Metabolomics in Hyperuricemia and Gout

Subjects: Biology

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Urate is one of the key metabolites of purine metabolism, and the overproduction of urate in the liver or decreased excretion in the kidney in humans may lead to elevated levels of urate in the circulation, termed hyperuricemia (HU). The formation of monosodium urate (MSU) crystals in the joint or surrounding tissues may trigger inflammatory responses and gout attacks, which is the most common inflammatory arthritis. In addition to gout, HU has also been associated with many other metabolic diseases, such as cardiovascular disease, obesity, diabetes, fatty liver diseases, kidney diseases, hypertension, and various cancers. Overwhelming evidence indicates that HU and gout lead to systematic metabolic alterations underlying these metabolic disorders. As one of the most powerful omics techniques, metabolomics systematically analyzes all small-molecule metabolites in a biological system that directly reflect the physiological and pathological conditions. Metabolomics has been increasingly employed in clinical and experimental research in HU and gout. Emerging studies have developed predictive models to differentiate HU from gout based on metabolomics and machine-learning algorithms.

gout metabolomics hyperuricemia mass spectrometry urate metabolism machine learning biomarkers

1. Introduction

Gout is a common chronic inflammatory disease of the joint caused by the deposition of monosodium urate crystals (MSU) [1][2]. Epidemiological data show that the incidence of gout varies widely from <1% to 6.8% around the world [3]. Clinical evidence suggests that persistent hyperuricemia (HU) is a prerequisite for gout [4], which is diagnosed as fasting blood urate levels over 420 μmol/L (7 mg/dL) in two separate days [5]. The increase of serum urate concentrations, HU, may be caused by multiple factors, such as upregulation of purine metabolism in the liver, increased dietary intake of purine, and/or impaired renal excretion. Gout flares are usually characterized by redness, swelling, heat, and pain in a single joint of the toes, ankles, and knee, and the inflammation resolves rapidly [6]. However, prolonged recurrent gout flares are often associated with the formation of tophi at the joint or extremity of the limb [7]. Urate lowering therapy (ULT) for HU or treatment of the inflammation by colchicine or non-steroidal anti-inflammatory drugs (NSAIDs) is the key to clinical gout management [8]. Gout and HU are often associated with multiple metabolic co-morbidities, including hypertension, kidney diseases, cardiovascular disease, non-alcoholic fatty liver diseases, and various cancers [9][10].

Metabolomics is a relatively new omics technology that systematically identifies and quantifies all small-molecule metabolites in an organism [11][12]. As the substrates and products of biological activities, metabolites are essential

for various cellular functions. Endogenous metabolites produced by the host organism and exogenous metabolites (xenobiotics) from microbiota, pharmaceuticals, or environmental chemicals constitute the entire metabolome, a collection of all metabolites. Emerging research has revealed that metabolites are not only the end products of biological processes, but also interact with the epigenome, transcriptome, and proteome [13][14][15][16]. For example, gout attacks triggered by MSU in the joints lead to systemic metabolic alterations. Previous studies found that MSU promotes GLUT1-mediated glycolysis that governs NLRP3 and interleukin-1β activation on macrophages [17]. In addition, MSU regulates a unique JNK-dependent macrophage metabolic and inflammatory response [18]. In the progression of gout from HU, dynamic metabolic changes are often observed as a result of interplays among genetic factors, physiological status, and environmental insults. In this regard, traditional biochemical approaches focusing on a single or several metabolites to investigate such a complicated pathogenesis process in gout or HU may be inadequate. Thus metabolomic alterations could directly or indirectly reflect physiological and pathological states [19]. Therefore, metabolomics has become a powerful tool to investigate metabolic processes and identify disease-related biomarkers [20]. In recent years, innovative developments in analytic and bioinformatic technologies have greatly expanded the analytical capabilities of metabolomics to cover increasingly more metabolites at the systems biology level. Mass spectrometry (MS)-based analytical platforms provide powerful resolving capabilities for metabolomics [21], while bioinformatics tools provide deeper and broader insights for biological research [22][23].

The past decade has witnessed tremendous progress in understanding the pathophysiology of HU and gout, in which metabolomics plays an indispensable role (**Figure 1**).

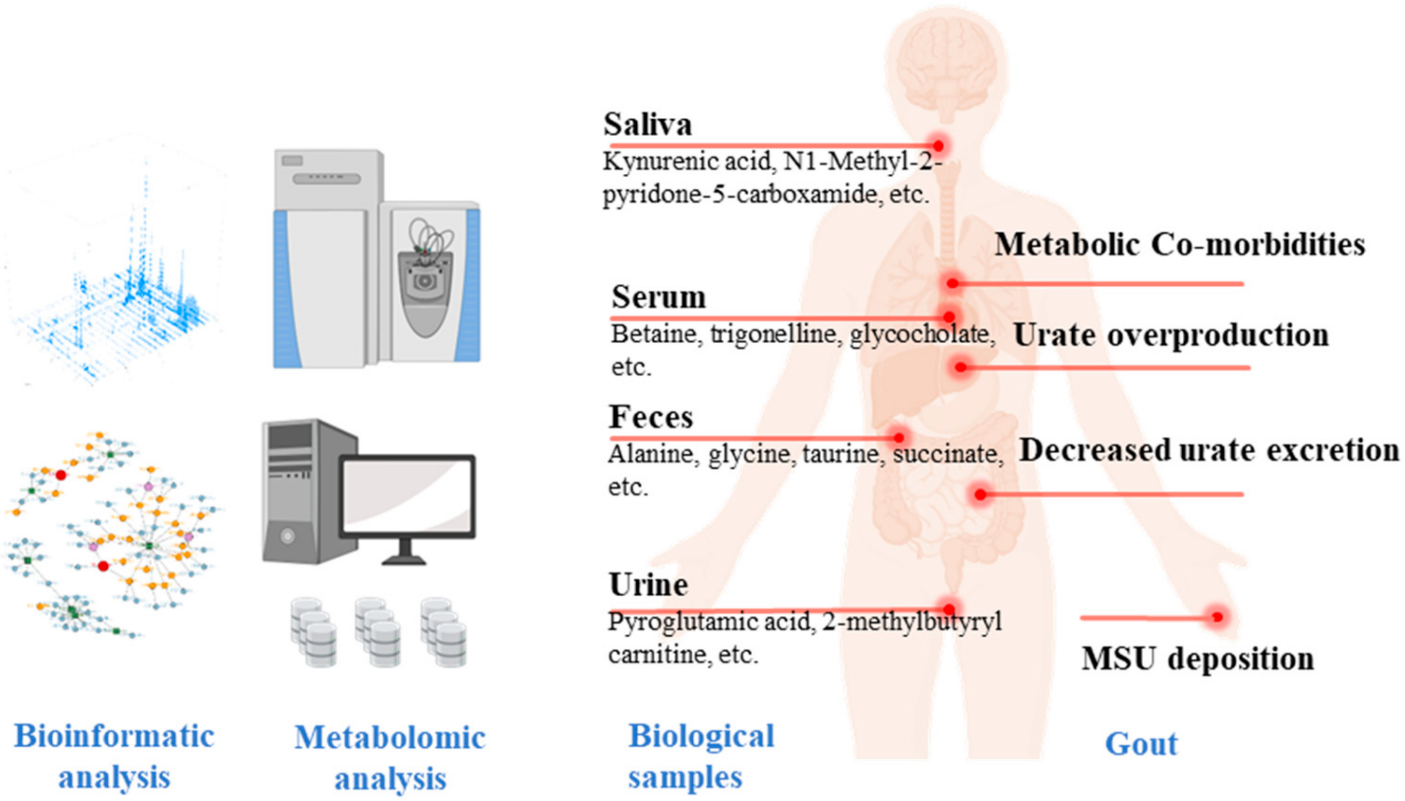

Figure 1. An overview of metabolomics studies in hyperuricemia and gout. Metabolomics studies are often performed using high-resolution mass spectrometry with multiple bioinformatics tools. In human clinical studies,

metabolomics studies use saliva, serum, feces, and urine samples. Examples of some differential metabolites are shown with the different biological sources in gout and HU studies. HU is caused by urate overproduction in the liver or decreased excretion in the kidney, and MSU deposition in the joints triggers gout attacks. HU and gout are associated with multiple metabolic co-morbidities.

2. Metabolic Profiling and Metabolite Biomarker Discovery in Clinical Populations with HU and Gout

In large-scale clinical research, metabolomics is routinely applied to profile metabolic alterations in an attempt to uncover the pathological mechanisms and discover potential metabolite biomarkers [20]. Although HU is the major cause of gout, only a small fraction of HU patients experience gout flares, while a majority of patients (90%) remain asymptomatic. Currently, there is no reliable clinical approach for predicting HU patients who will develop gout. Metabolomics provides a novel tool to reveal metabolic alterations between HU and gout and has the potential to identify metabolite biomarkers for predicting the progression of HU to gout. In this regard, metabolomics has been performed in gout and HU patients to systematically profile their metabolic characterization compared to healthy controls. Metabolites involved in amino-acid metabolism, lipid metabolism, purine metabolism, and energy metabolism have been discovered to be significantly different from healthy control in serum [24][25][26][27]. Other metabolic differences between HU and gout have been reported in recent metabolomics research, such as arginine biosynthesis, glycine, serine, and threonine metabolism, as well as some lipid metabolism [25][28]. Interestingly, some metabolites, such as those involved in tryptophan metabolism, neuroactive ligand-receptor interaction, and nitrogen metabolism pathway, were found to change at sequential gout progression stages [29], suggesting a role in the pathogenesis from HU to gout. The metabolomic observations in serum have been summarized in **Figure 2** to provide a better overview of the altered pathways from controls to HU and gout. Similar alterations were observed in urine and fecal samples from gout patients [27][30][31]. Together, these global metabolomics profiles observed in metabolomics studies may reflect a pathological state and help to understand the underlying pathogenesis of HU and gout.

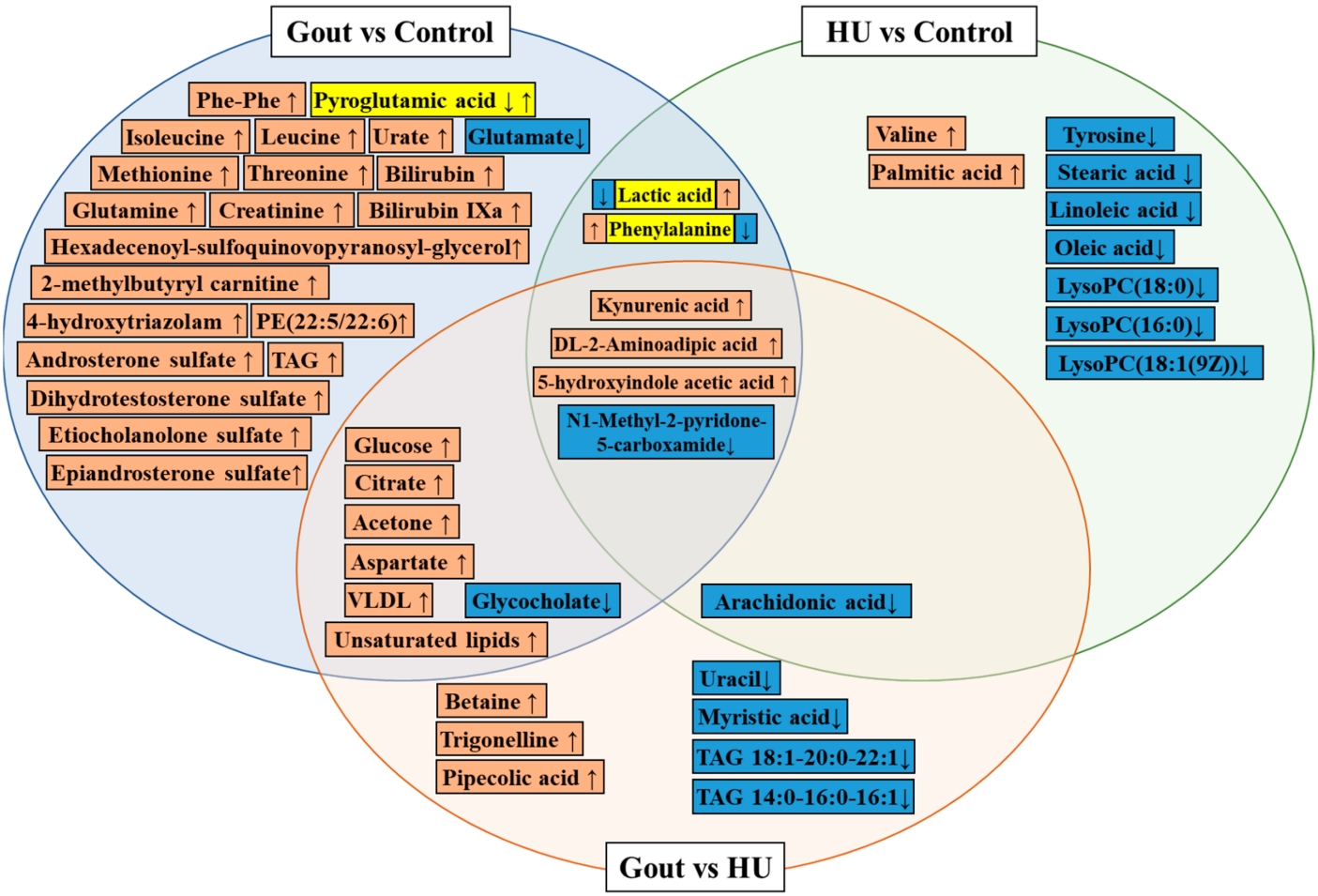

Figure 2. Venn diagram shows the up- and down-regulated metabolite biomarkers in serum of controls, HU, and gout. The orange text box denotes an increase in patients; the blue text box denotes a decrease in patients; and the yellow text box denotes metabolites reported by different articles with inconsistent trends.

To discover potential metabolite biomarkers for gout, especially in asymptomatic HU cohorts with a high risk of gout, it is essential to include large clinical cohorts in metabolomics studies with proper statistical models. In recent research, up to 547 cases were included, and multiple machine-learning-based models were applied to select the most predictable biomarkers from untargeted metabolomics data [29][32]. Apart from the traditional gout indicator, urate, several novel biomarkers, such as pyroglutamic acid, glycocholate, lactic acid, glutamate, bilirubin, 2-methyl butyryl carnitine, isoxanthopterin, and kynurenic acid, were identified. Notably, some of these reports validated their selected biomarkers in separate validation cohorts [27][29][32][33] or by other absolute quantitative approaches [29][33][34], which show a better potential for translation into an early clinical diagnosis in gout.

3. Multi-Omics and Big Data

The combination of metabolomics and other omics could provide comprehensive and novel insights into clinical and experimental research. Genotype-dependent metabolic phenotypes, such as urate and SLC2A9, 10-nonadecenoate (19:1n9)/10-undecenoate (11:1n1) and CYP4A, are discovered by analysis of GWAS with

untargeted metabolomics [35][36]. There is a lack of aldehyde dehydrogenase 16A1 (ALDH16A1), which was discovered to be associated with serum urate levels and gout in humans [37] by potential interactions with hypoxanthine phosphoribosyltransferase 1 (HPRT1) [38]. In mice kidneys, significant changes in the transcriptional levels of cellular lipid metabolic genes and urate transporters as well as lipid profiles were consistent with metabolomics data [39], suggesting a potential role of ALDH16A1 and lipid metabolism in gout. Emerging data suggest that gut microbiota participate in purine and urate metabolism [40], crosstalk with the host immune system [41], and affect intestinal urate excretion [42][43]. Thus, in feces from gout patients, a combination of microbiome and metabolome analyses revealed an up-regulation of microbiota, such as *Bacteroides*, *Porphyromonadaceae Rhodococcus*, *Erysipelatoclostridium* and *Anaerolineaceae*, as well as altered metabolites involved in urate excretion, purine metabolism, and inflammatory responses [31]. A prospective cohort analysis of 105,703 UK Biobank (UKB) participants by targeted NMR metabolomics identified glycoprotein acetylation as a biomarker positively associated with the risk of incident gout and validated in 4804 non-overlapping participants [44].

4. Metabolomics in Experiment Models

Recently, an MSU crystal-induced gouty arthritis (GA) rat model was established by injecting an MSU crystals suspension (20 mg/mL) in the dorsal side of the right ankle. In this model, rat serum metabolomics profiling found that arachidonic acid, sphingolipid, and glycerophospholipid metabolism were significantly changed [45]. Consistently, in studies based on high fructose combined with a potassium-oxonate (HFCPO)-induced HU rat model, researchers used untargeted plasma metabolomics to find significant alterations between the HU group and control group. The differential metabolites included acylcarnitine and amino-acid-related metabolites [46]. In addition, urine samples have been widely used in metabolomics studies. In potassium-oxonate-induced HU rats, researchers used ^{1}H NMR and LC-MS to conduct nontargeted metabolomics studies in plasma and urine samples. They discovered 21 metabolites in plasma and urine to be closely related to HU, such as pyruvate, lactate, creatine, glycine, lysophosphatidylcholine (LysoPC), and phosphatidylcholine (PC) [47]. LC-MS using multiple reaction monitoring modes has been applied to detect the macrophage metabolic changes in an acute gouty peritonitis mouse model after MSU stimulation. They found that IL-37 might alter the macrophage polarization status by metabolic reprogramming, primarily by reducing TCA and several amino acids and oligopeptides [48].

Medical treatments for HU and gout, such as ULT and anti-inflammatory therapies, may have a significant impact on systematic alterations in metabolism. A recent study using CE-TOFMS metabolomics tested the hypothesis that Xanthine oxidoreductase (XOR) inhibitors exert organ-protective effects [49]. The metabolomics focused on the renal metabolites in a rat model of renal I/R and found that XOR inhibitors can preserve tissue-specific concentrations of some high-energy phosphates, such as ATP and ADP.

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