Chapter 3

Metabolomics of bipolar disorder

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3.1 Metabolomics in psychiatric disorders

Metabolomics is the profiling of small molecule metabolites with less than 1500 Da that can characterize a particular biological system's current phenotype. It is frequently implemented to understand the pathophysiological processes involved in disease progression and search for new prognostic or diagnostic biomarkers of many disorders (Davies et al., 2014; Holmes, Wilson, & Nicholson, 2008; Quinones & Kaddurah-Daouk, 2009a). Therefore the metabolome is considered to be a chemical reflection of a molecular phenotype (Gowda et al., 2008; Mamas, Dunn, Neyses, & Goodacre, 2011). Besides, it has an advantage over other "omics" techniques in that it directly samples the metabolic changes in an organism. Metabolomics integrates information from changes at the gene, transcript, protein level, and posttranslational modifications. It is also thought to be a useful link between the genotype-phenotype gap (Gowda et al., 2008; Holmes et al., 2008; Mamas et al., 2011; Quinones & Kaddurah-Daouk, 2009).

There are two main approaches for studying metabolomics: targeted metabolomics, which measures a selected set of metabolites, and untargeted metabolomics, which assesses metabolites in an unbiased manner (Vivanco et al., 2011). The "metabolic profile" is an example of a targeted approach, which focuses on the identification (i.e., oxidation-reduction potential, mass/charge ratio) and quantification of predetermined groups of metabolites with similar physicochemical properties (i.e., acidic amino acids and carbohydrates) or participating in the same biochemical pathway (i.e., glycolysis, oxidation, or citric acid cycle) (Beckonert et al., 2007). In this strategy, the

Biomarkers in Bipolar Disorders. DOI: https://doi.org/10.1016/B978-0-12-821398-8.00006-0

hypothesis about a metabolite profile, which might be altered in response to a specific gene mutation, disease progression, pharmacotherapy, or diet intervention, is given beforehand (Beckonert et al., 2007; Kaddurah-Daouk & Krishnan, 2009).

"Metabolic fingerprinting" is an untargeted, hypothesis-free approach, which is not driven by any preliminary assumption. It aims to define changes in the whole metabolome occurring at a specific state in a cell, tissue, or organism, with no previous knowledge of metabolites that should be determined. The fingerprint can be termed as the unique metabolic pattern which characterizes the biological system under particular conditions (Ellis, Dunn, Griffin, Allwood, & Goodacre, 2007). The main scope of metabolic fingerprinting is to identify and qualify as many metabolites as possible in biological fluids, frequently using a comparative analysis of two subject groups (i.e., disease vs. control, disease vs. another disease). It is a promising tool in studies focused on disease diagnosis and prognosis (Ellis et al., 2007).

Currently, three primary analytical techniques are commonly used for nontargeted and targeted metabolomics studies: liquid chromatography-mass spectroscopy (LC-MS), gas chromatography-mass spectroscopy (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy (Lindon & Nicholson, 2008). Each technique has its advocates and possesses its unique features. However, no single method can provide adequate coverage of the entire human metabolome in any given biological sample at this time (Williams et al., 2006). Nowadays, metabolomics is becoming a dominant approach in systems biology research and is widely used as a new tool for holistic diagnosis in clinical and biomedical studies due to the continuous development of advanced analytical techniques and bioinformatics, providing feasible metabolites determination and identification in complex biological samples (e.g., blood, urine, or tissue extracts) (Gowda et al., 2008; Mamas et al., 2011). The use of metabolomics in examining novel biomarkers in different clinical areas is based on the hypothesis that diseases disrupt biochemical pathways, leading to a metabolic fingerprint characteristic of the disease's site and state (Lindon, Holmes, & Nicholson, 2003) or after exposure to a stimulus-specific environment, such as a specific drug (Quinones & Kaddurah-Daouk, 2009). For example, in developing asymptomatic conditions, a metabolic fingerprint might occur much earlier than any particular symptom. In fact, many different disorders can disrupt the metabolism and result in long-lasting changes that can be captured as metabolic signatures (Kaddurah-Daouk & Krishnan, 2009), including cardiovascular and coronary artery disease (Brindle et al., 2002; Sabatine et al., 2005), preeclampsia (Crocker, Kenny, Thornton, Szabo, & Baker, 2005), type 2 diabetes (Van Doorn et al., 2007), and liver, ovarian, and breast cancers (Odunsi et al., 2005; Xiaohui, Jingqing, & Peng, 2005; Yang et al., 2004). Specifically, metabolomics has been applied not only for disease diagnosis but also for drug discovery (Gomase, Changbhale, Patil, & Kale, 2008),

pharmacometabolomics/personalized medicine (Nicholson, 2006), nutrigenomics (Ryan & Robards, 2006), and metabolic engineering/biotechnology areas (Buchholz, Hurlebaus, Wandrey, & Takors, 2002).

In psychiatry, understanding the molecular causes and finding appropriate therapies are challenging research tasks; "omics" technologies have been used to elucidate the molecular mechanisms underlying brain dysfunction in a hypothesis-free manner (Turck & Filiou, 2015). Several key metabolite biosignatures related to psychiatric disorders have been reported, including alterations in levels of essential polyunsaturated fatty acids, lipid peroxidation, creatinine, and glutamate metabolites (Davison, O'Gorman, Brennan, & Cotter, 2018). All in all, the application of metabolomics in the understanding of neuropsychiatric disorders will likely enable the identification of multiple interacting pathways (Kaddurah-Daouk & Krishnan, 2009).

3.2 Biomarkers in bipolar disorder

Many studies have suggested theories to explain BD's etiology, including genetic factors, monoamine neurotransmission changes, neuroplasticity, neuroinflammation, autoimmunity, chronobiology, oxidative stress, and mitochondrial dysfunction (Goes, 2016; Isgren et al., 2015; Iwamoto, Bundo, & Kato, 2005; Konradi, Sillivan, & Clay, 2012; Quiroz, Gray, Kato, & Manji, 2008; Sousa, 2014; Van Enkhuizen et al., 2015). In fact, BD's pathophysiology is known to be multifactorial and complex, with its treatment remaining poorly understood (Scola & Andreazza, 2014). Accordingly, increased accuracy in the early diagnosis of BD is key to improving the clinical course and treating patients with this disorder. However, ongoing studies have not provided the evidence base for a diagnostic test for BD with routine clinical use (Haenisch et al., 2016). Indeed, BD diagnosis in early stages and predominantly depressive symptoms remains challenging (Brietzke et al., 2016). Identifying specific biomarkers could overcome these challenges, provide relevant information on BD's pathophysiology, and help establish the correct diagnosis and treatment (Brand, Moller, & Harvey, 2015).

Although BD's etiology remains unclear, several studies examining neuroimaging, genetics, epigenetics, and peripheral markers have provided essential insights into BD's pathophysiologic path (Frey et al., 2013). The following is a general overview of the current state of BD biomarkers and the main potential biomarkers highlighted in the literature: (1) brain imaging studies suggest that brain connectivity within the prefrontal cortex and the limbic system could be of specific interest in the search for BD biomarkers (Benedetti et al., 2011; Versace et al., 2010); (2) polymorphisms in the calcium voltage-gated channel subunit alpha1 C (*CACNA1C*; Ferreira et al., 2008; Liu et al., 2011; Schulze et al., 2009; Scott et al., 2009; Sklar et al., 2008, 2011; Stahl et al., 2019), ankyrin-3 (*ANK3*; Ferreira et al., 2008; Liu et al., 2011; Schulze et al., 2009; Scott et al., 2009; Sklar et al., 2008, 2011; Stahl et al., 2019), aryl hydrocarbon

receptor nuclear translocator-like protein 1 (*ARNTL*; Le-Niculescu et al., 2009), and brain-derived neurotrophic factor (*BDNF*; Geller et al., 2004) genes are among the main findings of genetic studies of patients with BD; (3) alterations in 5-methylcytosine levels at the *BDNF* and catechol-O-methyltransferase (*COMT*) promoters are one of the most consistent findings in the epigenetic studies of patients with BD (Abdolmaleky et al., 2006; Carlberg et al., 2014; D'Addario et al., 2012a; Dell'Osso et al., 2014; Duffy et al., 2019; Schröter et al., 2019; Strauss et al., 2013); (4) oxidative stress, specifically lipid peroxidation and DNA damage, is a potential peripheral marker; (5) peripheral BDNF levels may be used for diagnosis as well as observation of illness progression (Chiou & Huang, 2019; Fernandes et al., 2011; Fujinami et al., 2008; Lin, 2009; Mansur et al., 2016; Södersten et al., 2014); and (6) interleukin (IL)-4 and tumor necrosis factor (TNF)-α have been identified as possible inflammatory targets of special relevance in BD (Brietzke, Stabellini, Grassi-Oliveira, & Lafer, 2011; Hope et al., 2011; Munkholm, Braüner, Kessing, & Vinberg, 2013; O'Brien, Scully, Scott, & Dinan, 2006; Rao, Harry, Rapoport, & Kim, 2010).

These studies evaluating biomarkers have pointed out many potential candidates and provided the background for identifying new potential targets for the study of vulnerability, disease expression, clinical course, and treatment response (Frey et al., 2013). These findings emphasize BD's complexity: developing a general biomarker is not expected since patients in different stages (e.g., mania, euthymia, or depression) may have different profiles. On the other hand, a set of different biomarkers could identify patients' subgroups and define a unique treatment for each stage of the disease, transforming the treatment into a more personalized approach (Kittel-Schneider et al., 2014). Currently, BD's diagnosis is based on information obtained by self-report and behavioral observations, which lacks substantial biological validation and often results in under- and misdiagnoses, impacting treatment decisions (Brown, Andreazza, & Young, 2014; Frey et al., 2013). This phenomenon may contribute to increased suicide risk and a poorer prognosis for BD patients (Elinson, Houck, & Pincus, 2007; Oquendo, Currier, & Mann, 2006).

Metabolomics has successfully identified novel biomarkers for neuropsychiatric disorders, such as schizophrenia and autism (Yang et al., 2013; Yap et al., 2010). Regarding BD, some studies have utilized metabolomics to understand BD better, and findings from these studies have begun to converge, as will be described below.

3.3 Metabolomics in bipolar disorder

3.3.1 Brain tissue and cerebrospinal fluid metabolomics analysis

Few metabolomics studies have been performed in brain tissues from BD patients thus far. Lan et al. (2009) using proton nuclear magnetic resonance (^{1}H N Mr), found that myo-inositol, creatine, glutamate, lactate, and

phosphocholine were increased in postmortem dorsolateral prefrontal cortex of BD patients, suggesting mitochondrial dysfunction in BD (Lan et al., 2009). Previous studies in vivo by ^{1}H Mrs and ^{31}P-Mrs have also shown an increase in myo-inositol levels in the frontal and temporal lobes, basal ganglia, and cingulate gyrus of patients unmedicated (Davanzo et al., 2001; Moore et al., 2000) and treated with lithium (Kato, Shioiri, Takahashi, & Inubushi, 1991; Kato, Takahashi, Shioiri, & Inubushi, 1993). These abnormalities were not seen in euthymic patients, possibly due to a normalizing treatment effect with lithium (Kato et al., 1993,1994). Of note, myo-inositol is a crucial component of the phosphatidylinositol second messenger system (PI-cycle). Alterations in PI-cycle activity has been suggested to be involved in BD's pathophysiology and response to treatment (Berridge, Downes, & Hanley, 1982). More specifically, lithium has been recommended to act primarily by lowering myo-inositol concentrations, termed the "inositol-depletion hypothesis" (Berridge et al., 1982; Berridge, Downes, & Hanley, 1989). These clinical findings have been generally supportive of the involvement of myo-inositol in BD and its treatment.

In another metabolomics study of postmortem brain samples from BD, SCZ, diabetes, or depressive patients, analyses using hydrophilic interaction liquid chromatography-high resolution mass spectrometry (HILIC-HRMS) were able to identify altered metabolites in psychiatric brains and patients with diabetes when compared to controls. Changes in the levels of the lipophilic amino acids leucine/isoleucine, proline, methionine, phenylalanine, and tyrosine, the neurotransmitters gamma-aminobutyric acid (GABA), and *N*-Acetylaspartylglutamic acid (NAAG), and the sugar metabolites sorbitol, gluconic acid, xylitol, ribitol, arabinotol, and erythritol were detected. This suggests that the abnormal metabolism of sugar and branched-chain amino acids may be a key element in the pathophysiology of BD, SCZ, and depression, reflecting the metabolic similarity between these psychiatric disorders and diabetes. In accordance to this, antidiabetic treatments may help manage BD, SCZ, and depression (Zhang et al., 2016).

Using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) analysis of cerebrospinal fluid (CSF) metabolomic from BD patients and controls, (Yoshimi et al., 2016) identified that isocitric acid concentration was significantly higher in BD patients than in healthy controls (Yoshimi, Futamura, Bergen, et al., 2016). The mRNA and protein expression of isocitrate dehydrogenase (IDH3A) were substantially lower in postmortem brain tissues from BD patients, which suggests that abnormal mitochondrial metabolism of isocitric acid by IDH3A in the citric acid cycle may play a crucial role in the pathogenesis and could potentially be a novel therapeutic target of BD (Yoshimi, Futamura, Bergen, et al., 2016).

As seen above, the studies from postmortem brain samples and CSF may contribute to clarify BD's pathophysiology. Also, they are ideal biological

samples for research on neuropsychiatric disorders. However, brain tissue biopsy and lumbar puncture samples cannot be practically obtained from all patients because of ethical and safety concerns. In comparison, blood and urine samples can be acquired at minimal risk and cost. Therefore metabolic analyses of blood and urine in BD patients and controls are of great value in discovering new biomarkers.

3.3.2 Blood and urine metabolomic analyses

Many studies have used a metabolomics approach to characterize the blood (Noriko Yoshimi et al., 2016; Kageyama et al., 2017; Ribeiro et al., 2017) and urinary (Zheng et al., 2013) profile of patients with BD and healthy controls to identify and validate biomarkers in BD (summarized in Table 3.1).

The urinary metabolomics approach has high applicability and can be analyzed using targeted and nontargeted analyses (Warrack et al., 2009). Using the GC-MS-based metabolomics of patients with BD and healthy controls, 37 key urinary metabolites have been identified as responsible for discriminating patients with BD and healthy controls through multivariate analysis. Of these 37 potential biomarkers of urinary metabolites, 2,4-dihydroxypyrimidine was identified as a potential diagnostic biomarker for BD from replicated data in an independent sample set of BD patients controls (Xu et al., 2014). Conversely, the 2,4-dihydroxypyrimidine urinary metabolite signature identified was significantly different from the metabolites identified by the NMR-based metabolomic approach in a previous study from the same group (Zheng et al., 2013). The authors explain that this difference may be due to varying methods of detecting the metabolites in each study, with this disparity between the two methods probably caused by the detection of specific hydrophobic metabolites by NMR (which are not detected by GC-MS) (Xu et al., 2014).

Interestingly, it has been suggested that the combined application of NMR and GC-MS spectroscopy can identify a more comprehensive panel of urinary metabolites than any isolated metabolic platform. For instance, the application of a dual platform (GC-MS and NMR spectroscopy) generates a panel of urinary metabolite biomarkers with similar specificity but greater sensitivity compared to a panel of metabolites resulting from a study using only GC-MS (Chen, Liu, et al., 2014). Subsequent studies using a combination of GC-MS and NMR platforms have also suggested the potential of 2,4,4-dihydroxypyrimidine as a highly accurate BD urinary biomarker (Chen et al., 2015; Chen et al., 2019; Chen, Liu, et al., 2014). The urinary concentration of 2,4 dihydroxypyrimidine, a glutamine formation-associated metabolite (Löffler, Fairbanks, Zameitat, Marinaki, & Simmonds, 2005), was consistently and significantly decreased in BD patients relative to healthy controls (Chen et al., 2019; Chen, Liu, et al., 2014; Xu et al., 2014) and to MDD patients (Chen et al., 2015) across studies, suggesting a significant

TABLE 3.1 Metabolites responsible for discriminating BD patients from healthy controls.

Tissue	Size sample	Analytical platform	Main findings	Reference
Postmortem brain	Training sample: BD/SCZ/depression (n = 24), diabetic (n = 8), and HC (n = 21).	HILIC-HRMS	Twenty-five differential metabolites were responsible for the discrimination between BD/SCZ/depression patients and HC using OPLS-DA, including leucine/isoleucine, orthophosphate, valine, proline, threonic acid isomer, phenylalanine, GABA, sarcosine, inosine, homoserine, sorbitol/mannitol/iditol/dulcitol, methionine, sn-glycero-3-phosphocholine, deoxy sedoheptulose phosphate, tryptophan, erythritol/threitol, D-Myo-Inositol, tyrosine, 1,2-cyclic phosphate, homocarnosine isomer, *N*-acetyl-aspartyl-glutamate, sedoheptulose gluconic acid, hexose, and 4-guanidinobutanoate.	Zhang et al. (2016)
Postmortem dorsolateral prefrontal cortex (Brodmann area 9).	Training sample: BD (n = 10) and HC (n = 10).	^{a}H NMR	Five differential metabolites were responsible for the discrimination between BD patients and HC using analysis of OPLS-DA, including Myo-inositol, creatine, glutamate, lactate, and phosphocholine polyunsaturated lipid.	Lan et al. (2009)
Serum	Training sample: BD (n = 54) and HC (n = 39).	CE-TOFMS	Eighteen differential metabolites were responsible for the discrimination between BD patients and HC. After the logistical regression model with AUC of 0.974, six urinary metabolites, including pyruvate, *N*-acetyl glutamate, α-ketoglutarate, β-alanine, serine, and arginine showed association with BD subjects.	Yoshimi, Futamura, Bergen, et al. (2016)

(*Continued*)

TABLE 3.1 (Continued)

Tissue	Size sample	Analytical platform	Main findings	Reference
Plasma	Training sample: BD (n = 6), and HC (n = 19) Test sample: BD (n = 16), and HC (n = 11)	CE-TOFMS	Citrulline was nominally significantly decreased in BD compared to HC, which was not replicated in an independent sample set.	Kageyama et al. (2017)
Serum	Training sample: BD (n = 14) and HC (n = 21).	LC-QTOFMS	Four metabolites classes were responsible for the discrimination between BD individuals compared to HC using analysis of OPLS-DA (VIP ≥ 1.5): phosphatidylinositol, glycerophospholipids glycerolipids, and sphingolipids.	Ribeiro et al. (2017)
Urine	Training sample: BD (n = 45) and HC(n = 61) Test sample: BD (n = 26) and HC (n = 33)	GC-MS	Thirty-seven metabolites were differentially expressed in individuals with BD compared to HC using OPLS-DA (VIP > 1). After replication in *Test set samples*, 2,4-dihydroxypyrimidine discriminated the individuals with BD from the HC with an AUC of 0.805 (cut-off: 1.468).	Xu et al. (2014)
Urine	Training sample: BD (n = 78) and HC (n = 43) Test sample: BD (n = 48) and HC (n = 28)	GC-MS and NMR	Twenty-six metabolites were differentially expressed in individuals with BD compared to HC using analysis of OPLS-DA (VIP > 1). After replication in *Test set samples*, β-alanine, azelaic acid, 2, 4-dihydroxypyrimidine, pseudouridine, and α-hydroxybutyrate demonstrated the most significant deviations between BD subjects and HC with AUC of 0.974 (cut-off: 0.508).	Chen, Liu, et al. (2014)

(*Continued*)

TABLE 3.1 (Continued)

Tissue	Size sample	Analytical platform	Main findings	Reference
Urine	Training sample: BD (n = 35), and HC (n = 55). Test sample: BD (n = 20), and HC (n = 55).	GC-MS and NMR	Thirteen differential metabolites were responsible for the discrimination between young and middle-aged BD patients during depressive episodes compared to HC using analysis of OPLS-DA (VIP > 1). After replication in *Test set samples*, isobutyric acid, formic acid, 2,4-dihydroxypyrimidine, azelaic acid, and sucrose with AUC of 0.974.	Chen et al. (2019)
Urine	Training sample: BD (n = 43), and HC (n = 78). Test sample: BD (n = 28), and HC (n = 48).	GC-MS and NMR	Ten metabolites were differentially expressed in individuals with BD compared to HC using analysis of OPLS-DA (VIP > 1). After replication in *Test set samples*, β-aminoisobutyric acid, phenylalanine, pyroglutamic acid, (R*, S*) 2,3-dihydroxybutanoic acid, α-hydroxybutyrate, 5-hydroxyhexanoic acid, adipic acid, arabitol, mannitol, methylmalonic acid, and formate.	Chen et al. (2015)

Abbreviations: *AUC*, Area under the curve; *BD*, bipolar disorder; *CE-TOF-MS*, capillary electrophoresis time-of-flight mass spectrometry; *CoA*, coenzima A; *CSF*, cerebrospinal fluid; *GC-MS*, gas chromatography mass spectrometry.
^{a}H NMR = proton nuclear magnetic resonance spectroscopy; HILIC-HRMS = hydrophilic interaction liquid chromatography-high resolution mass spectrometry; LC-QTOF-MS = liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer; MDD = major depressive disorder; MS = mass spectrometry; MRC = metabolomics resource core; NMR = Nuclear magnetic resonance; OPLS-DA = orthogonal partial least-squares discriminant analysis; VIP = variable importance of projection.

glutamine metabolic disturbance in BD. In accordance with this hypothesis, previous studies have also found alterations in glutamine levels in postmortem brains and plasma from BD patients (Lan et al., 2009; Sussulini et al., 2009), suggesting a significant role for glutamine in the BD onset.

In addition to 2,4-dihydroxypyrimidine, (Chen, Liu, et al., 2014; Chen, Zhang, et al., 2014) found other candidate urinary metabolites for biomarkers in BD. Urinary metabolites have been identified as metabolites derived from GC-MS (azelaic acid, β-alanine, and pseudouridine) and a metabolite derived from NMR (α-hydroxybutyrate), which have been validated in an independent cohort (Chen, Liu, et al., 2014). Increased levels of urinary α-hydroxybutyrate (Chen, Liu, et al., 2014; Zheng et al., 2013) and β-alanine (Chen, Zhang, et al., 2014) have also been demonstrated in BD patients compared to healthy controls and MDD patients (Chen et al., 2015). In contrast, decreased levels of β-alanine in the serum of BD patients have also been reported by Yoshimi, Futamura, Bergen, et al. (2016) and Yoshimi, Futamura, Kakumoto, et al. (2016), although the precise mechanisms underlying the role of β-alanine synthesis/metabolism in BD pathogenesis are currently unknown (Yoshimi, Futamura, Kakumoto, et al., 2016). On the other hand, the increase in α-hydroxybutyrate levels may be related to increased oxidative stress in BD patients (Zheng et al., 2013).

Using GC-MS and NMR-based metabolomics, the same group reported 13 key urinary metabolites responsible for discriminating BD patients during a depressive episode from healthy controls in both a training and test sample. Meanwhile, a metabolite-metabolite interaction network showed that differential metabolites could interact with each other directly or through one metabolite, and most metabolites had a close relationship with carbohydrate metabolism (methylmalonic acid, L-lactic acid, isobutyric acid, and fructose) and energy metabolism (hydroxylamine and formic acid).

Finally, after a multivariate logistic regression, they found that the most significant deviations between BD and healthy controls could be explained by few urinary metabolites, of which the urinary concentrations of azelaic acid, isobutyric acid, formic acid, and sucrose were significantly increased in patients with BD compared to healthy controls (Chen et al., 2019). These different metabolites further indicate that energy homeostasis might be disturbed in depressed BD patients (Chen et al., 2019).

Blood serum and plasma are easily accessible and highly informative biofluids, making them ideal for the precocious detection of a wide range of diseases, including BD (Zhang, Sun, & Wang, 2012). In one study, serum-based metabolomics using the CE-TOFMS platform and multiple logistic regression analysis was proposed as diagnostic methods for BD patients. They reported changes in amino acid metabolism (arginine, alanine, and serine) and metabolites of the citric acid and urea cycle, such as the pyruvate, α-ketoglutarate, and *N*-acetylglutamic acid, whose levels were increased in BD patients. These five metabolites were independently associated with BD, suggesting that abnormalities in amino acid metabolism, urea, and the citric acid cycle in the mitochondria may play a role in BD's pathogenesis (Yoshimi, Futamura, Kakumoto, et al., 2016).

In another study, serum metabolomics employing ultra-performance LC-MS was used to analyze the untargeted lipid extracted from BD patients' serum samples and healthy controls. Sphingolipids and glycerolipids proportions have been found to be increased, and glycerophospholipids and phosphatidylinositol decreased in patients compared to the controls. The authors suggest that changes in glycerophospholipids and phosphatidylinositol metabolic pathways can lead to other metabolic dysfunctions (Ju & Greenberg, 2003), possibly causing diverse biochemical alterations in BD patients and reinforcing the role of lipid abnormalities in BD (Ribeiro et al., 2017).

Plasma metabolomics using CE-TOFMS has also been proposed to identify diagnostic biomarkers from drug-free patients with BD and control subjects. The only candidate metabolite identified by multivariate logistic regression analysis of BD was citrulline; however, these results were not replicated in an independent sample set of medicated BD patients. Citrulline levels have been nominally significantly decreased in drug-free BD patients, but not reduced in medicated patients (Kageyama et al., 2017). Citrulline, an amino acid synthesized from L-arginine by NO synthase (NOS), is present in the mitochondria (Ghafourifar & Richter, 1997). It has been proposed that citrulline might be a biomarker of mitochondrial dysfunction in BD, and its supplementation may constitute an effective therapy for this disorder (Atkuri et al., 2009; El-Hattab, Emrick, Chanprasert, Craigen, & Scaglia, 2014).

Altogether, in the metabolomics studies mentioned above, the majority of the key BD metabolites and their connected pathways seem to center on a few common themes, including (1) mitochondrial/energy metabolism; (2) oxidative stress; (3) amino acid metabolism and; (4) lipid metabolism (Fig. 3.1).

3.3.3 Pharmacometabolomics of bipolar disorder

Metabolomics application to studies of drug responses and the identification of biomarkers has recently evolved in the new field of "pharmacometabolomics" (Quinones & Kaddurah-Daouk, 2009). Indeed, LC-MS, GC-MS, and NMR-omics in urine, serum, and plasma of patients with BD compared to control subjects mentioned above have been found to be distinct metabolic signatures. Furthermore, a few metabolic signatures before or after lithium and antipsychotic treatments have been identified in biofluids of patients with BD, as well.

A plasma metabolomics analysis of BD patients who had been treated with ketamine showed differences in molecules between responsive and non-responsive patients due to alterations in the mitochondrial β-oxidation of fatty acids. The authors reported that these differences were not produced by ketamine administration per se but by the dysregulation in disease-related

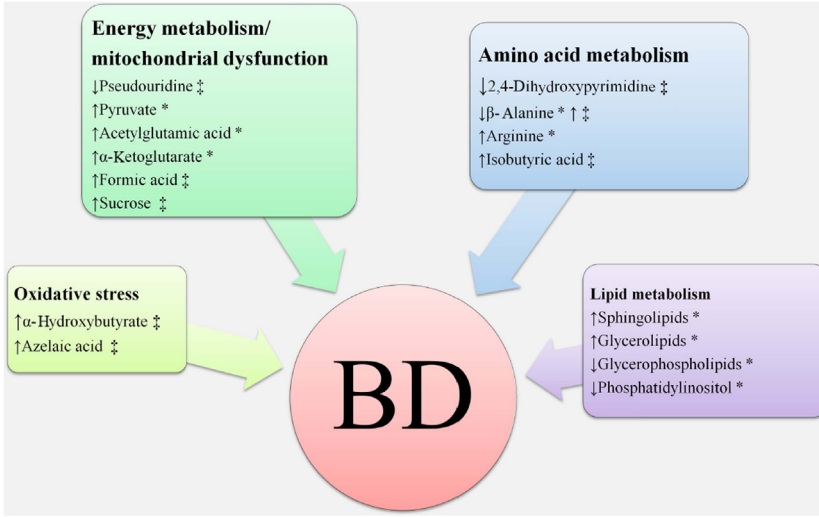

FIGURE 3.1 Schematic representation of metabolites associated with bipolar disorder (BD), as detected by metabolomics methods. ‡ urine; *blood.

mitochondrial function (Quiroz et al., 2008; Scaini et al., 2020; Villaseñor et al., 2014). Therefore metabolomics screening can help predict the pharmacological response and be used as a potential approach for individualizing ketamine therapy (Villaseñor et al., 2014).

In another study, an untargeted serum metabolomics profiling of BD subjects treated with second-generation antipsychotics (SGA) was compared to BD subjects treated with lithium using multivariate methods. As a result, levels of seven metabolites determined from serum were significantly increased in patients on SGA, named linoleic acid, 5-hydroxy indole acetic acid, cyclamic acid, glyoxal, alpha-tocopherol, *N*-acetylglutamic acid, and pyroglutamic acid. Top influential metabolite features were associated with several pathways, including linoleic acid metabolism, pyruvate, and glucose metabolism. It was also suggested that the variation in each of these metabolic pathways that influence glucose metabolism was significantly associated with SGAs (Burghardt, Evans, Wiese, & Ellingrod, 2015).

One of the most prevalent nonpsychiatric comorbidities in patients with BD is metabolic syndrome (Czepielewski, Filho, Brietzke, & Grassi-Oliveira, 2013). The main drivers are thought to include an unhealthy lifestyle and the use of antipsychotic medications, rather than the disease itself (Vancampfort et al., 2016). In this context, pharmacometabolomics could be used to predict comorbidities, such as metabolic syndrome. Some authors have postulated that metabolomics techniques associated with bioinformatics platforms would help to reliably predict trajectories of psychiatric and metabolic symptoms (Frank et al., 2018).

All in all pharmacometabolomics is expected to play a pivotal role in the development of personalized medicine (Nicholson, 2006). This field is based on the notion that given the uniqueness of each individual's DNA, there seems to be a parallel uniqueness in an individual's metabolic and state that defines how well one will respond to a particular treatment. Indeed, a better understanding of the biochemical variation of response to medications and the availability of biomarkers predictive of response would enable physicians to better select the right drug for their patients (in other words, personalized therapy) (Quinones & Kaddurah-Daouk, 2009). Pharmacometabolomics may also be used as a research strategy to inform pharmacogenomics, which aims to characterize how genetic variants affect individual responses to medications and hence, probabilities of beneficial or adverse effects (Ji et al., 2011). Pharmacometabolomics is sensitive to both the genetic and environmental influences that determine the basal metabolic fingerprint of an individual, as these will also influence the outcome of pharmacological intervention (Quinones & Kaddurah-Daouk, 2009). Collectively, data from the literature demonstrate the high potential of metabolomics to identify biomarkers and metabolic signatures of treatment response.

3.4 Future directions of metabolomics as a tool for biomarker discovery and clinical implications

Metabolomics refers to the global study of metabolism, which is an emerging field that has the potential to improve our understanding of the molecular mechanisms of disease (Sethi & Brietzke, 2015). In complex conditions such as BD, disruption of metabolism and long-lasting metabolic signatures can be identified using metabolomic platforms. An in-depth understanding of global perturbations in biochemical pathways and treatment with drugs could provide valuable insights into mechanisms, drug effects, and drug response variation and provide needed prognostic, diagnostic, and surrogate biomarkers.

It is important to note that research on BD metabolomics at this stage represents only the first step toward developing a metabolic signature as some limitations still need to be overcome. The first issue is the heterogeneity of participants in most studies, with the sample sizes for most studies so far being relatively small. Participants have varied both within and between groups in many variables, including medication status, disease subtype (e.g., BD I/BD II), age, stage of disease (prediagnostic, first depressive episode, established patients), and symptomology (e.g., mania/depression). BD patients during different episodes might also have distinct symptoms and metabolic phenotypes. For instance, previous studies showed that the BDNF levels were significantly decreased in manic and depressed BD patients compared to euthymic BD patients (Cunha et al., 2006). However, few previous metabolomics studies have considered the different mood states of BD in

their analyses. The molecular mechanisms of the selected metabolites, which can distinguish BD patients in different disease stages, may potentially reveal new insights into BD's cyclic and progressive nature (Haenisch et al., 2016).

Other potentially critical confounding variables such as diet, lifestyle, and smoking status, also need to be considered in metabolomics analyses. Appropriate matching of BD patients and analyses controlling for age, sex, ethnic background, and many other factors must be considered carefully. Moreover, Wood (2014) suggests that it is necessary to establish a normal range for metabolites in metabolomics studies due to patient populations' heterogeneity (Wood, 2014). Finally, metabolomics could provide biochemical labels to discriminate BD from other psychiatric disorders present in a similar fashion, such as depression and schizophrenia. To date, these biomarkers have been confirmed solely by comparing BD patients with healthy controls. Some of the inconsistent findings in the studies could relate to these differences.

In addition, some particular limitations and precautions related to metabolomics techniques need to be taken into account. For instance, sensitivity (the ability of diagnostic tests to correctly identify people who have the disease) and specificity (the ability of a test to determine all patients without disease) of the models is an issue that remains to be addressed in metabolomics studies. For a biomarker to be useful in diagnosing any condition, it should have a sensitivity and specificity of $>85\%$. Moreover, some difficulties exist in interpreting results from the biomarker studies published to date in this field. Several studies that measured multiple different metabolites did not account for false discovery rates, resulting in false-positive findings.

Technical and biological variability also need to be taken into account in metabolomic measurements. The contribution of technical variability to overall variability can be estimated a priori by testing the repeatability of the workflow employed (Filiou et al., 2015; Russell & Lilley, 2012). Biological variability is more challenging to estimate a priori. Specifically, each metabolomics experiment should be able to answer the question, "Which of the observed alterations in metabolite levels are disease-relevant, and which are within the range of biological variability across distinct subjects?" Numerous studies have reported fold changes that lie within the technical variability range and these results are typically not accurate (Stoop et al., 2010; Turck & Filiou, 2015). Taken together, all of these parameters should be taken into account to establish clear-cut fold change thresholds for reporting meaningful results.

Importantly, increased metabolite levels do not necessarily imply an increased pathway activity or vice versa, and this should be taken into account when interpreting metabolomics findings. To get insights into pathway activity levels, specific activity assays for the pathway/process under investigation should be performed (Turck & Filiou, 2015). Also, the information acquired through metabolomics analyses does not specify whether the changes in metabolite abundance are a cause or a consequence of the disease pathology. Thus it is unknown whether affected pathways in different

psychiatric diseases represent common causal mechanisms or nonspecific effects manifestation of the disease. Targeted methodological approaches may be needed to answer these questions (Turck & Filiou, 2015).

Perhaps the most serious issue that needs to be addressed in the "omics" field is the implementation of the acquired results. Especially in the realm of psychiatric disorders, there is admittedly a discontinuity between technical advances in the instrumentation and the performance of the acquired knowledge about disease understanding and the development of effective treatments (Hyman, 2012). Although an enormous amount of raw data is continuously being generated, validation and follow-up studies for metabolomics findings are still rare, which may be due to a lack of highly accurate alternative methods to validate data. What is also needed for clinical translation are consortia and initiatives to approach psychiatric disorders from a multidisciplinary perspective (Turck & Filiou, 2015).

To overcome some of these issues, large cohort studies are needed to compare BD with different diseases (e.g., depression, schizophrenia), BD subtypes, and disease stages, including those who are "at-risk," to validate and test the specificity of these markers. *Meta*-analyses would also be beneficial; however, it may be challenging to obtain a large amount of data for more specifically defined groups from previous studies due to the heterogeneity between subject groups and even within groups.

Finally, combining metabolomics with imaging and other "omics" approaches might be powerful ways to achieve these goals. Metabolomics can enable the mapping of early biochemical alterations in disease and hence provide an opportunity to develop predictive biomarkers that can trigger previous interventions (Sethi & Brietzke, 2015). The inclusion of metabolomics in all drug discovery and drug development steps will also become a routine as biochemistry is essential to understanding how drugs work and their safety and efficacy profiles.

References

Abdolmaleky, H. M., Cheng, K.-H., Faraone, S. V., Wilcox, M., Glatt, S. J., Gao, F., ... Thiagalingam, S. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human Molecular Genetics*, *15*(21), 3132–3145. Available from http://www.ncbi.nlm.nih.gov/pubmed/16984965.

Atkuri, K. R., Cowan, T. M., Kwan, T., Ng, A., Herzenberg, L. A., Herzenberg, L. A., ... Enns, G. M. (2009). Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(10), 3941–3945. Available from https://pubmed.ncbi.nlm.nih.gov/19223582/.

Barderas, M. G., Laborde, C. M., Posada, M., De La Cuesta, F., Zubiri, I., Vivanco, F., & Alvarez-Llamas, G. (2011). Metabolomic profiling for identification of novel potential biomarkers in cardiovascular diseases. *Journal of Biomedicine and Biotechnology*. Available from https://pubmed.ncbi.nlm.nih.gov/21274272/.

Beckonert, O., Keun, H. C., Ebbels, T. M. D., Bundy, J., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2007). Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nature Protocols*, *2*(11), 2692–2703. Available from https://pubmed.ncbi.nlm.nih.gov/18007604/.

Benedetti, F., Absinta, M., Rocca, M. A., Radaelli, D., Poletti, S., Bernasconi, A., ... Filippi, M. (2011). Tract-specific white matter structural disruption in patients with bipolar disorder. *Bipolar Disorders*, *13*(4), 414–424. Available from https://onlinelibrary.wiley.com/doi/full/10.1111/j.1399-5618.2011.00938.x.

Berridge, M. J., Downes, C. P., & Hanley, M. R. (1982). Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochemical Journal*, *206*(3), 587–595. Available from https://pubmed.ncbi.nlm.nih.gov/7150264/.

Berridge, M. J., Downes, C. P., & Hanley, M. R. (1989). Neural and developmental actions of lithium: A unifying hypothesis. *Cell*, *59*, 411–419. Available from https://pubmed.ncbi.nlm.nih.gov/2553271/.

Brand, S., Moller, M., & Harvey, B. (2015). A review of biomarkers in mood and psychotic disorders: A dissection of clinical vs. preclinical correlates. *Current Neuropharmacology*, *13*(3), 324–368. Available from https://pmc/articles/PMC4812797/?report = abstract.

Brietzke, E., Rosa, A. R., Pedrini, M., Noto, M. N., Kapczinski, F., & Scott, J. (2016). Challenges and developments in research of the early stages of bipolar disorder. *Revista Brasileira de Psiquiatria. Associacao Brasileira de Psiquiatria*, 329–337. Available from https://pubmed.ncbi.nlm.nih.gov/27533022/.

Brietzke, E., Stabellini, R., Grassi-Oliveira, R., & Lafer, B. (2011). Cytokines in bipolar disorder: Recent findings, deleterious effects but promise for future therapeutics. *CNS Spectrums*, *16*(7), 157–168. Available from https://core/journals/cns-spectrums/article/cytokines-in-bipolar-disorder-recent-findings-deleterious-effects-but-promise-for-future-therapeutics/E676BDBF4DB8F0CE4EF5C99A90928524.

Brindle, J. T., Antti, H., Holmes, E., Tranter, G., Nicholson, J. K., Bethell, H. W. L., ... Grainger, D. J. (2002). Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. *Nature Medicine*, *8*(12), 1439–1445. Available from https://pubmed.ncbi.nlm.nih.gov/12447357/.

Brown, N. C., Andreazza, A. C., & Young, L. T. (2014). An updated meta-analysis of oxidative stress markers in bipolar disorder. *Psychiatry Research*, *218*(1–2), 61–68. Available from https://pubmed.ncbi.nlm.nih.gov/24794031/.

Buchholz, A., Hurlebaus, J., Wandrey, C., & Takors, R. (2002). Metabolomics: Quantification of intracellular metabolite dynamics. *Biomolecular Engineering*, *19*(1), 5–15. Available from https://pubmed.ncbi.nlm.nih.gov/12103361/.

Burghardt, K. J., Evans, S. J., Wiese, K. M., & Ellingrod, V. L. (2015). An untargeted metabolomics analysis of antipsychotic use in bipolar disorder. *Clinical and Translational Science*, *8*(5), 432–440.

Carlberg, L., Scheibelreiter, J., Hassler, M. R., Schloegelhofer, M., Schmoeger, M., Ludwig, B., ... Schosser, A. (2014). Brain-derived neurotrophic factor (BDNF)—Epigenetic regulation in unipolar and bipolar affective disorder. *Journal of Affective Disorders*, *168*, 399–406.

Chen, C., Zhang, C., Cheng, L., Reilly, J. L., Bishop, J. R., Sweeney, J. A., ... Liu, C. (2014). Correlation between DNA methylation and gene expression in the brains of patients with bipolar disorder and schizophrenia. *Bipolar Disorders*, *16*(8), 790–799.

Chen, J. J., Liu, Z., Fan, S. H., Yang, D. Y., Zheng, P., Shao, W. H., ... Xie, P. (2014). Combined application of NMR- and GC-MS-based metabonomics yields a superior urinary biomarker panel for bipolar disorder. *Scientific Reports*, *4*, 1–6.

Chen, J. J., Xie, J., Zeng, L., Zhou, C. J., Zheng, P., & Xie, P. (2019). Urinary metabolite signature in bipolar disorder patients during depressive episode. *Aging (Albany NY)*, *11*(3), 1008–1018. Available from https://pmc/articles/PMC6382435/?report = abstract.

Chen, J. J., Zhou, C. J., Liu, Z., Fu, Y. Y., Zheng, P., Yang, D. Y., … Xie, P. (2015). Divergent urinary metabolic phenotypes between major depressive disorder and bipolar disorder identified by a combined GC-MS and NMR spectroscopic metabonomic approach. *Journal of Proteome Research*, *14*(8), 3382–3389.

Chiou, Y. J., & Huang, T. L. (2019). Brain-derived neurotrophic factor (BDNF) and bipolar disorder. *Psychiatry Research*, *274*, 395–399. Available from https://pubmed.ncbi.nlm.nih.gov/30852433/.

Crocker, I. P., Kenny, L. C., Thornton, W. A., Szabo, C., & Baker, P. N. (2005). Excessive stimulation of poly(ADP-ribosyl)ation contributes to endothelial dysfunction in pre-eclampsia. *British Journal of Pharmacology*, *144*(6), 772–780. Available from https://pubmed.ncbi.nlm.nih.gov/15778700/.

Cunha, A. B. M., Frey, B. N., Andreazza, A. C., Goi, J. D., Rosa, A. R., Gonçalves, C. A., … Kapczinski, F. (2006). Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. *Neuroscience Letters*, *398*(3), 215–219.

Czepielewski, L., Filho, L. D., Brietzke, E., & Grassi-Oliveira, R. (2013). Bipolar disorder and metabolic syndrome: A systematic review. *Revista Brasileira de Psiquiatria. Associacao Brasileira de Psiquiatria*, 88–93. Available from https://pubmed.ncbi.nlm.nih.gov/23567607/.

D'Addario, C., Dell'Osso, B., Palazzo, M. C., Benatti, B., Lietti, L., Cattaneo, E., … Altamura, A. C. (2012). Selective DNA methylation of BDNF promoter in bipolar disorder: Differences among patients with BDI and BDII. *Neuropsychopharmacology*, *37*(7), 1647–1655. Available from https://pmc/articles/PMC3358733/?report = abstract.

Davanzo, P., Thomas, M. A., Yue, K., Oshiro, T., Belin, T., Strober, M., & McCracken, J. (2001). Decreased anterior cingulate myo-inositol/creatine spectroscopy resonance with lithium treatment in children with bipolar disorder. *Neuropsychopharmacology*, *24*(4), 359–369. Available from https://pubmed.ncbi.nlm.nih.gov/11182531/.

Davies, S. K., Ang, J. E., Revell, V. L., Holmes, B., Mann, A., Robertson, F. P., … Skene, D. J. (2014). Effect of sleep deprivation on the human metabolome. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(29), 10761–10766. Available from https://pubmed.ncbi.nlm.nih.gov/25002497/.

Davison, J., O'Gorman, A., Brennan, L., & Cotter, D. R. (2018). A systematic review of metabolite biomarkers of schizophrenia. *Schizophrenia Research*, *195*, 32–50. Available from https://doi.org/10.1016/j.schres.2017.09.021.

Dell'Osso, B., D'Addario, C., Carlotta Palazzo, M., Benatti, B., Camuri, G., Galimberti, D., … Altamura, A. C. (2014). Epigenetic modulation of BDNF gene: Differences in DNA methylation between unipolar and bipolar. *Journal of Affective Disorders*, *166*, 330–333. Available from https://www.sciencedirect.com/science/article/abs/pii/S0165032714003085?via%3Dihub.

Duffy, A., Goodday, S. M., Keown-Stoneman, C., Scotti, M., Maitra, M., Nagy, C., … Turecki, G. (2019). Epigenetic markers in inflammation-related genes associated with mood disorder: A cross-sectional and longitudinal study in high-risk offspring of bipolar parents. *International Journal of Bipolar Disorders.*, *7*(1).

El-Hattab, A. W., Emrick, L. T., Chanprasert, S., Craigen, W. J., & Scaglia, F. (2014). *Mitochondria: Role of citrulline and arginine supplementation in MELAS syndrome*, *International Journal of Biochemistry and Cell Biology* (48, pp. 85–91). Available from https://pubmed.ncbi.nlm.nih.gov/24412347/.

Elinson, L., Houck, P., & Pincus, H. A. (2007). Working, receiving disability benefits, and access to mental health care in individuals with bipolar disorder. *Bipolar Disorders*, *9*(1–2), 158–165.

Ellis, D. I., Dunn, W. B., Griffin, J. L., Allwood, J. W., & Goodacre, R. (2007). Metabolic fingerprinting as a diagnostic tool. *Pharmacogenomics*, 1243–1266. Available from https://pubmed.ncbi.nlm.nih.gov/17924839/.

Fernandes, B. S., Gama, C. S., Maria Ceresér, K., Yatham, L. N., Fries, G. R., Colpo, G., … Kapczinski, F. (2011). Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: A systematic review and meta-regression analysis. *Journal of Psychiatric Research*, *45*(8), 995–1004.

Ferreira, M. A. R., O'Donovan, M. C., Meng, Y. A., Jones, I. R., Ruderfer, D. M., Jones, L.… Welcome Trust Case Control Consortium. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nature Genetics*, *40*(9), 1056–1058. Available from https://pubmed.ncbi.nlm.nih.gov/18711365/.

Filiou, M. D., Soukupova, M., Rewerts, C., Webhofer, C., Turck, C. W., & Maccarrone, G. (2015). Variability assessment of 15N metabolic labeling-based proteomics workflow in mouse plasma and brain. *Molecular BioSystems*, *11*(6), 1536–1542. Available from https://pubmed.ncbi.nlm.nih.gov/25782008/.

Frank, E., Maier, D., Pajula, J., Suvitaival, T., Borgan, F., Butz-Ostendorf, M., … Oresic, M. (2018). *Platform for systems medicine research and diagnostic applications in psychotic disorders—The METSY project*. *European Psychiatry* (50, pp. 40–46). Available from https://pubmed.ncbi.nlm.nih.gov/29361398/.

Frey, B. N., Andreazza, A. C., Houenou, J., Jamain, S., Goldstein, B. I., Frye, M. A., … Young, L. T. (2013). Biomarkers in bipolar disorder: A positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. *Australian and New Zealand Journal of Psychiatry*, 321–332. Available from https://pubmed.ncbi.nlm.nih.gov/23411094/.

Fujinami, A., Ohta, K., Obayashi, H., Fukui, M., Hasegawa, G., Nakamura, N., … Ohta, M. (2008). Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: Relationship to glucose metabolism and biomarkers of insulin resistance. *Clinical Biochemistry*, *41*(10–11), 812–817. Available from https://pubmed.ncbi.nlm.nih.gov/18402781/.

Geller, B., Badner, J. A., Tillman, R., Christian, S. L., Bolhofner, K., & Cook, E. H. (2004). Linkage disequilibrium of the brain-derived neurotrophic factor Val66Met polymorphism in children with a prepubertal and early adolescent bipolar disorder phenotype. *American Journal of Psychiatry*, *161*(9), 1698–1700. Available from https://pubmed.ncbi.nlm.nih.gov/15337662/.

Ghafourifar, P., & Richter, C. (1997). Nitric oxide synthase activity in mitochondria. *FEBS Letters*, *418*(3), 291–296. Available from https://pubmed.ncbi.nlm.nih.gov/9428730/.

Goes, F. S. (2016). *Genetics of bipolar disorder: Recent update and future directions*, *Psychiatric Clinics of North America* (39, pp. 139–155). Available from https://pubmed.ncbi.nlm.nih.gov/26876324/.

Gomase, V., Changbhale, S., Patil, S., & Kale, K. (2008). Metabolomics. *Current Drug Metabolism*, *9*(1), 89–98. Available from http://www.eurekaselect.com/openurl/content.php?genre = article&issn = 1389-2002&volume = 9&issue = 1&spage = 89.

Gowda, G. A. N., Zhang, S., Gu, H., Asiago, V., Shanaiah, N., & Raftery, D. (2008). Metabolomics-based methods for early disease diagnostics. *Expert Review of Molecular Diagnostics*, 617–633. Available from https://pubmed.ncbi.nlm.nih.gov/18785810/.

Haenisch, F., Cooper, J. D., Reif, A., Kittel-Schneider, S., Steiner, J., Leweke, F. M., ... Bahn, S. (2016). Towards a blood-based diagnostic panel for bipolar disorder. *Brain, Behavior, and Immunity*, *52*, 49–57.

Holmes, E., Wilson, I. D., & Nicholson, J. K. (2008). Metabolic phenotyping in health and disease. *Cell*, 714–717. Available from https://pubmed.ncbi.nlm.nih.gov/18775301/.

Hope, S., Dieset, I., Agartz, I., Steen, N. E., Ueland, T., Melle, I., ... Andreassen, O. A. (2011). Affective symptoms are associated with markers of inflammation and immune activation in bipolar disorders but not in schizophrenia. *Journal of Psychiatric Research*, *45*(12), 1608–1616.

Hyman, S. E. (2012). Revolution stalled. *Science Translational Medicine. American Association for the Advancement of Science*, 155cm11. Available from http://www.ScienceTranslationalMedicine.org.

Isgren, A., Jakobsson, J., Pålsson, E., Ekman, C. J., Johansson, A. G. M., Sellgren, C., ... Landen, M. (2015). Increased cerebrospinal fluid interleukin-8 in bipolar disorder patients associated with lithium and antipsychotic treatment. *Brain, Behavior, and Immunity*, *43*, 198–204. Available from https://pubmed.ncbi.nlm.nih.gov/25451615/.

Iwamoto, K., Bundo, M., & Kato, T. (2005). Altered expression of mitochondria-related genes in post-mortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Human Molecular Genetics*, *14*(2), 241–253. Available from https://pubmed.ncbi.nlm.nih.gov/15563509/.

Ji, Y., Hebbring, S., Zhu, H., Jenkins, G. D., Biernacka, J., Snyder, K., & Weinshilboum, R. M. (2011). Glycine and a glycine dehydrogenase (GLDC) SNP as citalopram/escitalopram response biomarkers in depression: Pharmacometabolomics-informed pharmacogenomics. *Clinical Pharmacology & Therapeutics*, *89*(1), 97–104. Available from https://pubmed.ncbi.nlm.nih.gov/21107318/.

Ju, S., & Greenberg, M. L. (2003). Valproate disrupts regulation of inositol responsive genes and alters regulation of phospholipid biosynthesis. *Molecular Microbiology*, *49*(6), 1595–1604. Available from https://pubmed.ncbi.nlm.nih.gov/12950923/.

Kaddurah-Daouk, R., & Krishnan, K. R. R. (2009). Metabolomics: A global biochemical approach to the study of central nervous system diseases. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 173–186. Available from https://pubmed.ncbi.nlm.nih.gov/18843269/.

Kageyama, Y., Kasahara, T., Morishita, H., Mataga, N., Deguchi, Y., Tani, M., ... Kato, T. (2017). Search for plasma biomarkers in drug-free patients with bipolar disorder and schizophrenia using metabolome analysis. *Psychiatry and Clinical Neurosciences*, *71*(2), 115–123. Available from http://www.ncbi.nlm.nih.gov/pubmed/27676126.

Kato, T., Shioiri, T., Murashita, J., Hamakawa, H., Inubushi, T., & Takahashi, S. (1994). Phosphorus-31 magnetic resonance spectroscopy and ventricular enlargement in bipolar disorder. *Psychiatry Research: Neuroimaging*, *55*(1), 41–50. Available from https://pubmed.ncbi.nlm.nih.gov/8047628/.

Kato, T., Shioiri, T., Takahashi, S., & Inubushi, T. (1991). Measurement of brain phosphoinositide metabolism in bipolar patients using in vivo 31P-MRS. *Journal of Affective Disorders*, *22*(4), 185–190. Available from https://pubmed.ncbi.nlm.nih.gov/1658103/.

Kato, T., Takahashi, S., Shioiri, T., & Inubushi, T. (1993). Alterations in brain phosphorous metabolism in bipolar disorder detected by in vivo 31P and 7Li magnetic resonance spectroscopy. *Journal of Affective Disorders*, *27*(1), 53–59. Available from https://pubmed.ncbi.nlm.nih.gov/8432961/.

Kittel-Schneider, S., Weigl, J., Volkert, J., Geßner, A., Schmidt, B., Hempel, S., ... Reif, A. (2014). Further evidence for plasma progranulin as a biomarker in bipolar disorder. *Journal of Affective Disorders*, *157*, 87–91. Available from https://pubmed.ncbi.nlm.nih.gov/24581833/.

Konradi, C., Sillivan, S. E., & Clay, H. B. (2012). Mitochondria, oligodendrocytes and inflammation in bipolar disorder: Evidence from transcriptome studies points to intriguing parallels with multiple sclerosis. *Neurobiology of Disease*, 37–47. Available from https://pmc/articles/PMC3117935/?report = abstract.

Lan, M. J., McLoughlin, G. A., Griffin, J. L., Tsang, T. M., Huang, J. T. J., Yuan, P., ... Bahn, S. (2009). Metabonomic analysis identifies molecular changes associated with the pathophysiology and drug treatment of bipolar disorder. *Molecular Psychiatry*, *14*(3), 269–279. Available from https://pubmed.ncbi.nlm.nih.gov/18256615/.

Le-Niculescu, H., Patel, S. D., Bhat, M., Kuczenski, R., Faraone, S. V., Tsuang, M. T., ... Niculescu, A. B., 3rd (2009). Convergent functional genomics of genome-wide association data for bipolar disorder: Comprehensive identification of candidate genes, pathways and mechanisms. *American Journal of Medical Genetics Part B Neuropsychiatric Genetics*, *150* (2), 155–181. Available from https://pubmed.ncbi.nlm.nih.gov/19025758/.

Lin, P. Y. (2009). State-dependent decrease in levels of brain-derived neurotrophic factor in bipolar disorder: A meta-analytic study. *Neuroscience Letters*, *466*(3), 139–143.

Lindon, J. C., Holmes, E., & Nicholson, J. K. (2003). *Peer reviewed: So what's the deal with metabonomics?* American Chemical Society. Available from https://pubs.acs.org/sharingguidelines.

Lindon, J. C., & Nicholson, J. K. (2008). Analytical technologies for metabonomics and metabolomics, and multi-omic information recovery. *TrAC Trends in Analytical Chemistry*, *27*(3), 194–204.

Liu, Y., Blackwood, D. H., Caesar, S., De Geus, E. J. C., Farmer, A., Ferreira, M. A. R.... Wellcome Trust Case-Control Consortium. (2011). Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. *Molecular Psychiatry*, 2–4. Available from https://pubmed.ncbi.nlm.nih.gov/20351715/.

Löffler, M., Fairbanks, L. D., Zameitat, E., Marinaki, A. M., & Simmonds, H. A. (2005). Pyrimidine pathways in health and disease. *Trends in Molecular Medicine*, 430–437. Available from https://pubmed.ncbi.nlm.nih.gov/16098809/.

Mamas, M., Dunn, W. B., Neyses, L., & Goodacre, R. (2011). The role of metabolites and metabolomics in clinically applicable biomarkers of disease. *Archives of Toxicology*, *85*(1), 5–17. Available from https://pubmed.ncbi.nlm.nih.gov/20953584/.

Mansur, R. B., Santos, C. M., Rizzo, L. B., Asevedo, E., Cunha, G. R., Noto, M. N., ... Brietzke, E. (2016). Brain-derived neurotrophic factor, impaired glucose metabolism, and bipolar disorder course. *Bipolar Disorders*, *18*(4), 373–378. Available from http://doi.wiley.com/10.1111/bdi.12399.

Moore, C. M., Breeze, J. L., Gruber, S. A., Babb, S. M., Frederick, B. D. B., Villafuerte, R. A., ... Renshaw, P. F. (2000). Choline, myo-inositol and mood in bipolar disorder: A proton magnetic resonance spectroscopic imaging study of the anterior cingulate cortex. *Bipolar Disorders*, *2*(3 PART 2), 207–216. Available from https://pubmed.ncbi.nlm.nih.gov/11249799/.

Munkholm, K., Braüner, J. V., Kessing, L. V., & Vinberg, M. (2013). *Cytokines in bipolar disorder vs. healthy control subjects: A systematic review and meta-analysis*, . *Journal of Psychiatric Research* (47, pp. 1119–1133). Available from https://pubmed.ncbi.nlm.nih.gov/23768870/.

Nicholson, J. K. (2006). Global systems biology, personalized medicine and molecular epidemiology. *Molecular Systems Biology*. Available from https://pubmed.ncbi.nlm.nih.gov/17016518/.

O'Brien, S. M., Scully, P., Scott, L. V., & Dinan, T. G. (2006). Cytokine profiles in bipolar affective disorder: Focus on acutely ill patients. *Journal of Affective Disorders*, *90*(2−3), 263−267. Available from https://pubmed.ncbi.nlm.nih.gov/16410025/.

Odunsi, K., Wollman, R. M., Ambrosone, C. B., Hutson, A., McCann, S. E., Tammela, J., … Alderfer, J. L. (2005). Detection of epithelial ovarian cancer using 1H-NMR-based metabonomics. *International Journal of Cancer*, *113*(5), 782−788. Available from https://pubmed.ncbi.nlm.nih.gov/15499633/.

Oquendo, M. A., Currier, D., & Mann, J. J. (2006). Prospective studies of suicidal behavior in major depressive and bipolar disorders: What is the evidence for predictive risk factors? *Acta Psychiatrica Scandinavica*, 151−158. Available from https://pubmed.ncbi.nlm.nih.gov/16889585/.

Quinones, M. P., & Kaddurah-Daouk, R. (2009). Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. *Neurobiology of Disease*, 165−176. Available from https://pubmed.ncbi.nlm.nih.gov/19303440/.

Quiroz, J. A., Gray, N. A., Kato, T., & Manji, H. K. (2008). Mitochondrially mediated plasticity in the pathophysiology and treatment of bipolar disorder. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 2551−2565. Available from https://pubmed.ncbi.nlm.nih.gov/18235426/.

Rao, J. S., Harry, G. J., Rapoport, S. I., & Kim, H. W. (2010). Increased excitotoxicity and neuroinflammatory markers in post-mortem frontal cortex from bipolar disorder patients. *Molecular Psychiatry*, *15*(4), 384−392. Available from https://pubmed.ncbi.nlm.nih.gov/19488045/.

Ribeiro, H. C., Klassen, A., Pedrini, M., Carvalho, M. S., Rizzo, L. B., Noto, M. N., … Sussulini, A. (2017). A preliminary study of bipolar disorder type I by mass spectrometry-based serum lipidomics. *Psychiatry Research*, *258*(August), 268−273.

Russell, M. R., & Lilley, K. S. (2012). Pipeline to assess the greatest source of technical variance in quantitative proteomics using metabolic labelling. *Journal of Proteomics*, *77*, 441−454. Available from https://pubmed.ncbi.nlm.nih.gov/23079070/.

Ryan, D., & Robards, K. (2006). *Metabolomics: The greatest omics of them all? Analytical Chemistry* (78, pp. 7954−7958). Available from https://pubs.acs.org/doi/abs/10.1021/ac0614341.

Sabatine, M. S., Liu, E., Morrow, D. A., Heller, E., McCarroll, R., Wiegand, R., … Gerszten, R. E. (2005). Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation*, *112*(25), 3868−3875. Available from https://pubmed.ncbi.nlm.nih.gov/16344383/.

Scaini, G., Valvassori, S. S., Diaz, A. P., Lima, C. N., Benevenuto, D., Fries, G. R., … Quevedo, J. (2020). Neurobiology of bipolar disorders: A review of genetic components, signaling pathways, biochemical changes, and neuroimaging findings. *Brazilian Journal of Psychiatry*, *42*(5), 536−551. Available from http://www.scielo.br/scielo.php?script = sci_arttext&pid = S1516-44462020005007202&tlng = en.

Schröter, K., Brum, M., Brunkhorst-Kanaan, N., Tole, F., Ziegler, C., Domschke, K., … Kittel-Schneider, S. (2019). Longitudinal multi-level biomarker analysis of BDNF in major depression and bipolar disorder. *European Archives of Psychiatry and Clinical Neuroscience*. Available from http://www.ncbi.nlm.nih.gov/pubmed/30929061.

Schulze, T. G., Detera-Wadleigh, S. D., Akula, N., Gupta, A., Kassem, L., Steele, J., … McMahon, F. J. (2009). Two variants in Ankyrin 3 (ANK3) are independent genetic risk

factors for bipolar disorder. *Molecular Psychiatry*, *14*(5), 487–491. Available from https://pubmed.ncbi.nlm.nih.gov/19088739/.

Scola, G., & Andreazza, A. C. (2014). Current state of biomarkers in bipolar disorder. *Current Psychiatry Reports*, 1–8. Available from https://link.springer.com/article/10.1007/s11920-014-0514-4.

Scott, L. J., Muglia, P., Kong, X. Q., Guan, W., Flickinger, M., Upmanyu, R., … Boehnke, M. (2009). Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(18), 7501–7506. Available from https://pubmed.ncbi.nlm.nih.gov/19416921/.

Sethi, S., & Brietzke, E. (2015). Omics-based biomarkers: Application of metabolomics in neuropsychiatric disorders. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *19*(3), 1–13.

Sklar, P., Ripke, S., Scott, L. J., Andreassen, O. A., Cichon, S., Craddock, N., … Purcell, S. M. (2011). Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. *Nature Genetics*, *43*(10), 977–985. Available from https://pmc/articles/PMC3637176/?report = abstract.

Sklar, P., Smoller, J. W., Fan, J., Ferreira, M. A. R., Perlis, R. H., Chambert, K., … Purcell, S. M. (2008). Whole-genome association study of bipolar disorder. *Molecular Psychiatry*, *13*(6), 558–569. Available from https://pubmed.ncbi.nlm.nih.gov/18317468/.

Södersten, K., Pålsson, E., Ishima, T., Funa, K., Landén, M., Hashimoto, K., & Agren, H. (2014). Abnormality in serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in mood-stabilized patients with bipolar disorder: A study of two independent cohorts. *Journal of Affective Disorders*, *160*, 1–9. Available from https://pubmed.ncbi.nlm.nih.gov/24709015/.

Sousa, R. T. D. E. (2014). Fisiopatologia do Transtorno de Humor Bipolar e efeito do tratamento com lítio: Enfoque em neuroproteção e função mitocondrial. 138.

Stahl, E. A., Breen, G., Forstner, A. J., McQuillin, A., Ripke, S., Trubetskoy, V.… Bipolar Disorder Working Group of the Psychiatric Genomics Consortium. (2019). Genome-wide association study identifies 30 loci associated with bipolar disorder. *Nature Genetics*, *51*(5), 793–803.

Stoop, M. P., Coulier, L., Rosenling, T., Shi, S., Smolinska, A. M., Buydens, L., … Luider, T. M. (2010). Quantitative proteomics and metabolomics analysis of normal human cerebrospinal fluid samples. *Molecular & Cellular Proteomics*, *9*(9), 2063–2075. Available from https://pmc/articles/PMC2938111/?report = abstract.

Strauss, J. S., Khare, T., de Luca, V., Jeremian, R., Kennedy, J. L., Vincent, J. B., & Petronis, A. (2013). Quantitative leukocyte BDNF promoter methylation analysis in bipolar disorder. *International Journal of Bipolar Disorders.*, *1*(1), 1–9.

Sussulini, A., Prando, A., Maretto, D. A., Poppi, R. J., Tasic, L., Banzato, C. E. M., & Arruda, M. A. Z. (2009). Metabolic profiling of human blood serum from treated patients with bipolar disorder employing 1H NMR spectroscopy and chemometrics. *Analytical Chemistry*, *81*(23), 9755–9763. Available from https://pubmed.ncbi.nlm.nih.gov/19863109/.

Turck, C. W., & Filiou, M. D. (2015). What have mass spectrometry-based proteomics and metabolomics (not) taught us about psychiatric disorders? *Molecular Neuropsychiatry*, *1*(2), 69–75.

Van Doorn, M., Vogels, J., Tas, A., Van Hoogdalem, E. J., Burggraaf, J., Cohen, A., & van der Greef, J. (2007). Evaluation of metabolite profiles as biomarkers for the pharmacological effects of thiazolidinediones in type 2 diabetes mellitus patients and healthy volunteers.

British Journal of Clinical Pharmacology, *63*(5), 562–574. Available from https://pubmed.ncbi.nlm.nih.gov/17488363/.

Van Enkhuizen, J., Janowsky, D. S., Olivier, B., Minassian, A., Perry, W., Young, J. W., & Geyer, M. A. (2015). The catecholaminergic-cholinergic balance hypothesis of bipolar disorder revisited. *European Journal of Pharmacology*, *753*, 114–126. Available from https://pmc/articles/PMC4318788/?report = abstract.

Vancampfort, D., Correll, C. U., Galling, B., Probst, M., De Hert, M., Ward, P. B., & Stubbs, B. (2016). Diabetes mellitus in people with schizophrenia, bipolar disorder and major depressive disorder: A systematic review and large scale meta-analysis. *World Psychiatry*, *15*(2), 166–174. Available from https://pubmed.ncbi.nlm.nih.gov/27265707/.

Versace, A., Almeida, J. R. C., Quevedo, K., Thompson, W. K., Terwilliger, R. A., Hassel, S., … Phillips, M. L. (2010). Right orbitofrontal corticolimbic and left corticocortical white matter connectivity differentiate bipolar and unipolar depression. *Biological Psychiatry*, *68*(6), 560–567. Available from https://pubmed.ncbi.nlm.nih.gov/20598288/.

Villaseñor, A., Ramamoorthy, A., Silva Dos Santos, M., Lorenzo, M. P., Laje, G., Zarate, C., … Wainer, I. W. (2014). A pilot study of plasma metabolomic patterns from patients treated with ketamine for bipolar depression: Evidence for a response-related difference in mitochondrial networks. *British Journal of Pharmacology*, *171*(8), 2230–2242.

Warrack, B. M., Hnatyshyn, S., Ott, K. H., Reily, M. D., Sanders, M., Zhang, H., & Drexler, D. M. (2009). Normalization strategies for metabonomic analysis of urine samples. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *877*(5–6), 547–552. Available from https://pubmed.ncbi.nlm.nih.gov/19185549/.

Williams, R., Lenz, E. M., Wilson, A. J., Granger, J., Wilson, I. D., Major, H., & Plumb, R. (2006). A multi-analytical platform approach to the metabonomic analysis of plasma from normal and zucker (fa/fa) obese rats. *Molecular BioSystems*, *2*(3–4), 174–183. Available from https://pubmed.ncbi.nlm.nih.gov/16880935/.

Wood, P. L. (2014). Mass spectrometry strategies for clinical metabolomics and lipidomics in psychiatry, neurology, and neuro-oncology. *Neuropsychopharmacology*, 24–33. Available from https://pmc/articles/PMC3857645/?report = abstract.

Xiaohui, F., Jingqing, B., & Peng, S. (2005). Diagnosis of breast cancer using HPLC metabonomics fingerprints coupled with computational methods. In *Annual international conference of the IEEE engineering in medicine and biology - proceedings* (pp. 6081–6084). Available from: https://pubmed.ncbi.nlm.nih.gov/17281650/.

Xu, X. J., Zheng, P., Ren, G. P., Liu, M. L., Mu, J., Guo, J., … Xie, P. (2014). 2,4-Dihydroxypyrimidine is a potential urinary metabolite biomarker for diagnosing bipolar disorder. *Molecular Biosystems*, *10*(4), 813–819.

Yang, J., Chen, T., Sun, L., Zhao, Z., Qi, X., Zhou, K., … Wan, C. (2013). Potential metabolite markers of schizophrenia. *Molecular Psychiatry*, *18*(1), 67–78. Available from https://pubmed.ncbi.nlm.nih.gov/22024767/.

Yang, J., Xu, G., Zheng, Y., Kong, H., Pang, T., Lv, S., & Yang, Q. (2004). Diagnosis of liver cancer using HPLC-based metabonomics avoiding false-positive result from hepatitis and hepatocirrhosis diseases. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences.*, *813*(1–2), 59–65.

Yap, I. K. S., Angley, M., Veselkov, K. A., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2010). Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. *Journal of Proteome Research*, *9*(6), 2996–3004.

Yoshimi, N., Futamura, T., Bergen, S. E., Iwayama, Y., Ishima, T., Sellgren, C., … Hashimoto, K. (2016). Cerebrospinal fluid metabolomics identifies a key role of isocitrate

dehydrogenase in bipolar disorder: Evidence in support of mitochondrial dysfunction hypothesis. *Molecular Psychiatry*, *21*(11), 1504–1510.

Yoshimi, N., Futamura, T., Kakumoto, K., Salehi, A. M., Sellgren, C. M., Holmén-Larsson, J., … Hashimoto, K. (2016). Blood metabolomics analysis identifies abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism in bipolar disorder. *BBA Clinical*, *5*, 151–158.

Zhang, A., Sun, H., & Wang, X. (2012). Serum metabolomics as a novel diagnostic approach for disease: A systematic review. *Analytical and Bioanalytical Chemistry*, 1239–1245. Available from https://pubmed.ncbi.nlm.nih.gov/22648167/.

Zhang, R., Zhang, T., Ali, A. M., Washih, M. A., Pickard, B., & Watson, D. G. (2016). Metabolomic profiling of post-mortem brain reveals changes in amino acid and glucose metabolism in mental illness compared with controls. *Computational and Structural Biotechnology Journal*, *14*, 106–116.

Zheng, P., Wei, Y. D., Yao, G. E., Ren, G. P., Guo, J., Zhou, C. J., … Xie, P. (2013). Novel urinary biomarkers for diagnosing bipolar disorder. *Metabolomics: Official Journal of the Metabolomic Society*, *9*(4), 800–808.