liver Disease Through Remodeling the Gut Microbiota and Modulating the Serum Metabolism. Front Pharmacol 2020;11:584090. doi:10.3389/fphar.2020.584090, PMID:33328987.
[110] Deng Y, Pan M, Nie H, Zheng C, Tang K, Zhang Y, *et al*. Lipidomic Analysis of the Protective Effects of Shenling Baizhu San on Non-Alcoholic Fatty Liver Disease in Rats. Molecules 2019;24(21):3943. doi:10.3390/molecules24213943, PMID:31683679.
[111] Xu T, Wang P, Zheng X, Yan Z, Li K, Xu J, *et al*. The therapeutic effects and mechanisms of Long Chai Fang on chronic hepatitis B. Ann Transl Med 2021;9(10):865. doi:10.21037/atm-21-1923, PMID:34164499.
[112] Li Z, Zhao Y, Cheng J, Xu L, Wen X, Sun Y, *et al*. Integrated Plasma Metabolomics and Gut Microbiota Analysis: The Intervention Effect of Jiawei Xiaoyao San on Liver Depression and Spleen Deficiency Liver Cancer Rats. Front Pharmacol 2022;13:906256. doi:10.3389/fphar.2022.906256, PMID:35924041.
[113] Cui L, Lu H, Lee YH. Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases. Mass Spectrom Rev 2018;37(6):772–792. doi:10.1002/mas.21562, PMID:29486047.
[114] Azad RK, Shulaev V. Metabolomics technology and bioinformatics for precision medicine. Brief Bioinform 2019;20(6):1957–1971. doi:10.1093/bib/bbx170, PMID:29304189.
[115] Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted Metabolomics Strategies-Challenges and Emerging Directions. J Am Soc Mass Spectrom 2016;27(12):1897–1905. doi:10.1007/s13361-016-1469-y, PMID:27624161.