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# Current State of the Art of High-Throughput Metabolomics

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## 1.1 Introduction

At present, a series of systems biology disciplines, such as genomics, proteomics, transcriptomics, metabolomics, and lipidomics have provided new methods for researchers to explore the pathogenesis of diseases. As an important component of systems biology, metabolomics can discover the metabolite biomarkers and various related metabolic pathways in bio-samples [1]. Metabolomics is usually used with nuclear magnetic resonance (NMR), mass spectrometry (MS), and chromatography, and it plays an important role in clinic, disease treatment, drug metabolism, plant research, agricultural, and nutrition research [2]. Due to the important position of metabolomics in the life science, a large number of domestic and foreign scholars have improved the innovation research of techniques and methods of metabolomics [3]. In this chapter, we show the recent advancements and current application as well as future development of metabolomics.

## 1.2 Metabolomics

In 1999, Dr. Jeremy Nicholson proposed the concept of metabolomics [4]. Metabolomics is a method to quantitatively analyze all the metabolites in bio-sample and to reveal the relationship between metabolites and pathological changes. It mainly focuses on the small-molecule metabolites with relative molecular weight of less than 1000 [5]. Its research process mainly includes sample preparation and analysis, data processing and analysis, and metabolic mechanism analysis. Metabolomics studies used analytical tools including NMR spectroscopy, liquid chromatography–mass spectrometry (LC–MS), and gas-chromatography–mass spectrometry (GC–MS) [6–9]. Due to the very complex and huge original data volume of metabolome, it cannot be analyzed by conventional methods. Instead, it often uses pattern recognition analysis, such as principal component analysis

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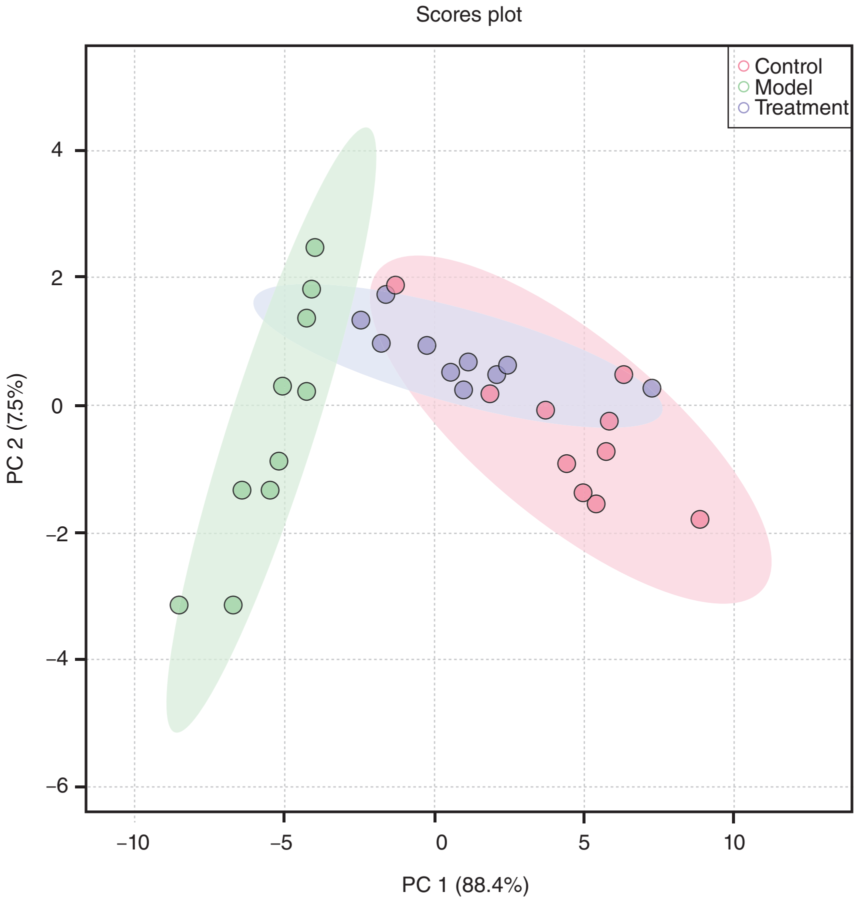

**Figure 1.1** Principal component analysis in high-throughput metabolomics.

(Figure 1.1) and loading (molecules) analysis (Figure 1.2), for screening metabolites (Figure 1.3) and for exploring the metabolic pathway (Figures 1.4 and 1.5) and the metabolic network change mechanism (Figure 1.6). Thus metabolomics analysis has formed a series analysis approaches including data extraction, data preprocessing, supervised and unsupervised pattern recognition, and biological information exploration [10–12].

## 1.3 Mass Spectrometry Technology

MS has become a widely used analytical tool for chemists and is increasingly used for metabolomics analysis. It plays a key role in the identification of structural information for metabolite molecules. With the advance of technology, MS combination with separation technology is gradually improving [13, 14]. Due to the high specificity and sensitivity, low sample consumption, fast analysis, and the advantages

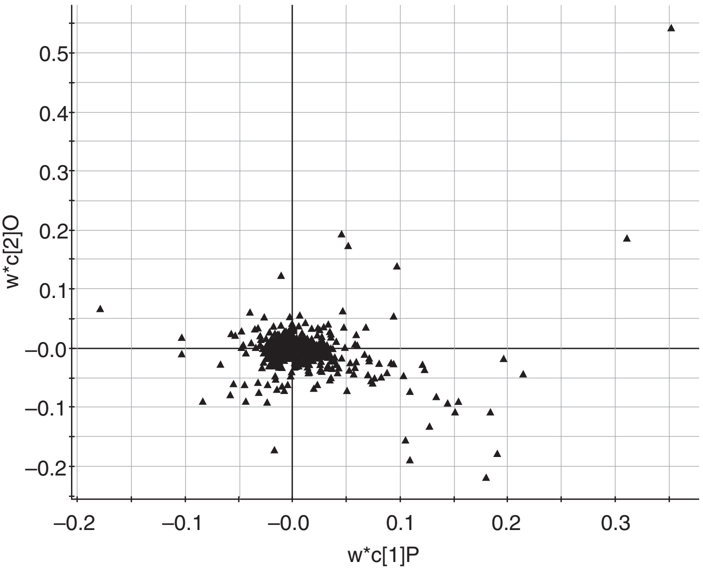

**Figure 1.2** The loading ion analysis for metabolomics.

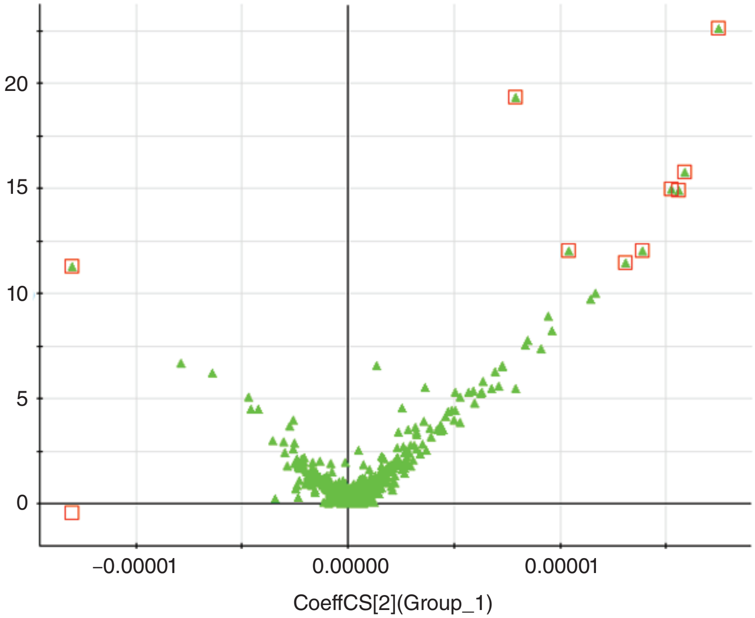

**Figure 1.3** The variable importance in projection for screening metabolites from large biological data sets.

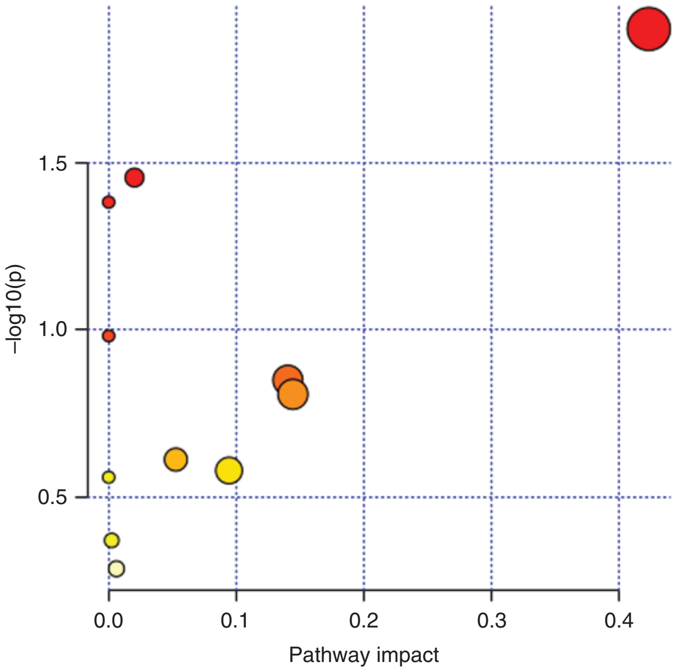

**Figure 1.4** Metabolic pathway analysis for the biologically meaningful metabolite sets.

of identification, mass spectrometer is widely used in metabolomics science, and it opens up a new gate for the rapid analysis of complex samples [15–17].

## 1.4 Recent Advancements and Applications

MS-based metabolomics methods have been used to reveal the disease diagnosis, drug effects, metabolic mechanism, toxins, and various diseases including cardiovascular disease, cancer, natural product discovery, toxicological effects, and nutrition [18–24].

### 1.4.1 Disease Diagnoses and Metabolism

The disease causes the pathophysiological changes and eventually result in corresponding metabolites and pathway changes. Some scholars have established a diagnostic method for diseases by detection of metabolites. Cholesterol sulfate and phospholipids are considered as novel biomarkers for atherosclerosis [25]. The levels of norvaline, 1,5-anhydroglucitol, and L-aspartic acid are linked with macroalbuminuric diabetic kidney disease [26, 27]. Abdulwahab et al. used MS-based metabolomics and revealed new associations between 37 proteins and T2DM and found the significant up-regulation of immunoglobulin [28]. Yang et al. found that there were 33 distinct metabolites in the urine of femoral head necrosis patients [29].

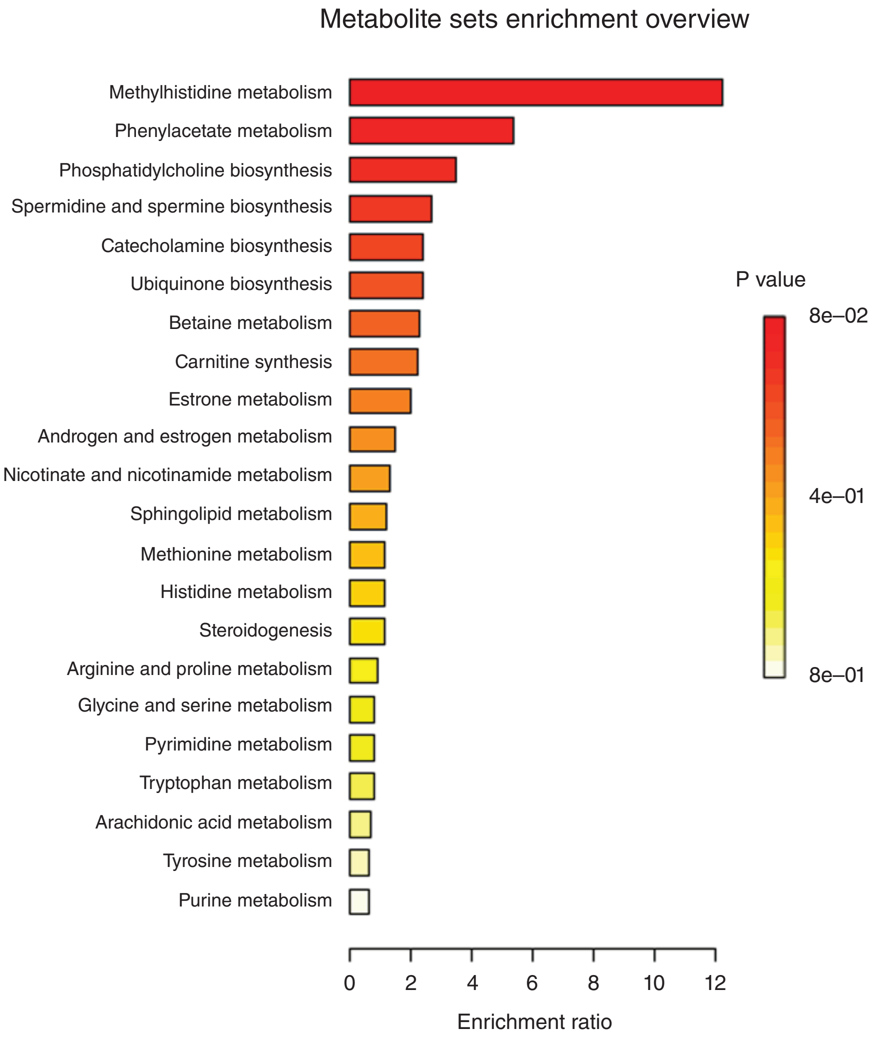

**Figure 1.5** Metabolite enrichment analysis for the biologically meaningful metabolite sets based on the libraries.

Lin et al. used MS to detect plasma metabolites and found that the alanine, aspartic acid, and carbamate were significantly different among all groups of respiratory distress patients [30]. Øvrehus et al. had found that early hypertensive nephrosclerosis showed disturbances in dopamine intrarenal biosynthesis [31]. Metabolic pathways such as sphingolipid, vitamin D-related compounds, and steroid precursors were discovered in glaucoma patients [32]. Dong et al. discovered the 31 metabolites between nonalcoholic fatty liver disease and nonalcoholic steatohepatitis [33]. Based on the metabolic method, Wang et al. found that the changes of acute ischemic stroke were mainly related to amino acid-related metabolism [34]. There are 30 different metabolites of gout patients, mainly involving tricarboxylic acid cycle, amino acid metabolism, and lipid metabolism [35]. A total of 12 metabolites,

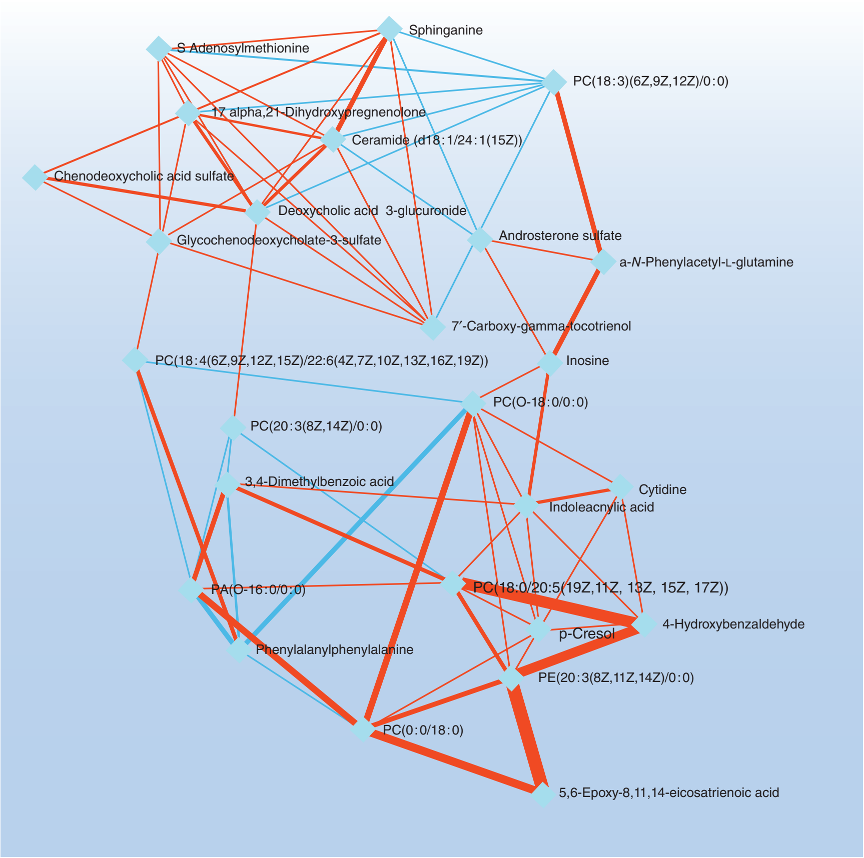

**Figure 1.6** Metabolic networks for the biologically meaningful metabolite sets performed with MetaboAnalyst online tool (www.metaboanalyst.ca).

mainly related to fatty acid metabolism, exerted significant changes in ischemic stroke [36]. Metabolomics research is of great significance for the disease diagnosis, to better understand the disease pathogenesis and to provide new evidence for the primary prevention of disease [37–43]. Cancer is a major health problem in the world, so understanding the metabolic causes of cancer and its influencing factors is important for disease treatment. Yang et al. used MS to explore the metabolic changes of ovarian and had identified 18 metabolites closely related to ovarian cancer [44]. Some scholars used LC/MS to study the metabolic changes of pancreatic cancer tissue and found that seven metabolites may be potential biomarkers [45]. Metabolic pathways are closely related to tumor growth, metastasis, and immune escape mechanism [46, 47].

### 1.4.2 Metabolic Mechanisms

The disorder of metabolic mechanism is one of the crucial factors affecting the disease, for clarifying the metabolic mechanism plays a crucial role in the treatment of the disease. Breviscapine mainly improves the metabolism of phospholipids by regulating the level of serotonin [48]. A total of 19 metabolites were found with the hypoglycemic effect of *Crassostrea gigas* polysaccharide and mainly involved in carbohydrate metabolism, amino acid metabolism, and purine metabolism [49]. Aloe emodin has a therapeutic effect on hyperlipidemia by regulating metabolic disorders [50]. Pang et al. had found that methotrexate could regulate the inflammatory-related metabolic networks [51]. Metabolic mechanism of disease research in metabolomics-based approach provided a new method for disease treatment [52–56].

### 1.4.3 Drug Efficacy

The efficacy evaluation is an essential requirement for discovering the effective constituents and therapeutic targets. Therefore, based on the metabolomics and other omics techniques, the innovative methods such as chinmedomics, fectomics, and functiomics could be established and used for evaluating the efficacy; discovering the chemicalome and metabolome; screening of active compounds, fectome and functiome; revealing the effective mechanism of drug, herbal medicine, and traditional medicine; etc. and seek to elucidate the therapeutic properties with modern techniques. Some scholars have found that the process of drug treatments could be uncovered by metabolic methods [57]. Bao et al. used UPLC/MS metabolomic method to rapidly identify the anticancer compounds in *Forsythia* and found that betulinic acid was the most effective anticancer compound. The forsythia extract can exert anti-inflammatory effect through acting on different metabolic pathways [58, 59]. A total of 27 potential biomarkers were discovered and related with effective mechanism of Xanthii Fructus for allergic rhinitis, which mainly involved glycerophospholipid and branched-chain amino acid metabolism [60]. Gross saponins of *Tribulus terrestris* fruit could regulate the multiple metabolic pathways to reverse the middle cerebral artery occlusion [61]. Gastrointestinal injury was mainly related to phenylalanine, arachidonic acid, and taurine. Fructus Gardenia affected function state of gastrointestinal via regulating a variety of metabolic pathways [62]. Metabolomics has a wide range of applications in drug efficacy, drug screening, and toxicity and effectiveness evaluations [63–68].

### 1.4.4 Nutrition

The application of metabolomics in the nutrition field is called nutritional metabolomics, which refers to the systematic study of the interaction between diet and organism metabolism. The changes of cucumber fruit metabolites under nano-Cu stress were examined by NMR and GC–MS and found that nano copper exposure had an effect on the metabolic profile of fruit metabolites [69]. UPLC–MS-based metabolomics strategy was used to explore the nutrient profiles of kale and found that the contents of components in different cabbage varieties were different [70]. MS technology was used to explore the differences of secondary metabolites and heavy metals content in dried ginger from different habitats.

It showed that abiotic stress due to metals may lead to differences in metabolite abundance [71]. GC–MS technology was used to investigate the metabolic changes of strawberry fruit caused by controlled atmosphere treatment and found that several metabolites were mainly related to the biologically active compounds [72]. These studies have provided the scientific basis for the regulation and balance of nutrition and diet [73–80].

### 1.4.5 Plant Biology

The metabolic characteristics of leaves and roots of soybean seedlings cultured under normal and excessive Mo conditions were analyzed by UPLC–MS/MS. 2-Oxo-arginine, L-nicotine, gluconic acid, D-gluconic acid, and citric acid play an important role in chelation of molybdenum and reducing its toxicity [81]. Yang et al. studied the metabolic responses of magnesium deficiency in soybean leaves and roots and found that there were considerable differences in carbon and nitrogen metabolic responses between leaves and roots under magnesium deficiency [82]. Allwood et al. had investigated the yield, grain quality, and response of grain metabolites to increase nitrogen application and found that nitrogen application had a significant effect on grain yield [83]. Zhang et al. performed metabolomic analysis on two wheat varieties with different induced nitrogen levels and revealed that flavonoids were considered as potential biomarkers under low nitrogen stress [84]. Metabolomics is increasingly utilized in agronomy and plant biology to understand the behavior of plants under different stress conditions [85–88].

## 1.5 Limitation

Although metabolomics technology is widely used, there are still many problems. The analytical techniques have not been able to achieve comprehensive qualitative and quantitative analysis of all metabolites in living organisms [89–94]. The instruments and equipment required for conducting the metabolomics research are expensive, and the proficiency of the operators is a high-level demand. The experiments have shown that various interference factors, such as age, gender, diet, temperature, and even changes in environment, could affect the metabolic profiles [95–102]. In the metabolomics data analysis, although there are several commonly used databases, the number of metabolites is currently not identified by the standard compound. Therefore, the metabolomics database needs to be further improved. In the process of data analysis, most methods are applied to linear data sets, and how they are used from nonlinear data is worth thinking deeply. Effective extraction of metabolites is still a faced problem [7, 103–110]. It requires mathematics and statistics to be fully borrowed and absorbed in metabolomics field [111–116]. Perfection of metabolomics still requires further efforts.

## 1.6 Conclusions and Future Perspectives

A better understanding of the disease-related mechanisms on the endogenous metabolism is an urgent need. Fortunately, the MS-based metabolomics has been used in various areas, providing scientific support for the discovery of endogenous metabolites. Advances in this field have enabled the discovery of numerous disease biomarkers and then provided insight into the disease pathogenesis [117–120]. It addresses the challenges of methods and applications for the discovery and identification for metabolite biomarkers, but the analytical instruments, techniques, and data acquisition or analysis require additional improvement. It is still challenging to establish a predictive model for diagnostic rationalization. Other challenges such as sample preparation, sample matrix, and residual effects can also lead to data variability. The challenge is usually accompanied by opportunities, and the current challenges are also huge opportunities for future development. With the improvement of methods, it is believed that metabolomics will be comprehensive, efficient, and even become a powerful tool to monitor disease from beginning to end.

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