

# Chapter 14

## Metabolomic Biomarkers in Mental Disorders: Bipolar Disorder and Schizophrenia



Melissa Quintero, Danijela Stanisic, Guilherme Cruz, João G. M. Pontes, Tássia Brena Barroso Carneiro Costa, and Ljubica Tasic

**Abstract** Psychiatric disorders are some of the most impairing human diseases. Among them, bipolar disorder and schizophrenia are the most common. Both have complicated diagnostics due to their phenotypic, biological, and genetic heterogeneity, unknown etiology, and the underlying biological pathways, and molecular mechanisms are still not completely understood. Given the multifactorial complexity of these disorders, identification and implementation of metabolic biomarkers would assist in their early detection and diagnosis and facilitate disease monitoring and treatment responses. To date, numerous studies have utilized metabolomics to better understand psychiatric disorders, and findings from these studies have begun to converge. In this chapter, we briefly describe some of the metabolomic biomarkers found in these two disorders.

**Keywords** Psychiatric illness · Mental disorders · Schizophrenia · Bipolar disorder · Biomarkers · Metabolomics

### 14.1 Introduction

Millions of people experience mental or neuropsychiatric disorders including bipolar affective disorder and schizophrenia, among others. These mental illnesses, usually observed in childhood, adolescence, and early adulthood, are characterized by

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M. Quintero · D. Stanisic · G. Cruz · T. B. B. C. Costa · L. Tasic (✉)  
Laboratory of Chemical Biology, Department of Organic Chemistry, Institute of Chemistry, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil  
e-mail: [ljubica@unicamp.br](mailto:ljubica@unicamp.br)

J. G. M. Pontes  
Laboratory of Microbial Chemical Biology, Institute of Chemistry, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil

a combination of abnormal thoughts, emotions, behaviors, and disturbed perception of reality, affecting not only the person with illness but also society. While not being completely understood in terms of their causes, the symptoms of mental illnesses are scientifically valid and well known. Treatments are readily available and include psychotherapy and medication and are effective for most people, but there are no specific tests that can be used as an add-on for diagnosis. As such, there are high levels of curiosity to understand if molecular biomarkers can assist in making clearer diagnostic decisions.

Biomarker research has been an extensive success in various medical fields so far, but using biomarkers to diagnose and predict treatment response for mental disorders is still a challenge [1]. Based on observations that a specific compound may be present or altered just in patients with a given mental disorder and not in healthy individuals, a valid biomarker can be found if successfully detected. However, the fundamental definition of a psychiatric disorder is based on subjective and/or behavioral criteria which are determined clinically, making it difficult to determine whether or not a person has a particular disorder [2].

The search for biomarkers for psychiatric disorders has a long history, with earlier studies investigating molecular markers, like platelet imipramine binding or cerebrospinal 5-hydroxyindoleacetic acid (5-HIAA) in people with depression [3], or behavioral markers such as smooth pursuit eye movements in people with schizophrenia [4]. Currently, global profiling approaches, such as metabolomics, are expected to be able to pinpoint discriminating molecules as clinical biomarkers, providing an overview of the metabolic status and global biochemical events associated with a particular cellular or biological system [5]. Metabolomics has the potential to scrutinize candidate markers that will improve the diagnosis of the diseases facilitating better patient prognoses and, thus, the development of novel therapeutic strategies [6]. Metabolic markers in diagnosis are thought to be one of the most interesting categories of biomarkers, given their role in physiological processes. Since a biomarker should be detected and measured in a sample obtained using noninvasive procedures, body fluids including plasma/serum, urine, saliva, and, to some extent, cerebrospinal fluid (CSF) are thought to be useful sources for biomarker monitoring [5]. Furthermore, the scientific synergy between biomarkers and metabolomics is important as metabolomics has been used in many instances to identify novel biomarkers which can lead to new and improved therapeutic strategies for many serious and life-threatening diseases. Preliminary metabolomic signatures and some biomarkers have already been described for schizophrenia and bipolar disorder [7–11], but the endophenotype specificities are still under discussion, and identification of illness-specific biomarkers capable of adding not only to the diagnosis process but for use in monitoring prognosis or clinical response to treatment is still lacking. We need to improve the understanding of the biological abnormalities in psychiatric illnesses across conventional diagnostic boundaries. This review focuses on metabolomic biomarkers for bipolar disorder and schizophrenia.

### 14.1.1 *Metabolomic Biomarkers*

The use of metabolomics in the examination for novel biomarkers in different clinical areas is based on the hypothesis that diseases cause disruption of biochemical pathways leading to a metabolic fingerprint characteristic of the site and nature of the disease [12]. The term “biomarker” or “biological marker” was first used in 1989 as a medical subject heading to mean “measurable and quantifiable biological parameters which serve as indexes for health- and physiology-related assessments such as disease risk, psychiatric disorders, environmental exposure and its effects, disease diagnosis, metabolic processes, substance abuse, epidemiologic studies etc.” In 2001 that definition was standardized and further defined. One of the earliest biomarker approaches in psychiatry [13] employed chromatography to detect a urinary metabolite, 3,4-dimethoxyphenylethylamine (later identified as *p*-tyramine [14]), which formed a controversial “pink spot” on paper chromatograms among those with schizophrenia but not in healthy individuals. Since then, genomic and transcriptomic approaches have dominated biomarker discovery efforts in psychiatric disorders. However, the global study of metabolites (i.e., metabolomics) emerged later as a promising approach for identification of potential diagnostic and treatment response biomarkers for psychiatric disorders [15].

Currently, there are several types of metabolomic biomarkers, which enable response prediction or dynamical description of both disease progression and treatment effectiveness. The integration between clinical metabolomics and pharmacology may allow the discovery of more meaningful biomarkers which could enable the development of individualized treatment methods. The success of this integration depends on the explicit consideration of study designs and data analysis techniques that can effectively quantify sources of biological variability [16].

To date, the most explored bioanalytical platforms in metabolomics research are nuclear magnetic resonance (NMR) and mass spectrometry (MS) [17]. The recent rapid development of a range of analytical platforms including gas chromatography, liquid chromatography techniques like high-performance liquid chromatography (HPLC) or ultra-performance liquid chromatography (UPLC), and capillary electrophoresis, and the use of these as hyphenated analytical methods coupled to MS or NMR, created new possibilities for biomarker research. Other techniques popular in metabolomic research include magnetic resonance imaging (MRI) and high-resolution magic angle spinning spectroscopy (HR-MAS). Bioanalytical platforms enable separation, detection, characterization, and quantification of metabolites, and then we can relate this information to altered metabolic pathways. Due to the complexity of the metabolome and the diverse properties of metabolites, no single analytical platform can detect all of the metabolites in a biological sample. The combined use of modern instrumental analytical approaches has helped to increase the coverage of detected metabolites that cannot be achieved by single-analysis techniques [18–20]. Integrated platforms have been frequently used to provide sensitive and reliable detection of thousands of metabolites in a biofluid sample (Fig. 14.1).

## BIOMARKERS: HOW DO WE FIND THEM?

Differences among samples are discovered and assigned to biomarkers by comparison with databases and molecular fingerprints.

## BIG DATA TREATMENTS

Acquired data are processed using multivariate statistical analyses.

## DATA ACQUISITION

The samples can be submitted to different bioanalyses by some techniques, such as, NMR, MS/MS, MRI (*in vivo*).

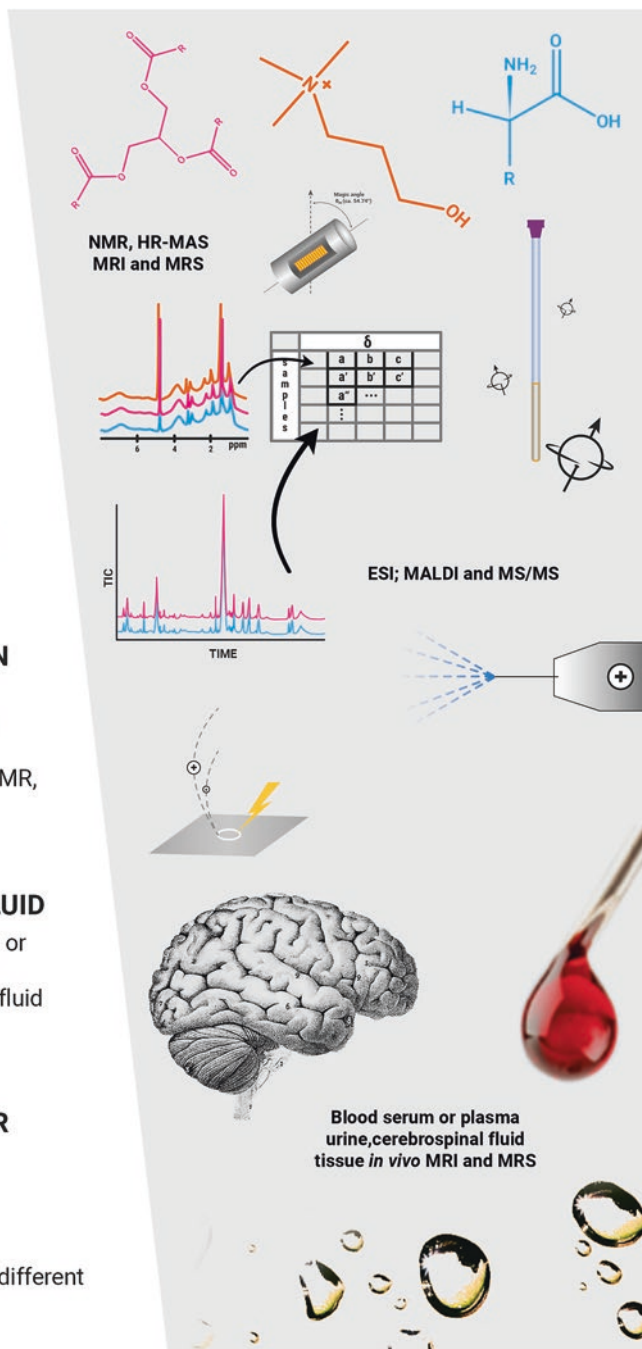
## TISSUE AND BIOFLUID

A tiny amount of tissue or biofluid, such as, urine, blood or cerebrospinal fluid is collected.

## MENTAL DISORDER DIAGNOSIS

Classified according to the ICD-10 indexes.

Similar symptoms, but different illnesses.



**Fig. 14.1** Workflow in untargeted metabolomic studies. After clinical evaluation and sample collection from at least two groups (e.g., disease and healthy), an appropriate analytical method or combination of more than one should be used for sample assessment and the resulting large amounts of data analyzed for differences that might point to mental disorder biomarkers

Bioanalytical platforms in metabolomics start from a comparative analysis among at least two groups of samples and rely on big data processing by chemometrics. The most used of the data processing techniques are multivariate statistical analyses such as principal component analysis (PCA) to explore the data and detect outliers [21, 22], partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) to classify the samples and identify the main metabolites responsible for the discrimination [23, 24], and logistic regression to evaluate the metabolites as biomarkers [25]. The list of tools available for spectral processing and data analysis are available in Alonso et al. [19].

## 14.2 Biomarkers in Psychiatric Disorders

### 14.2.1 Metabolomic Biomarkers in Bipolar Disorder

Bipolar disorder is chronic and recurrent disorder that affects around 1% of the global population [26–28], leading to disabilities in young people (mostly between 18 and 44 years of age), such as cognitive and functional impairment, and increased mortality particularly from suicide and cardiovascular disease [29, 30]. Bipolar disorder is characterized as biphasic moods that include depression and mania (in some cases hypomania) and which occur as recurrent episodes of changes in behavior of individuals. There are several subclassifications of this disorder including bipolar I, bipolar II, cyclothymia, and other atypical forms, depending on severity and duration of depressive and manic episodes [29]. The diagnostic error still leads to erroneous treatment, increasing patient suffering, and, therefore, greater suicide risk [31].

Bipolar disorder is a major cause of comorbidity because it directly affects the productivity of people making the disease a socioeconomic problem, besides being a major cause of death, and 25% of those affected by the disease attempt suicide with 11% success [30, 32–34]. Bipolar disorder is a historically known disease with well-documented epidemiology [30]. In the early nineteenth century, it was believed that the cause of most diseases was a problem of uric acid metabolism. By exerting influence on common diseases such as gout, high blood pressure, and renal calculus, it was also attributed to dementia, schizophrenia, mania, depression, and bipolar disorder [31]. Lithium was discovered as a chemical element in the same century. In the tests performed with its salts, mainly lithium carbonate, it was found that it is capable of dissolving crystals of uric acid. Based on this information, psychiatrists started prescribing lithium carbonate ( $\text{Li}_2\text{CO}_3$ ) for mental disorders. This study could be considered as one of the first metabolomic research instances. Other studies showed that  $\text{Li}^+$  was effective against bipolar disorder [31, 35, 36].

Bipolar disorder biomarkers have been studied in blood, serum, urine, and plasma by  $^1\text{H}$  NMR [10, 37–39], by gas chromatography (GC)-MS [38, 40, 41], and through in vivo brain imaging experiments [42–44]. Table 14.1 lists some of metabolites that are altered for this type of mental disorder including

**Table 14.1** Specific biomarkers related to bipolar disorders

Biomarkers	Sample type	Analytical platforms
Acetate-choline [39]	Human blood serum	NMR
Acetone [10, 45]	Female urine	NMR
<i>N</i> -Acetyl-aspartate [46]	Brain	MRS
<i>N</i> -Acetyl-aspartate (NAA)/PCr-Cr [47]	White matter	MRI
<i>N</i> -Acetyl-aspartyl-glutamate [46]	Brain	MRS
<i>N</i> -Acetyl-aspartyl-glutamic acid [37]	Human blood serum	NMR
<i>N</i> -Acetyl-glutamic acid [48]	Serum	CE-TOFMS
<i>N</i> -Acetyl-phenylalanine [37]	Human blood serum	NMR
Adipic acid [10]	Urine	GC-MS
$\beta$ -Alanine [10, 38, 48]	Urine	<sup>1</sup> H-NMR, GC-MS, CE-TOFMS
$\gamma$ -Aminobutyric acid (GABA) [42, 49]	Brain, temporal lobes	MRS, MRI
$\beta$ -Amino- <i>isobutyric</i> acid [10]	Urine	GC-MS
Amygdalin [37]	Human blood serum	NMR
Arabitol [10]	Urine	GC-MS
Arginine [48]	Serum	CE-TOFMS
Azelaic acid [10, 38]	Urine	NMR, GC-MS
Choline [9, 37, 45]	Human blood serum, urine, male urine	NMR
Citrulline [50]	Plasma of drug-free patients	CE-TOFMS
Creatine [42–44, 46]	Brain, white and gray matter, cerebrospinal fluid	MRS, MRI
Formate [10, 45]	Male urine	NMR
Glutamate [39, 42–44]	Brain, human blood serum, white and gray matter, cerebrospinal fluid	MRS, MRI, NMR
Glutamine [37, 42–44]	Human blood serum, brain, white and gray matter, cerebrospinal fluid	NMR, MRS, MRI
Glycine [10]	Urine	GC-MS
Glycerol-phosphocholine + phosphocholine (GPC + PC) [47]	White matter	MRI
Glycolate [10]	Urine	NMR
5-HIAA [51]	White matter	HPLC, DTI
5-HT serotonin neurotransmitter [51]	White matter	HPLC, DTI
4-Hydroxybenzoic acid [10]	Urine	GC-MS
$\alpha$ -Hydroxybutyrate [9, 10, 38, 45]	Urine	NMR, GC-MS
5-Hydroxy-hexanoic acid [10]	Urine	GC-MS
D-Hydroxy-pyrimidine [10, 38, 41]	Urine	NMR, GC-MS
<i>Isobutyrate</i> [9]	Urine	NMR
<i>Isocitric</i> acid ( <i>isocitrate</i> ) [52]	CFS (rats)	CE-TOFMS
$\alpha$ -Ketoglutaric acid [37]	Human blood serum	NMR

(continued)

**Table 14.1** (continued)

Biomarkers	Sample type	Analytical platforms
Kynurenine, kynurenic acid and 3-hydroxy-kynurenine [51]	White matter	HPLC, DTI
Lactate[10]	Urine	NMR
Lipids and lipoamide [37, 39]	Human blood serum	NMR
Mannitol [10]	Urine	GC-MS
Methylmalonic acid [10]	Urine	GC-MS
<i>N</i> -Methyl-nicotinamide [45]	Urine	NMR
<i>Myo</i> -inositol [37, 39, 53]	Human blood serum	NMR
Oxalacetate [45]	Female urine	NMR
Phenylalanine [10]	Urine	GC-MS
Phosphatidic acid (44:4) [54]	Human blood serum	UHPLC-MS
Phosphatidic acid (48:8 (OH)) [54]	Human blood serum	UHPLC-MS
Phosphatidylethanolamine (42:5) [54]	Human blood serum	UHPLC-MS
Phosphatidylglycerol (32:4 (OH)) [54]	Human blood serum	UHPLC-MS
Phosphatidylinositol (40:3) [54]	Human blood serum	UHPLC-MS
Phosphocreatine-creatine (PCr-Cr) [44, 47]	White matter and gray matter, cerebrospinal fluid	MRI
Propionate [10]	Urine	NMR
Pseudouridine [38]	Urine	NMR, GC-MS
Pyruvate	Serum	CE-TOFMS
Pyroglutamic acid [10]	Urine	GC-MS
Serine [48]	Serum	CE-TOFMS
Triacylglycerol (42:3) [54]	Human blood serum	UHPLC-MS
Tryptophan [51]	White matter	HPLC, DTI
5-Hydroxyindole-3-acetic acid (5-HIAA) [51]	White matter	HPLC, DTI
5-Hydroxytryptamine (5-HT) serotonin neurotransmitter [51]	White matter	HPLC, DTI

$\alpha$ -hydroxybutyrate, choline, *isobutyrate*, and *N*-methylnicotinamide which differentiate patients with bipolar disorders from healthy individuals, while propionate, formate, 2,3-dihydroxybutanoic acid, phenylalanine, 2,4-dihydroxypyrimidine, and  $\beta$ -alanine were differentiated in patients with bipolar disorder and mild cognitive disorders [9, 41, 55].

In some studies, such as  $^1\text{H-NMR}$  analysis of anterior cingulate cortex of brain tissue, a higher concentration of *myo*-inositol in patients with bipolar disorder was found and when patients underwent treatment with lithium-based drugs, the concentration of *myo*-inositol was decreased considerably [53]. These data were reinforced by the metabolomic analysis of blood serum [37]. *Myo*-inositol is synthesized from special plasma membrane lipids by inositol monophosphatase type II (IMPase II), which is a candidate enzyme for  $\text{Li}^+$  since it is inhibited by  $\text{Li}^+$  in tests using pharmacologically relevant concentrations in vitro [56]. *Myo*-inositol acts as a secondary messenger



and is responsible for immobilizing calcium ( $\text{Ca}^{2+}$ ) stored inside the cell, which acts as a prosthetic agent of other enzymes such as hexokinase. In general, *myo*-inositol is able to initiate several metabolic paths vital to cellular functions [57]. The action of  $\text{Li}^+$  may be linked to the competitive inhibition of inositol monophosphatase II, which would lead to a decrease in *myo*-inositol concentrations in neurons, which depend on this enzyme to synthesize it since *myo*-inositol does not cross the blood-brain barrier [58].

On the other hand, glutamate, glutamine, creatine, and the ratios among these metabolites are the most cited in the literature, and NMR, MRI, LC-MS, and HPLC techniques have been used for their detection [37, 39, 42–44]. Glutamate is one of the most abundant neurotransmitters in the brain, as a precursor of glutamine,  $\gamma$ -aminobutyric acid (GABA), and glutathione. It is one of the structural components of proteins and an intermediate metabolite [59–61]. The differences between metabolites indicated by Sethi et al. [37] lead to conclusion that some possible biomarkers for bipolar disorder are  $\alpha$ -ketoisovaleric acid,  $\alpha$ -ketoglutaric acid, *N*-acetyl-aspartyl glutamic acid, *N*-acetyl-phenylalanine, and glutamine, which were detected or altered only in bipolar disorder patients, while *N*-acetyl-alanine was detected only in controls [37]. *N*-acetyl-aspartate, *N*-acetyl-aspartyl-glutamate, creatine, and phosphocreatine are some of the metabolites suggested by Haarman et al. [46] as the levels of these were decreased in the left hippocampus after MRS quantification using LC Model. The authors hypothesized that these results are a consequence of diminished neuronal integrity in this region. *N*-acetyl-aspartate is the second most abundant substance in the central nervous system, formed in mitochondria from acetyl-CoA and aspartate. Phosphocreatine and creatine are important buffers in the brain and muscle tissues, providing energy and a constant ATP concentration, and a decrease in their concentrations might be due to cell death [46].

A lipidomic study in blood serum employing ultra-HPLC (UHPLC) coupled with high-resolution MS (HRMS) identified lipid profiles of bipolar disorder patients and healthy individuals. Sphingolipids and glycerolipids were increased, while glycerophospholipids were decreased in patient serum samples. Of the lipids with greater differential proportions in the groups, the authors concluded that phospholipid biosynthesis is the most altered pathway in bipolar disorder patients [54].

Recently, Soeiro-de-Souza et al. [43] investigated and compared healthy individuals with bipolar disorder I and II patients, who were treated with  $\text{Li}^+$ , anticonvulsants, and antipsychotics. The authors carried out brain imaging using magnetic resonance spectroscopy and quantified glutamate, glutamine, and creatine and their ratios after treatment. One of their conclusions was that bipolar disorder patients and controls had the same volume of white matter but lower volumes of anterior cingulate cortex and a higher volume of CSF. Also, the change in the concentration of glutamate was observed in treated patients. Moreover, they found a lower ratio of glutamate/creatine during medication and a constant glutamine/creatine ratio. By comparing bipolar subjects with healthy individuals, the higher glutamate/creatine and glutamine/creatine ratios were observed after omitting medications, which might be indicative that their increments were not caused by medication use [43]. The concentration of glutamine was high, and the glutamate/glutamine ratio and



glutamate concentrations were lower in the patient group when compared to the healthy controls. Pairing the groups by age and gender, lower glutamate concentrations and glutamate/glutamine ratios were observed in the bipolar patients [42].

Zheng et al. [9] examined urinary samples using a NMR-based metabolomic method and found that choline, *isobutyrate*,  $\alpha$ -hydroxybutyrate, and *N*-methylnicotinamide could be good urinary metabolite biomarkers for bipolar disorder (AUC of 0.89 for training samples and 0.86 in the sample test). They found that the increase of  $\alpha$ -hydroxybutyrate was derived from elevated levels of  $\alpha$ -ketobutyrate, which is consequence of a higher conversion rate of cystathionine to cysteine. The altered levels of choline in bipolar patients' urine could be due to the abnormalities in the acetylcholine neurotransmission precursor mechanism and abnormal phospholipid metabolism [9, 62]. The higher concentration of *isobutyrate* in urine is a result of a lower blood concentration of valine, which can influence an increase in concentration of catecholamines and serotonin. The *N*-methylnicotinamide concentration was decreased in bipolar subjects being an end product of nicotinamide processing in the tryptophan-NAD<sup>+</sup> pathway. Tryptophan is a precursor of kynurenine and serotonin metabolism, and the decrease in metabolites of nicotinic acid leads to an increase in kynurenine metabolism [9]. Five metabolites were specified as potential biomarkers (pseudouridine,  $\beta$ -alanine,  $\alpha$ -hydroxybutyrate, 2,4-dihydropyrimidine, and azelaic acid) in bipolar disorder by a combined <sup>1</sup>H-NMR/GC-MS approach [38]. Previous studies from the same group suggested that 2,4-dihydropyrimidine, *N*-methylnicotinamide, choline, *isobutyrate*, and  $\alpha$ -hydroxybutyrate are as possible metabolites for diagnosis of bipolar disorder using urine samples examined by GC-MS analysis [9, 38, 41].

Another study that connects kynurenic acid and metabolites from kynurenine metabolism to bipolar disorder is from Poletti et al. [51]. Kynurenine and kynurenic acid were determined from bipolar disorder patient plasma using HPLC and diffusion tensor imaging (DTI) in an evaluation of white matter microstructure. There is a hypothesis that serotonergic turnover in bipolar disorder could influence the white matter of brain microstructure as that changes in myelination and integrity of myelin can influence signal speed and communication with different brain areas [63–65]. Through application of DTI, it was possible to confirm the hypothesis in a homogeneous sample of patients with bipolar disorder and to link kynurenine acid and 5-hydroxyacetic acid with changes in white matter microstructure. The result demonstrated an inefficient turnover of serotonin, but this did not affect tryptophan, despite the lower concentration of the latter in blood samples [51].

### 14.2.2 *Metabolomic Biomarkers in Schizophrenia*

Schizophrenia is a chronic psychiatric disorder with a heterogeneous genetic and neurobiological background that influences early brain development and is expressed as a combination of psychotic symptoms such as hallucinations and delusions, along with organizational, motivational, and cognitive dysfunctions [66, 67].

It starts in late adolescence or early adulthood and affects approximately 0.5–1% of the world population with high heritability [68].

Abnormalities in neurotransmission have provided the basis for theories on the pathophysiology of schizophrenia. Most of these theories center on either an excess or a deficiency of neurotransmitters such as dopamine, serotonin, and glutamate. Other theories implicate aspartate, glycine, and  $\gamma$ -aminobutyric acid (GABA) as part of the neurochemical imbalance in schizophrenia [69]. Another theory for the symptoms of schizophrenia involves the activity of glutamate, the major excitatory neurotransmitter in the brain. This theory has arisen in response to the finding that phencyclidine and ketamine, both noncompetitive NMDA/glutamate antagonists, could induce schizophrenia-like symptoms [70]. However, there is not a consensus on whether there is an increase [71, 72] or a decrease [73, 74] of these metabolite concentrations. Metabolites, like tele-methylhistamine (t-MH), were detected by GC-MS in CSF samples of schizophrenia patients. Other studies showed that t-MH is associated with an increase of histaminergic activity and desensitization of neural tissues due to decreases of histamine H1 and H2 receptors and an increase of histamine concentrations [75].

Several metabolomic studies have also pointed to some metabolic abnormalities (Table 14.2). He et al. [8] demonstrated differences in amino acid and lipid metabolism in medicated and non-medicated schizophrenia patients when compared to the control group. Subsequent analyses of these potentially relevant metabolites as well as analysis of known schizophrenia risk genes identified the glutamine and arginine signaling pathways as possible risk factors. Another study [71] raised the possibility that there are at least two different schizophrenia-related risk pathways, and these are involved in glucoregulation and proline metabolism. In addition to glutamine and arginine metabolism, amino acids altered in plasma or CSF of schizophrenia patients have been linked to nitrogen compound biosynthetic processes. The finding of changes in certain lipids, fatty acids, and amino acids has implicated phospholipid synthesis [85]. The use of metabolic profiles in CSF from drug-naïve patients compared with matched controls found elevated glucose concentrations in patients, whereas the serum glucose concentration showed no differences [7]. Lipid analysis in plasma from patients with schizophrenia compared with that of healthy individuals revealed significantly lower concentrations of lipids [87]. Potential metabolite markers consisted of several fatty acids and ketone bodies, and the presumed upregulated fatty acid catabolism may result from an insufficiency of glucose supply in the brains of patients with schizophrenia [72]. Also, glycine and serine are amino acids that have frequently been reported as markers of schizophrenia due to their significant changes in the metabolic profile among patients and healthy individuals [84, 88, 89].

Noninvasive analyses of schizophrenia patient expired breaths indicated a higher level of alkanes when compared to healthy individuals. The level of ethane and pentane, which cross the pulmonary alveolar membrane, is result of dead cells due to peroxidation of cell membrane lipids. Other alkanes such as butane were not detected. Furthermore, pentane is not a specific biomarker for schizophrenia since it

**Table 14.2** Specific biomarkers related to schizophrenia

Biomarkers	Sample type	Analytical platforms
Acetoacetate [72]	Urine	GC-TOFMS and <sup>1</sup> H-NMR
Acetone [72]	Urine	GC-TOFMS and <sup>1</sup> H-NMR
<i>N</i> -Acetyl-aspartate [76]	Serum	GC-MS
<i>Cis</i> -aconitic acid [72]	Urine	GC-TOFMS
Allantoin [77]	Serum	GC-MS
2-Amino adipic acid [72]	Urine	GC-TOFMS
α-Aminobutyrate (AABA) [72]	Serum Urine	GC-TOFMS
γ-Aminobutyric acid (GABA) [77]	Blood	LC-ESI/MS/MS
Arachidonic acid [78]	Serum	HPLC-MS HPLC fluorescence
Arginine [8]	Plasma	MS
Aspartic acid [40, 72, 76]	Serum Peripheral blood mononuclear cells (PBMC)	GC-MS GC-TOFMS
Benzoic acid [40]	PBMC	GC-MS
Betaine [77]	Blood and plasma	CE-TOFMS
1,3-Bisphosphoglycerate [76]	Serum	GC-MS
Carbon disulfide [79]	Breath	GC-MS
Catechol [72]	Urine	GC-TOFMS
Cholesterol [76, 80]	Serum	GC-MS
Citrate [7, 72, 76]	Serum CSF	GC-MS GC-TOFMS and <sup>1</sup> H-NMR
Citric acid [55]	PBMC	GC-MS
Creatine [77]	Blood and plasma	CE-TOFMS
Creatinine [40]	PBMC	GC-MS
Cystine [72]	Serum	GC-TOFMS
6-Deoxy-mannofuranose [80]	Serum	GC-MS
Dihydroxyacetone phosphate [55]	PBMC	GC-MS
2,3-Dihydroxybutanoic acid [72]	Urine	GC-TOFMS
3,4-Dihydroxyphenylacetic acid (DOPAC) [77]	Blood	LC-ESI/MS/MS
3,4-Dimethoxyphenethylamine (3,4-DMPEA) [81]	Urine	MS
Dopamine (DA) [40, 77]	PBMC Blood	GC-MS LC-ESI/MS/MS
Eicosanoic acid [80]	Serum	GC-MS
Eicosenoic acid [72]	Serum	GC-TOFMS
Erythrose [76]	Serum	GC-MS
Ethane [82]	Breath	GC-MS
2-Ethyl-3-hydroxypropionic acid [72]	Urine	GC-TOFMS

(continued)

**Table 14.2** (continued)

Biomarkers	Sample type	Analytical platforms
Fructose [55]	PBMC	GC-MS
Fructose 6-phosphate [55]	PBMC	GC-MS
Fumaric acid [40]	PBMC	GC-MS
Galactose oxime [80]	Serum	GC-MS
Glucose [7, 55, 72, 76]	PBMC Urine CSF Serum	GC-MS, GC-TOFMS and <sup>1</sup> H-NMR
Glucose 6-phosphate [55]	PBMC	GC-MS
Gluconic acid [77]	Blood and plasma	CE-TOFMS
Glucuronic acid [76]	Serum	GC-MS
Glutamate [72, 77, 78, 83]	Blood, plasma, urine	CE-TOFMS; LC-ESI/MS/MS GC-TOFMS HPLC-MS
Glutamine [8]	Plasma CSF	MS
γ-Glutamylcysteine [78]	Serum	HPLC-MS HPLC fluorescence
Glutathione [78]	Serum	HPLC-MS HPLC fluorescence
Glyceraldehyde-3-phosphate [55]	PBMC	GC-MS
Glycerate [72]	Serum	GC-TOFMS
Glycerate 3-phosphate [55]	PBMC	GC-MS
Glycerol [40, 76]	PBMC Serum	GC-MS
Glycerol 3-phosphate [55]	PBMC	GC-MS
Glycine [76, 84]	Plasma Serum	GC-MS
Glycoamine [72]	Urine	GC-TOFMS
Heptadecanoic acid [80]	Serum	GC-MS
Hexadecanoic acid (palmitic acid) [72, 76]	Serum	GC-MS GC-TOFMS
Histidine [8]	Plasma	MS
Homoserine [40]	PBMC	GC-MS
Homovanillic acid (HVA) [77]	Blood	LC-ESI/MS/MS
Hydroxyacetic acid [72]	Urine	GC-TOFMS
3-Hydroxyadipic acid [72]	Urine	GC-TOFMS
2-Hydroxybutyrate [72]	Serum Urine	GC-TOFMS
3-Hydroxybutyrate [72, 78]	Serum Urine	GC-TOFMS and <sup>1</sup> H-NMR; HPLC-MS
2-Hydroxyethyl palmitate [40]	PBMC	GC-MS

(continued)

**Table 14.2** (continued)

Biomarkers	Sample type	Analytical platforms
5-Hydroxyindole-3-acetic acid (5-HIAA) [77]	Blood	LC-ESI/MS/MS
Hydroxylamine [40]	PBMC	GC-MS
3-Hydroxysebacic acid [72]	Urine	GC-TOFMS
5-Hydroxytryptamine (5-HT) [77, 78]	Blood Serum	LC-ESI/MS/MS HPLC-MS HPLC fluorescence
Inositol [40]	PBMC	GC-MS
Isoleucine [72]	Urine	GC-TOFMS
$\alpha$ -Ketoglutarate [76]	Serum	GC-MS
L-Kynurenine [78]	Serum	HPLC-MS HPLC fluorescence
Lactate [7, 55, 72, 76, 78]	PBMC Serum CSF	GC-MS; GC-TOFMS; <sup>1</sup> H-NMR; HPLC-MS
Lactobionic acid [76]	Serum	GC-MS
Linoleic acid [72, 76, 78]	Serum	HPLC-MS HPLC fluorescence GC-MS GC-TOFMS
Malate [72]	Serum	GC-TOFMS
Maltose [40]	PBMC	GC-MS
3-Methoxy-4-hydroxyphenylglycol (MHPG) [77]	Blood	LC-ESI/MS/MS
<i>Tele</i> -methylhistamine [85, 86]	CSF	GC-MS
Methyl phosphate [40]	PBMC	GC-MS
<i>Myo</i> -inositol [72, 76]	Serum	GC-MS GC-TOFMS
Norepinephrine (NE) [77]	Blood	LC-ESI/MS/MS
Octadecanoic acid (stearic acid) [72, 76]	Serum	GC-MS GC-TOFMS
(9Z)-Octadec-9-enoic acid (oleic acid) [72, 80]	Serum	GC-MS GC-TOFMS
Octanoic acid [40]	PBMC	GC-MS
Ornithine [8]	Plasma	MS
2-Oxoglutarate [72]	Serum	GC-TOFMS
1-Oxoproline [80]	Serum	GC-MS
PC ae C38:6 [8]	Plasma	MS
Pentadecanoic acid [80]	Serum	GC-MS
Pentane [79, 82]	Breath	GC-MS
4-Pentenoic acid [72]	Urine	GC-TOFMS
Phenylalanine [72]	Serum	GC-TOFMS
Pipecolic acid [72]	Urine	GC-TOFMS

(continued)

**Table 14.2** (continued)

Biomarkers	Sample type	Analytical platforms
2-Piperidinecarboxylic acid [80]	Serum	GC-MS
Pyroglutamic acid (5-oxoproline) [40, 72]	PBMC Urine Serum	GC-MS GC-TOFMS
Pyruvate [55, 72]	PBMC Serum	GC-MS GC-TOFMS
Ribose 5-phosphate [55]	PBMC	GC-MS
Serine [72, 78, 84]	Plasma, serum	GC-MS, GC-TOFMS, HPLC-MS
Sorbitol [40]	PBMC	GC-MS
Suberic acid [72]	Urine	GC-TOFMS
Succinic acid [55]	PBMC	GC-MS
Tetradecanoic acid [72]	Serum	GC-TOFMS
Threonic acid [72]	Urine	GC-TOFMS
Threonine [78]	Serum	HPLC-MS HPLC fluorescence
$\alpha$ -Tocopherol [40]	PBMC	GC-MS
$\gamma$ -Tocopherol [40, 76]	Serum PBMC	GC-MS
-Tryptophan [76, 78]	Serum	HPLC-MS HPLC fluorescence GC-MS
L-Tyrosine [78]	Serum	HPLC-MS HPLC fluorescence
Uric acid [76]	Serum	GC-MS
Valine [40, 72]	PBMC Urine	GC-MS GC-TOFMS
Vanillylmandelic acid (VMA) [77]	Blood	LC-ESI/MS/MS

has been reported to occur in other diseases such as heart attack, rheumatoid arthritis, and nutritional deficiency [79, 82].

Another biomarker that has been studied is trimethylglycine (also known as betaine) since it participates in homocysteine metabolism. More specifically, it participates in the conversion of homocysteine to methionine which is mediated by betaine-homocysteine methyltransferase in human tissues. Koike et al. [83] observed a decrease of betaine levels in schizophrenia patients and consequently an increase of the homocysteine concentration in the brain, which can lead to a high oxidative stress and a neural damage. In addition to betaine, hydroxylamine, pyroglutamic acid,  $\gamma$ -tocopherol, and  $\alpha$ -tocopherol have been listed as markers of the increase in oxidative stress in schizophrenia patients [83]. The higher concentrations of hydroxylamine in patients compared to healthy individuals suggest a higher accumulation of reactive oxygen species (ROS) in patients with schizophrenia, while lower tocopherol concentrations indicate a decrease in antioxidative defense [40].

As already stated, biomarker candidates found for BD and schizophrenia are numerous, but these have still not been tested in geographically different environments and using greater number of samples as means of validation. In order to apply these in clinical research, there must be coordinated efforts around the globe to verify which of the cited biomarkers are applicable and universal. It is also expected that this will help to expand research in modern and multidisciplinary psychiatry and design biomarker-based user friendly tests that can be performed as simple clinical trials with easy to read and interpretable data.

### 14.3 Samples, Methodology, and Techniques: Concerns

To guarantee the quality of the data and the biological interpretations, it is important to properly select the sample type, the method of sample preparation, and the pre-processing procedures. Another critical issue is the selection of an appropriate control group [90, 91]. When immediate analysis is not feasible, to prevent changes in the composition of the samples, it is necessary to store them at  $-80^{\circ}\text{C}$ , but cycles of freezing and thawing should be avoided [90, 92–94].

The cellular components of the blood (erythrocytes, leukocytes, and platelets) can be excluded using centrifugation, obtaining the plasma or serum. The supernatant portions of these are derived with or without the addition of anticoagulants, respectively. Plasma is composed of a variety of substances such as proteins, peptides, and electrolytes. The precipitation of proteins can be carried out with trichloroacetic acid (TCA) and cold methanol to reduce their potential interference in the analysis [90].

Plasma, serum, and CSF are naturally buffered. However, the pH in urine samples can vary and should be controlled by addition of a sodium phosphate buffer ( $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ ) [90–93].

The sodium salt of 3-trimethylsilylpropionic acid (TSP) should not be used as a NMR standard reference in samples containing high concentrations of proteins. An alternative is the use of formate or internal standards, such as the lactate signal (3H, d, 1.33 ppm,  $^3J = 7$  Hz) in plasma or serum samples [94, 95].

Analysis of urine samples presents several advantages compared to blood or CSF, since urine collection is easy and noninvasive and enables more extensive sampling frequency. Furthermore, the sample preparation is less complex due to the absence, or low level, of proteins and peptides [90, 92, 96]. However, it is important to define the time of urine collection since the concentration of metabolites can vary during the day due to dietary, lifestyle, and pharmaceutical interference [90, 92].

The collection of CSF is invasive, and, therefore, routine sampling and longitudinal studies are not desirable. One of the main concerns of using CSF is the potential of blood contamination during the sampling process. There are some known protocols to decrease blood contamination in CSF [90, 93].

Numerous methods of sample preparation prior to MS-based analysis have been reported. These are diverse extraction protocols that will lead to the observation of dif-



ferent fractions in the metabolite profile [90, 94]. However, the signals are usually filtered using specific pulse sequences in NMR-based analysis instead of applying sample preparation techniques, such as protein precipitation or ultrafiltration [94–97].

For further information, there are many described procedures for collection and preparation of serum [91, 94, 95, 97], plasma [91, 94], urine [91, 92, 94], and CSF [98] samples. Barbosa et al. described the detailed procedures involved in serum lipidomics [97].

### 14.3.1 *Softwares and Databases*

NMR spectral processing can be performed using Topspin or MestreNova software. The Chenomx commercial package provides a tool that can be used from the point of spectral processing to metabolite identification. For data statistical analysis, there are free online platforms including PRIME [99] and MetaboAnalyst [100] and free or commercial software, such as R, MVAPACK [101], MATLAB, Statistica, and the Unscrambler. The open-access databases Human Metabolome Database (HMDB) [102], Biological Magnetic Resonance Data Bank (BMRB) [103], and Madison-Qingdao Metabolomics Consortium Database (MMCD) [104] can be used to assist in the spectral assignments of metabolites. A list and discussion about the database and software for NMR metabolomics are available in the literature [105].

Current software available for processing mass spectra includes MassHunter (Agilent<sup>®</sup>), Thermo<sup>™</sup> Xcalibur<sup>™</sup> (LC-MS), MestreNova (NMR and MS), and others. Some specific software for both GC-MS and LC-MS preprocessing includes MetAlign<sup>™</sup> [106], MZmine 2 (LC-MS) [107], XCMS [108], and SpectConnect (GC-MS) [109].

The statistical analysis of mass spectra data can be performed using the Agilent MassHunter Profinder (data extraction) and Mass Profiler Professional (MPP-Agilent<sup>®</sup>). Data mining can also be performed, and chemometric analysis can be used for analysis of volatile organic compounds in GC-MS analysis. Finally, ANOVA analysis and unsupervised statistical methods can also be applied such as PCA and HCA [110].

Some software is also available that can be used for image data processing generated from imaging mass spectrometry (IMS) such as BioMap (Novartis) or flexImaging (Bruker Daltonics), and the FlexAnalysis system (Bruker Daltonics) can be used for quantification.

### 14.3.2 *NMR × MS*

Standard NMR pulse sequences frequently used in metabolomic studies are the water suppression pulse sequence Watergate [111, 112], T<sub>2</sub>-edited CPMG pulse sequence for filtering out signals of larger molecules [113, 114], and diffusion-ordered spectroscopy (DOSY)-edited pulse sequence to detect assemblies of lipids [94, 95, 97].

The development of high-resolution  $^1\text{H}$  magic angle spinning (MAS) NMR spectroscopy has extended the capability of metabolomic studies since this makes it possible to analyze intact tissues without a pre-treatment step. The experiments are the same ones as those used in liquid state NMR metabolomics [12, 94, 96, 115].

Since each technique has its strengths and limitations, the combination of NMR and MS techniques for metabolic fingerprinting and profiling is growing and showing the capacity to improve results by covering a more comprehensive range of metabolites [94, 116, 117]. This can be done using hyphenated techniques such as HPLC-NMR-MS, in which parallel NMR and MS analyses are performed using a splitting of the eluting HPLC fraction. Another alternative is to aliquot the sample for analysis by NMR and MS separately. Some authors have already applied this approach in bipolar disorder and schizophrenia metabolomic studies.

Generally, when it is necessary to perform analyses in which the samples need to be analyzed over a period of time, NMR spectroscopy is chosen because of the greater reproducibility of the resulting spectra, while GC-MS or LC-MS are commonly used when it is necessary to achieve a higher sensitivity. However, this requires high purity of samples and sometimes laborious preparation of the metabolic components, such as derivatization of the samples [118]. Furthermore, the testing of the range of molecules analyzed according to their functional groups will be conditioned to the extraction solvent that is used in preparation of the samples.

In addition to the possibility of analyzing biofluids and the preparation care already mentioned previously, tissue analysis by IMS has been explored as in the work of Matsumoto et al. [119]. IMS is a technique that has allowed the analysis of complex surfaces of samples (in 2D or 3D) and can result in the display of hundreds to thousands of compounds per sample surface [120, 121].

## 14.4 Conclusion

Bipolar disorder and schizophrenia are chronic mental disorders that affect the population and can notably interfere with the normal life of a person. Modern medicine still struggles with the accurate diagnosis and treatment of these diseases. Recent and promising metabolomic discoveries in mental disorders will help in the selection of appropriate drug treatments, improve human health significantly, and ameliorate the quotidian effects on individuals. Acetone, *N*-acetyl-aspartate, GABA, creatine, glutamine, glutamate, glycine, 5-hydroxyindole-3-acetic acid (5-HIAA), myo-inositol,  $\alpha$ -ketoglutarate, L-kynurenine, lactate, phenylalanine, pyruvate, and tryptophan are the most frequently occurring biomarkers cited in the literature as being altered in bipolar disorder and schizophrenia patients [10, 37, 39, 40, 42, 43, 45–47, 49, 51, 53, 55, 72, 76–78]. Both disorders have some similar symptoms and share some common metabolites, but the question is whether these metabolites originate from the same source and metabolic pathway. It is anticipated that further studies will help to determine this.

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