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Peripheral biomarkers allow differential diagnosis between schizophrenia and bipolar disorder

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ABSTRACT

Schizophrenia (SCZ) and bipolar disorder (BD) are severe mental disorders that pose important challenges for diagnosis by sharing common symptoms, such as delusions and hallucinations. The underlying pathophysiology of both disorders remains largely unknown, and the identification of biomarkers with potential to support diagnosis is highly desirable. In a previous study, we successfully discriminated SCZ and BD patients from healthy control (HC) individuals by employing proton magnetic resonance spectroscopy (^{1}H-NMR). In this study, ^{1}H-NMR data treated by chemometrics, principal component analysis (PCA) and supervised partial least-squares discriminant analysis (PLS-DA), provided the identification of metabolites present only in BD (as for instance the 2,3-diphospho-D-glyceric acid, *N*-acetyl aspartyl-glutamic acid, monoethyl malonate) or only in SCZ (as isovaleryl carnitine, pantothenate, mannitol, glycine, GABA). This may represent a set of potential biomarkers to support the diagnosis of these mental disorders, enabling the discrimination between SCZ and BD, and among these psychiatric patients and HC (as 6-hydroxydopamine was present in BD and SCZ but not in HC). The presence or absence of these metabolites in blood allowed the categorization of 182 independent subjects into one of these three groups. In addition, the presented data suggest disturbances in metabolic pathways in SCZ and BD, which may provide new and important information to support the elucidation and/or new insights into the neurobiology underlying these mental disorders.

1. Introduction

Schizophrenia (SCZ) and bipolar disorder (BD) are severe mental disorders that may cause important disabilities, functional impairments and premature death (Ketter et al., 2004; Buoli et al., 2017; van Rheenen et al., 2017) with important associated burden (Grover et al., 2015; Zhou et al., 2016; Correll et al., 2017; Esan et al., 2017). Both disorders are determined by a complex interaction between genetic and environmental factors, and the underlying pathophysiology has not been thoroughly elucidated to date (van Winkel et al., 2010; Demjaha et al., 2012; Forstner et al., 2017). Significant scientific research efforts have been focused on the identification of biomarkers for diagnosis or on the discovery of new molecular targets that could be employed for therapy and/or risk management in clinics. In addition, these biomarkers may have also the potential to assist the elucidation of putative novel molecular pathway(s) underlying these mental disorders.

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Abbreviations

NMR	Nuclear Magnetic Resonance spectroscopy
BD	Bipolar Disorder
SCZ	Schizophrenia
DA	Dopamine
HC	Healthy Controls
SD	Standard Deviation
PANSS	Positive and Negative Syndrome Scale
SCID-I	Structured Clinical Interview for DSM-IV-Axis I
DSM	Diagnostic and Statistical Manual of Mental Disorders, 4th edition
YMRS	Young Mania Rating Scale
HDRS	Hamilton Depression Rating Scale
CGI	Clinical Global Impression
GAF	Global Assessment of Functioning
GLM	General Linear Model
HSQC	Heteronuclear Single Quantum Coherence spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
BMRB	Biological Magnetic Resonance Data Bank
HMDB	Human Metabolome Database
PABA	4-aminobenzoic acid
PCA	Principal Component Analysis
PLS-DA	Partial Least-Squares Discriminant Analysis
VIP	Variable Importance in Projection
GABA	gamma aminobutyric acid
NAA	*N*-acetyl-aspartate
NAAG	*N*-acetyl aspartyl-glutamic acid
6-OHDA	6-hydroxidopamine
TAGs	triacylglycerols
FAs	fatty acids
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Sciences
NMDA	*N*-methyl-D-aspartate
LC-MS	Liquid Chromatography-Mass Spectrometry
SGAs	Second Generation Antipsychotics

The current diagnostic criteria for psychiatric diagnosis are still based on clinical phenomenology and rely on psychiatrist's judgment based on standard clinical interview applications and on inaccurate reports from patients or caretakers. Several medical tests, including neuroimaging, blood and genetic tests, are employed, but mostly aiming to exclude other medical conditions that could potentially give rise to similar clinical symptoms (Mehdizadeh et al., 2016; Jani et al., 2016). Therefore, clinical psychiatric interviews are still considered the gold standard for clinical diagnosis. However, molecular biomarkers clearly have the potential to be helpful not only for diagnosis and to assist adequate pharmacological intervention, but may also potentially contribute in generating diseases biological models to support personalized treatments (Grande et al., 2014). In previous studies, we have demonstrated that it is possible to discriminate SCZ (Tasic et al., 2017a) and BD (Sethi et al., 2017) subjects from HC individuals, and SCZ patients from crack-cocaine users (Tasic et al., 2017b), all matched for sex and age.

Although without an *a priori* hypothesis regarding which molecular differences would be expected among the groups studied, we did expect to find changes in compounds that could be linked to SCZ and BD etiology or to pathophysiological pathways underlying each or both diseases.

Therefore, our main aim was to verify if the metabolomics approach based on proton NMR (^{1}H NMR) analysis of patient blood serum samples could allow the correct and precise discrimination of individuals into three groups of study, namely, SCZ, BD, and HC. In addition, we also aimed to identify the metabolites that are differentially present among these groups, which can be helpful in achieving a better understanding of the pathway(s) affected in each studied group.

2. Methods

2.1. Ethics

This study was approved by the Research Ethics Committee of Universidade Federal de São Paulo (UNIFESP) [CEP Nº. 0305/12]. All participants provided written informed consents prior to their enrollment in this study. Clinical and laboratory investigations were strictly conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Subject enrolment and psychiatric assessment

Fifty-four (54) patients with schizophrenia (SCZ) and sixty-eight (68) euthymic outpatients with BD type 1 (BD) aged between 18 and 65 years took part in this cross-sectional study with a unique visit to the clinics and no information on blinding differential procedures for the clinical diagnostic procedure.

For psychiatric diagnosis assessment, all individuals underwent a clinical interview employing the Structured Clinical Interview for DSM-IV Axis 1 (SCID-I) applied by trained psychiatrists. The Positive and Negative Syndrome Scale (PANSS) and the Global Assessment of Functioning Scale (GAF) (Bressan et al., 1998; Levine and Rabinowitz, 2007) were further assessed for SCZ patients. A minimum time threshold of 6 months in which severe symptoms were maintained was required for inclusion in the SCZ group. Manic and depressive symptoms severities were assessed using the Young Mania Rating Scale (YMRS) and the Hamilton Depression Rating Scale (HDRS)-17, respectively (Hamilton, 1967; Young et al., 1978). Only euthymic individuals, defined as not fulfilling DSM-IV criteria for a current mood episode and concomitant presence of HDRS and YMRS scores lower than 8, were included in the research. All patients (from SCZ and BD groups) were under a stable medication treatment regimen (defined as taking the same medications at the same doses for a minimum of two months prior to inclusion in this research).

The healthy control (HC) group consisted from 60 healthy volunteers who had no current or lifelong history of mental disorders according to the SCID-1, no history of prescribed psychotropic medications, and negative family history of a major psychiatric disorder (defined as unipolar depression, BD, SCZ or suicide in any first-degree relatives). The HC volunteers were recruited by inviting blood donors and subjects from a center for employment assistance.

The exclusion criteria for all groups were as follows: (*i*) acute and chronic unstable medical conditions (which include any metabolic disease or syndrome as obesity, diabetes, dyslipidemia, among others), (*ii*) comorbidity with substance abuse or drug dependence, (*iii*) pregnancy (suspected or confirmed) and/or breastfeeding, and (*iv*) familiar history of mental illness.

2.3. Blood collection

All blood samples were taken in the morning (between 8 and 10 a.m.) after at least 12 h of fasting. Blood was drawn into Vacutainer tubes and immediately allowed to clot for at least 30 min, and not more than 40 min, at room temperature before being centrifuged at 1500 × *g*, for 5 min. The serum collected from the supernatant was transferred into clean polypropylene tubes, which were stored at −80 °C. The maximum period of storage before the assays was up to two weeks.

2.4. NMR spectroscopy analyses: ^1H NMR, T_2-edited ^1H NMR and 2D NMR

For nuclear magnetic resonance spectroscopy analyses, serum samples were thawed and centrifuged at 12,300 × *g*, for 10 min at 4 °C to separate any precipitate. Aliquots of 250 μL of the supernatants were diluted with 250 μL of deuterated water (D_2O) and placed into 5.0-mm diameter NMR tubes. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded as three independent measurements for each sample using a Bruker 600 NMR spectrometer (Bruker Advance III, Bruker GmBH, Rheinestennen, Germany), applying the pulse sequence WATERGATE (p3919 gp) over 128 ns, at a standard spectral width (12 kHz) and a temperature of 25 °C (Piotto et al., 1992).

T_2-edited NMR spectra were recorded using the CPMG (Carr-Purcell-Meiboom-Gill) sequence (Meiboom and Gill, 1958), where a fixed total spin-spin relaxation delay $2n\tau$ of 100 ms was used to attenuate the broad NMR signals from slowly tumbling molecules, such as proteins and lipids, while retaining those from low-molecular-weight compounds and some lipid components. For each spectrum, 128 transients were acquired into 32,000 data points with a spectral width of 12 kHz.

Aiming to confirm the assignments made from ^{1}H and T_2-edited NMR spectra, blood serum samples were also examined using 2D [^{1}H–^{13}C] HSQC (Heteronuclear Single Quantum Coherence) and HMBC (Heteronuclear Multiple Bond Correlation). For each 2D spectrum, 256 increments with 64 transients per increment were collected and extended to 4 k data points. The signal assignments were based on the literature and/or databases, such as the Biological Magnetic Resonance Data Bank (BMDB) (Ulrich et al., 2008) and the Human Metabolome Database (HMDB) (Wishart et al., 2007). The samples were assigned on the T_2-edited spectra and confirmed by the correlations on the [^{1}H–^{13}C] HSQC data.

Using MestreNova software, the spectra were manually baseline and phase corrected, normalized by the sum, and the CH_3-lactate signal (δ 1.33, 3H, ^3J = 7.0 Hz) was used as the chemical shift reference.

2.5. Chemometrics of ^1H NMR spectral data

^{1}H-NMR water suppressed spectra, ranging from 1.00 ppm to 4.40 ppm and 6.50–8.00 ppm, were transported into a matrix, and chemometrics analyses were performed using MATLAB (MathWorks, Natick, MA, USA) software. The spectra were aligned using interval Correlation Optimized shifting (icoshift) (Savorani et al., 2010), and all variables were autoscaled. Therefore, we first performed Principal Component Analysis (PCA) (Rasmus and Smilde, 2014) to visualize our dataset and identify outliers, and then four Partial Least-Squares Discriminant Analysis (PLS-DA) models were constructed to evaluate the metabolic differences among the three studied groups (Berrueta et al., 2007), namely, (1) HC × SCZ × BD; (2) HC × *x* SCZ; (3) HC × BD; and (4) SCZ × BD. For PLS-DA, each sample was taken as the mean of the three ^{1}H NMR spectra; 70% of the data were used to build the models and the remaining 30% of the data were left to validate the models.

2.6. Statistical and comparative analysis

For statistical analysis, the Gaussian distribution using the Kolmogorov-Smirnoff tests for the total sample and in each comparison group verified the variable distribution. Chi-square was adopted for categorical variables, such as age and gender. To measure the mean differences for non-categorical variables, ANOVA one-way test with Bonferroni post-hoc comparison test and unpaired Student's *t*-test was performed. Data analyses were performed using the Statistical Package

Table 1
Clinical and demographic characteristics of the samples.

	HC (N = 60)	SCZ (N = 50)	BD (N = 45)	Test value	*p*-value
Age in years; mean (SD)	36.0 (10.5)	35.4 (9.5)	46.6 (9.3)	15.1[a]	0.001**
Sex Female; N (%)	42 (70)	24 (48)	36 (78)	10.1[b]	0.006*
Ethnicity Caucasian; N (%)	28 (71)	16 (64)[#]	26 (66)	0.4[b]	0.806
Years of education; mean (SD)	12.7 (3.0)	11.6 (2.7)[#]	10.5 (3.8)	5.1[a]	0.007*
BMI in mg/m2; mean (SD)	26.8 (5.2)	–	29.1 (7.3)	−1.8[c]	0.068
Current smoking; N (%)	7 (11.6)	7 (28)[#]	12 (26)	1.9[b]	0.385
Positive and Negative Syndrome Scale; mean (SD)	–	63.1 (14.0)[#]	–	–	–
Young Mania Rating Scale; mean (SD)	0.4 (1.0)	–	3.8 (6.1)	17.2[c]	0.001**
Hamilton Depression Rating scale; mean (SD)	1.4 (1.8)	–	7.7 (8.1)	31.1[c]	0.001**
General functioning assessment (symptoms); mean (SD)	88.7 (8.3)	–	67.5 (17.3)	66.1[c]	0.001**
General functioning assessment (functioning); mean (SD)	87.4 (5.8)	45.3 (13.2)[#]	64.4 (20.5)	85.3[a]	0.001**
Age at onset in years; mean (SD)	–	22.4 (6.7)[#]	32.4 (11.6)	−3.8[c]	0.001**
Duration of illness in years; mean (SD)	–	13.3 (6.9)[#]	19.1 (11.3)	−2.3[c]	0.024*
Number of total mood episodes; mean (SD)	–	–	9.3 (8.5)	–	–
Number of past manic episodes; mean (SD)	–	–	5.2 (5.5)	–	–
Number of past hypomanic episodes; mean (SD)	–	–	0.6 (2.8)	–	–
Number of past depressive episodes; mean (SD)	–	–	3.7 (4.3)	–	–
Age of first use of mood stabilizer; mean (SD)	–	–	31.9 (11.2)	–	–
Number of Medication used, mean (SD)		1.3 (0.4)	3.1 (1.4)		
Valproic acid, N (%)	–	–	15(33)	–	–
Fluoxetine, N (%)	–	–	5 (11)	–	–
Lithium, N (%)	–	–	21 (46)	–	–
Clonazepan, N (%)	–	–	12 (26)	–	–
Risperidone, N (%)	–	4 (15)[#]	16 (35)	–	–
Olanzapine, N (%)	–	10 (38)[#]	6 (13)	–	–
Quetiapine, N (%)	–	3 (11)[#]	11 (24)	–	–
Haloperidol, N (%)	–	5 (19)[#]	3 (6)	–	–
Clozapine, N (%)	–	10 (38)[#]	–	–	–
Others, N (%)	–	2 (7)[#]	28 (62)	–	–
Do not know, N (%)	–	1 (3)[#]	6 (13)	–	–

*$p \leq 0.05$.
**$p \leq 0.001$.
[a] ANOVA one-way test.
[b] Chi-square test.
[c] Student's *t*-test; [#]n = 26.

for Social Sciences (SPSS) Version 14.0. The significance threshold was established as $p < 0.05$.

3. Results

Blood serum samples of individuals from three groups, namely schizophrenia (SCZ), bipolar disorder (BD), and healthy control (HC), totaling 182 subjects (Table 1), were submitted to three independent Nuclear Magnetic Resonance (NMR) experiments, yielding a total of 546 ^{1}H NMR spectra. In addition, for thirty (30) randomly chosen blood serum samples from the individuals (12 SCZ, 7 BD, and 11 HC), T_2-edited ^{1}H NMR spectra were recorded, and seven Heteronuclear Single Quantum Coherence spectroscopy (HSQC) [^{13}C, ^{1}H] (2 SCZ, 2 BD, and 3 HC) were collected. Examples of the obtained results are illustrated in Figs. 1 and 2.

As shown in Fig. 1, ^{1}H NMR average spectra present only small differences among the samples from the three studied groups, *i.e.,* SCZ, BD and HC. These differences are better observed in the T_2-edited ^{1}H NMR spectra, in which the greatest differences, attributed to the aliphatic metabolites (0.60–4.40 ppm, Fig. 2) and aromatic compounds (6.50–8.00 ppm, Fig. 2), could be noticed mainly in two spectral regions.

When comparing the average T_2-edited ^{1}H NMR spectra (Supplementary Material Fig. S1, and amplified regions in Fig. 2), the most pronounced differences are observed in 6.50–8.00 ppm region, in which phenylalanine (Phe) peaks appear as more prominent in the BD group, while tyrosine (Tyr) and histidine (His) peaks seem to be altered and less abundant in the SCZ group. In addition, these last two amino acid peaks are also differentially abundant in the BD group compared to the HC group individuals. Interestingly, differences in the 0.60–4.40 ppm region are also noticed, as glucose levels seem to be altered and more prominent in SCZ and BD groups compared to the HC volunteers' group.

Additionally, higher lipid levels were also observed in SCZ and BD compared to HC individuals, while amino acid levels are higher only in the BD group. There are also differences in the concentrations of lactate (more intense in the SCZ group) and the exclusive presence of gamma aminobutyric acid (GABA) in the blood serum samples of the SCZ group (Supplementary Material Fig. S2, and subtraction of medium spectra between each pair of groups, SCZ-HC, BD-HC and SCZ-BD).

By PCA (Principal Component Analysis), five outliers were found (Figs. S3 and S4 of Supplementary Material). As shown in Fig. 3A, it was possible to successfully classify the SCZ (green squares) and HC individuals (blue diamonds) using only the variables from the aforementioned spectral regions (*i.e.*, 0.60–4.40 ppm and 6.50–8.00 ppm). Similarly, the applied variables were sufficient for the discrimination of BD (red circles) from the HC samples (Fig. 3C). Moreover, it is important to mention that although group discrimination is satisfactory, as shown in Table 1, some subjects were misclassified.

Positive results were obtained in all executed PLS-DA (Partial Least-Squares Discriminant Analysis) classification models, but we may highlight as being the most representative, those for the SCZ and HC groups (Fig. 3A and Table 2), with accuracy values of 1.000 for SCZ and HC classes in the test set. We can also highlight the PLS-DA classification model of SCZ, BD and HC (Fig. 4B and Table 2), with accuracy values of 0.806, 0.886 and 0.839, respectively, in the test set. Thus, we might point to the blood serum NMR-based metabolomics as a suitable method to assist psychiatric evaluation in the differential diagnosis of these two severe diseases along with additional accurate discrimination from HC.

The most important variables in PLS-DA were identified through the analysis of the variable importance in projection (VIP). The important variables that contributed to the discrimination among the groups are those that presented VIP scores > 1 (Mehmood et al., 2012; Almeida et al., 2013).

Analysis of VIP graphs (Fig. 3C, D, 4C and 4D), as well as the 2D-NMR and T_2-edited ^{1}H NMR data, allowed the identification of metabolites listed in Table 3 and in Table S1 (Supplementary Material) to be the most important for the discrimination of these three groups. Some metabolites were exclusively observed in the SCZ group, while others were present only in the BD group.

4. Discussion

Slight variations in metabolome, for comparison between healthy and ill individuals, are highly informative. Metabolomic profiling studies in mental illnesses have been reported (Kaddurah-Daouk and Krishnan, 2009; Zhang et al., 2016), although relatively limited if

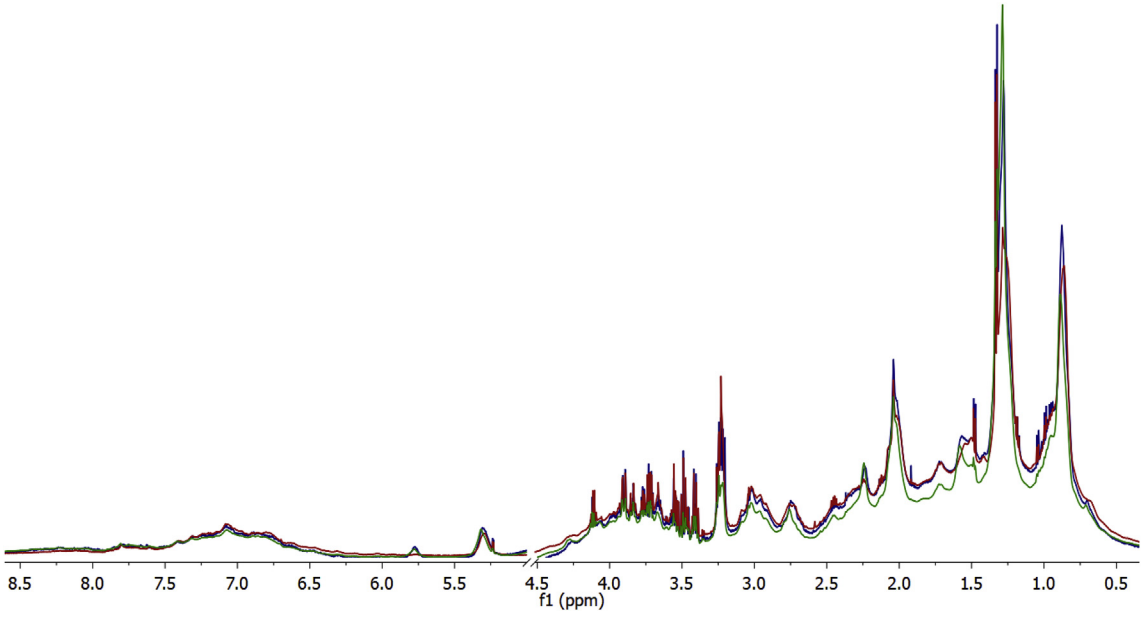

Fig. 1. ^{1}H NMR average spectra (600 MHz) of blood serum samples. Serum from schizophrenia (SCZ, green) or bipolar disorder (BD, red) patients, and healthy control volunteers (HC, dark blue). Representative spectra for each group is reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

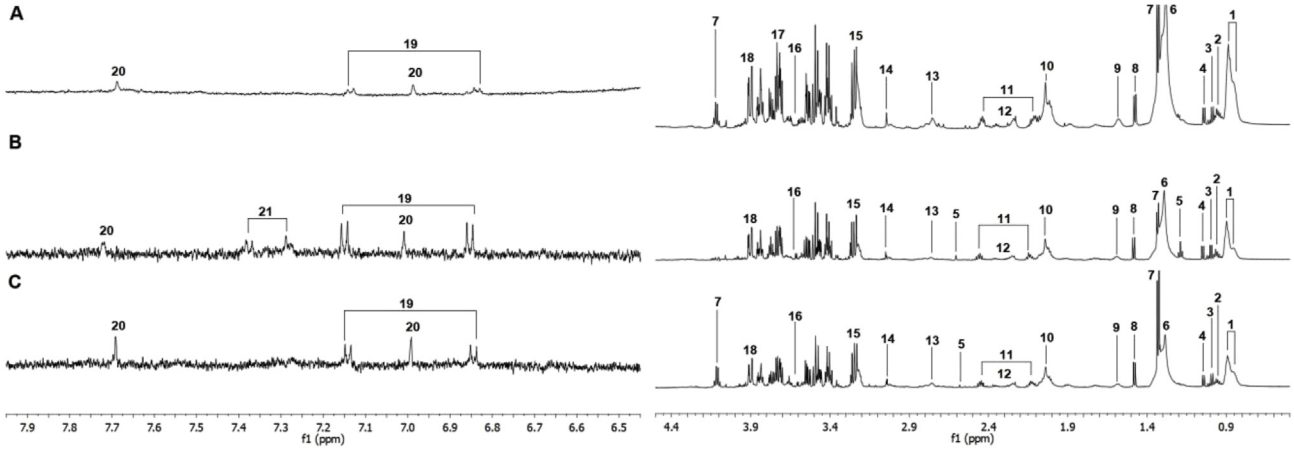

Fig. 2. Amplified regions of the T_2-edited ^{1}H NMR spectra in: 0.60–4.40 ppm, and 6.50–8.00 ppm, with the identification of the major peaks corresponding to aliphatic and aromatic compounds, where: 1- LDL and VLDL; 2- Leu; 3- Ile; 4- Val; 5- ethanol; 6- Lipids; 7- Lactate; 8- Ala; 9- FAs; 10- FAs; 11- Gln; 12- FAs; 13- FAs; 14- Creatine; 15- GPCho, PCho and Cho; 16- *myo*-Inositol; 17- Gly; 18- Glc; 19- Tyr; 20- His; 21- Phe. Serum from schizophrenia (A) or bipolar disorder (B) patients, and healthy control volunteers (C). Representative spectra for each group is reported.

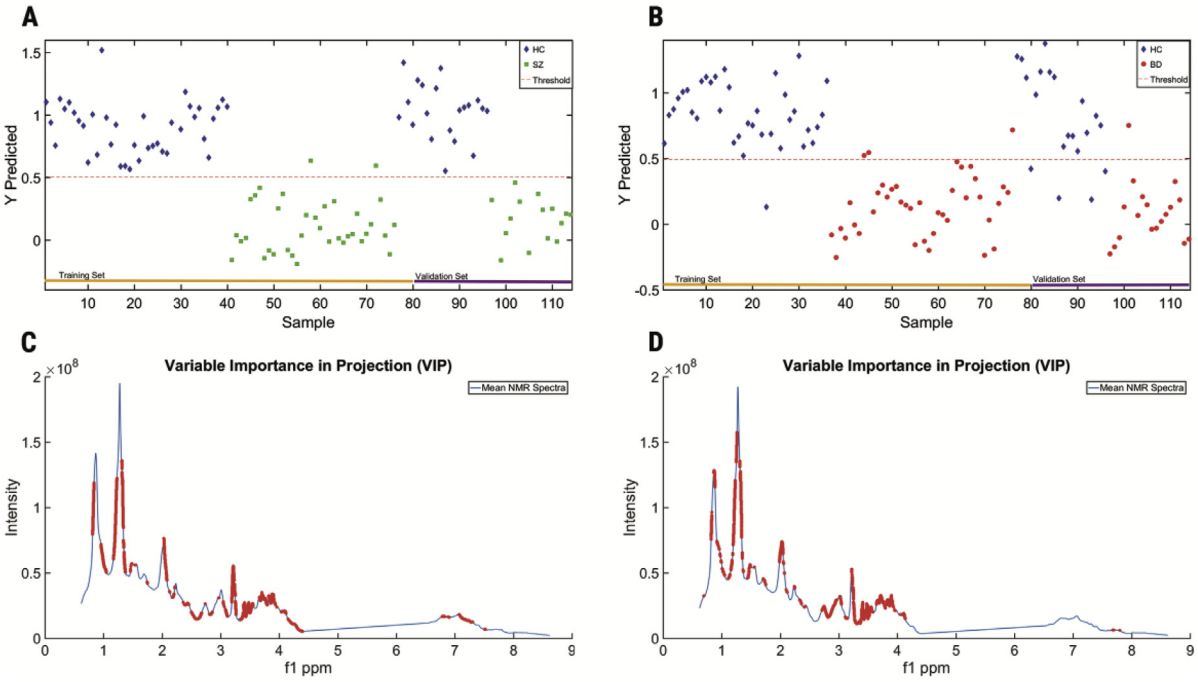

Fig. 3. Illustration of the results obtained in PLS-DA analyses of ^{1}H NMR spectra data from the blood serum samples from schizophrenia (SCZ, green squares) and bipolar disorder (BD, red circles) patients, and healthy control group (HC, blue diamonds). The PLS-DA prediction plots for (**A**) SCZ and HC, and (**B**) BD and HC groups. In all cases, we have used two thirds of samples to construct the models (training set, shown in orange-yellow line) and one third of samples to test (validation set, shown in purple line). The PLS-DA plots of variable importance in projections (VIP) obtained for (**C**) SCZ and HC, and (**D**) BD and HC models, noting that the red points represent variables with high importance. All PLS-DA were constructed by combining variables from the two ^{1}H NMR spectral regions: 0.60–4.40 ppm and 6.50–8.00 ppm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

compared to cancer or obesity (Shaffer et al., 2017; Li et al., 2017; Zhan et al., 2018). A recent review reported numerous biomarkers for schizophrenia (SCZ) or bipolar disorder (BD), identified from metabolomic studies of urine, blood serum or plasma, cerebrospinal fluid, brain white and gray matter, breath and/or peripheral blood mononuclear cells (Quintero et al., 2019). Identification of plasma biomarkers in patients with SCZ or BD using metabolome analysis is described by several authors, including us (Quinones and Kaddurah-Daouk, 2009; Xuan et al., 2011; Orešič et al., 2011; He et al., 2012; Yang et al., 2013; Tasic et al., 2017a, 2017b; Sethi et al., 2017), but most of the published data did not identify any specific compounds associated with psychosis (Wood et al., 2003). Metabolites as glycine, PABA (4-aminobenzoic acid), GABA (gamma aminobutyric acid) and pantothenate allowed the discrimination of SCZ from HC (Tasic et al., 2017a), while key metabolites for discrimination of BD from HCs included lipoamide, α-ketoglutaric acid, α-ketovaleric acid, and amino acids and derivatives, as the L-glutamine, *N*-acetyl-L-phenylalanine and *N*-acetylaspartyl glutamic acid (NAAG) (Sethi et al., 2017). However, to the best of our knowledge, none of these previous studies successfully identified biomarkers that allow the discrimination of closely related mental disorders, as SCZ and BD.

The blood serum ^{1}H-NMR signature differences observed among SCZ, BD and HC are not surprising, as they are potentially expected outcomes due to altered metabolic pathways. The serum metabolomics

Table 2
Sensitivity and specificity values obtained in PLS-DA overall results exposed in Fig. 3 (***SCZ* × *HC*** and ***BD* × *HC)*** and Fig. 4 (***SCZ* × *BD*** and ***SCZ* × *BD* × *HC***).

Groups	*Parameters*	*SCZ*	*BD*	*HC*
SCZ* × *HC	Sensitivity (Train)	0.944	---	1.000
	Specificity (Train)	1.000	---	0.944
	Sensitivity (CV)	0.889	---	0.949
	Specificity (CV)	0.949	---	0.889
	Sensitivity (Test)	1.000	---	1.000
	Specificity (Test)	1.000	---	1.000
BD* × *HC	Sensitivity (Train)	---	0.925	0.972
	Specificity (Train)	---	0.972	0.925
	Sensitivity (CV)	---	0.875	0.944
	Specificity (CV)	---	0.944	0.875
	Sensitivity (Test)	---	0.944	0.800
	Specificity (Test)	---	0.800	0.944
SCZ* × *BD	Sensitivity (Train)	0.967	1.000	---
	Specificity (Train)	1.000	0.967	---
	Sensitivity (CV)	0.933	0.914	---
	Specificity (CV)	0.914	0.933	---
	Sensitivity (Test)	1.000	0.867	---
	Specificity (Test)	0.867	1.000	---
SCZ* × *BD* × *HC	Sensitivity (Train)	0.912	0.886	0.825
	Specificity (Train)	0.947	0.905	0.899
	Sensitivity (CV)	0.853	0.743	0.800
	Specificity (CV)	0.867	0.797	0.841
	Sensitivity (Test)	0.933	0.875	0.850
	Specificity (Test)	0.833	0.743	0.835

Train - Training samples
CV - Cross Validation samples
Test - Test samples
** The specificity and the sensitivity are two of the mostly used criteria for assessing the performance of diagnostic tests. The *sensitivity* is a measure of how well the model is able to correctly classify samples of the class of cases, while the *specificity* measures how well the model can predict samples from the class of controls. These values range from 1 (perfect discrimination between classes) and 0 (0.5 and lower usually means poor discrimination).

differences observed within each group did not show to be potentially influenced by the polypharmacy regimen adopted by SCZ or BD patients (Table 1), although we recognize that the limited number and high variability of used drug among the samples which were also

Table 3
The list of metabolites from the blood serum samples that were identified as the most important in accordance to PLS-DA models, which had the highest VIP values. Symbols (+) or (−) attributed to schizophrenia (SCZ), bipolar disorder (BD) and healthy control (HC) were attributed to altered concentrations of metabolites – for higher or lower/absent, respectively.

Metabolite	HC	BD	SCZ
N-acetyl-D-mannosamine	+	-	-
2,3-diphospho-D-glyceric acid	-	+	-
N-acetyl aspartyl-glutamic acid (NAAG)	-	+	-
monoethyl malonate	-	+	-
6-hydroxydopamine (6-OHDA)	-	+	+
isovaleryl carnitine	-	-	+
pantothenate	-	-	+
mannitol	-	-	+
glycine	-	-	+
gamma aminobutyric acid (GABA)	-	-	+

collected in different days and clinical facilities do not allow us to definitely clarify this matter. For additional confirmation of the minimum effect of the medication on each individual metabolome, a PCA plot with samples labelled according to the pharmacotherapy was also performed showing only a non-significant trend of clustering (Supplementary Material Figs. S5 and S6). Larger sample cohort with lower degree of polypharmacy may contribute to increase the interpretation of the pharmacotherapy effect, and we recognize this as a limitation of the present study, since, as mentioned, it was not possible to adjust the present metabolome analyses for medication effects due to the limited sample size and high polypharmacy characteristic of the present cohort.

More importantly, the key metabolites with known association with mental disorders and included in the most altered spectral regions among the three groups and/or between SCZ and BD individuals compared to the HC volunteers, are shortly presented as follow.

N-acetyl-aspartate (NAA) is a primary marker of neuronal integrity and function, and it is also a recognized biomarker of neuronal mitochondrial dysfunction in BD. NAA can be converted to NAAG, and

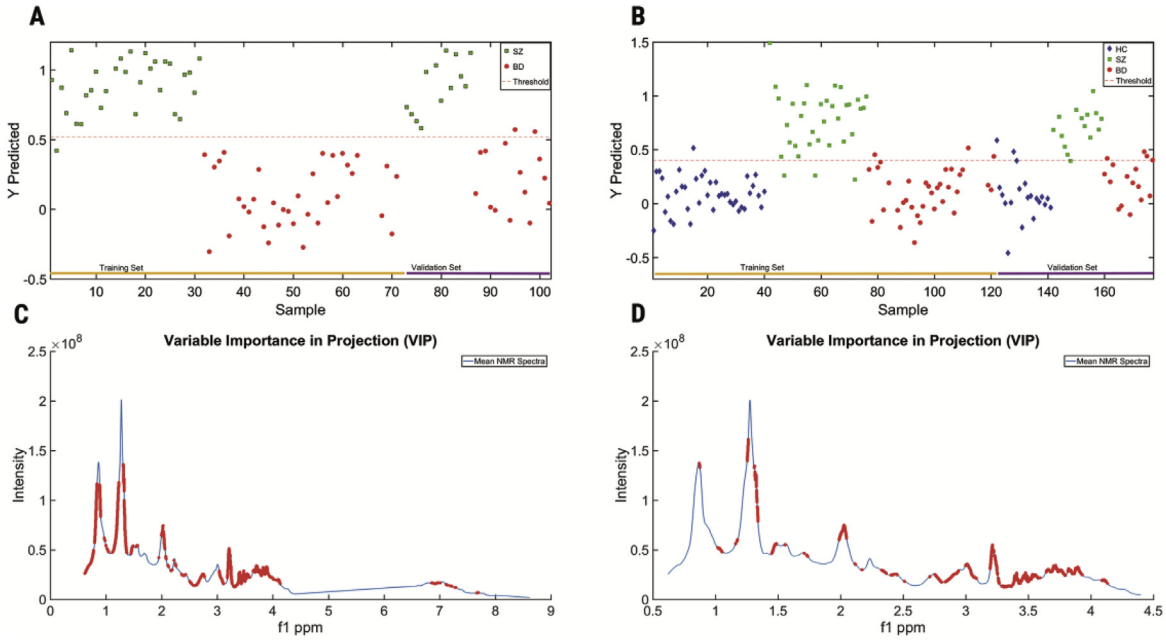

Fig. 4. Illustration of the results obtained in PLS-DA analyses of ^{1}H NMR spectra data from the blood serum samples from schizophrenia (SCZ, green squares) and bipolar disorder (BD, red circles) individuals, and healthy control group (HC, blue diamonds). The PLS-DA prediction plots for (**A**) SCZ and BD groups, using 70% of the samples to construct the model (training set, shown in orange-yellow line) and 30% to test (validation set, shown in purple line); and (**B**) analysis of three groups (namely SCZ, BD and HC) classifications. The PLS-DA plots of variable importance in projections (VIP) obtained for (**C**) SCZ and BD, and (**D**) SCZ, BD and HC models, noting that the red points represents variables with high importance. All PLS-DA were constructed by combining variables from the one (SCZ × BD × HC, the first, 0.60–4.40 ppm) or two (SCZ × BD) ^{1}H NMR spectral regions: 0.60–4.40 ppm and 6.50–8.00 ppm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

high level of NAA and NAAG is important to the central nervous system (CNS), while their levels changes are associated with different neurological diseases (Stork and Renshaw, 2005; Benarroch, 2008). In agreement with our present data, reductions in NAA have been described in the brains of SCZ patients (Renshaw et al., 1995; Fukuzako et al., 1995; Yurgelun-Todd et al., 1996; Bertolino et al., 1998; Nudmamud et al., 2003). In addition, ^{1}H NMR spectroscopy analysis of the brain in bipolar patients treated with lithium(I) had revealed elevated NAA in the basal ganglia region compared to normal volunteers (Sharma et al., 1992). Interestingly, the efflux of intracellular NAA subsequent to sustained cellular swelling was reported in impaired blood-brain barrier and cytotoxic edema conditions (Taylor et al., 1995); and this phenomenon could explain the presence of NAA in the blood serum of BD patients, as we report here for the first time.

Gamma aminobutyric acid (GABA) is an important metabolite that has functions similar to those of glutamic acid neurotransmitter (van Kammen et al., 1982). The variation of GABA levels has been reported as increasing, decreasing or remaining unaltered in SCZ, depending on the disease phase or cerebral region analyzed (Marsman et al., 2014; Chiapponi et al., 2016). Variation of brain GABA level was reported in BD (Brady et al., 2013), and characterization of psychotic and BD by cortical GABA markers was demonstrated (Volk et al., 2016). In addition, the role of GABA neurons in SCZ have been extensively addressed in the last few years (Glausier and Lewis, 2017). In our present study, in accordance with these reports, GABA was present in SCZ individuals, and its presence in blood serum was important for the discrimination of SCZ and BD herein.

Notably, the neurotransmitters GABA and NAAG were also identified in a metabolomic profiling study of post-mortem brains from subjects diagnosed with SCZ or BD (Zhang et al., 2016). More importantly, NAAG has effects on metabotropic and NMDA (*N*-methyl-D-aspartate) glutamate receptors, and it is therefore associated with both glutamatergic and GABAergic neurons, with role as a marker for the integrity and activity of these neurons (Neale et al., 2000). Interestingly, in this study, GABA was present in SCZ, but not in BD and HCs, while NAAG was present in BD, but not in SCZ and HCs (Table 2), suggesting a specific disturbance of neuronal function in SCZ compared to BD. Correlation of NAA reduction with disease duration was reported to be indicative of a degenerative process (Ende et al., 2000), and the potential influence of neuronal damage extent or dysfunction due to axonal loss in the presence or absence of GABA or NAAG in our SCZ and BD individuals will be further analyzed in the near future.

The 6-hydroxidopamine (6-OHDA) was identified here in the blood serum samples of BD and SCZ patients. Stein and Wise (1971) have previously proposed 6-hydroxidopamine (6-OHDA) as a possible causal agent of SCZ, and herein, this molecule was identified in the blood serum of BD and SCZ patients, but not in HCs.

Isovaleryl carnitine is a neurochemical biomarker produced as a consequence of the accumulation of isovaleric acid, which is a metabolite harmful to the CNS (Vockley and Ensenauer, 2006). Interestingly, we have previously described this biomarker in SCZ patients (Tasic et al., 2017a), which was also identified by behavioral metabolomics analysis in methamphetamine sensitization and impaired metabolic flexibility imposed by atypical antipsychotics (Albaugh et al., 2012; Adkins et al., 2013).

Additionally, we could highlight some other general differences in the blood serum metabolomes of SCZ and BD patients compared to HC volunteers, as for instance, the triacylglycerols (TAGs) that are energy storage molecules decomposed by lipolysis after long starvation and energy deprivation period, and that can afterwards be used by the tissues as energy source. Furthermore, the higher concentrations of amino acids as alanine, glutamine and glutamate detected in the SCZ and BD individuals blood serum, may suggest possible disturbed use of glucuronic amino acids via degradation and deamination processes. In fact, bioenergetic abnormalities in SCZ (Steiner et al., 2014; Pillinger et al., 2017) and the influence of antipsychotics in glucose metabolism have been reported by others (Quinones and Kaddurah-Daouk, 2009; Ballon et al., 2014), and their contributions to metabolic dysfunctions are now recognized (Volpato et al., 2013; Freyberg et al., 2017). Indeed, important differences were observed in glucose concentrations (higher in BD individuals), lipid levels (higher in BD), and aromatic compounds levels (higher in SCZ). While higher amounts of lipids and remarkably higher *N*-acyl amino acids, as well as some peptides, were observed in BD serum samples, while SCZ individuals conversely show blood serum metabolite alterations characteristic of long-term neural receptors activation, which may be suggestive of putative inability to inactivate neurotransmitters. Because of this inappropriate switch in peripheral fuel utilization, plasma/serum blood and brains, glycine levels were also reported to be higher in SCZ patients compared to HC (Hashimoto, 2010; Mehdizadeh et al., 2016). In addition, accumulation of metabolites harmful to the CNS was noticed in SCZ, suggesting possible general neurotoxic effects that could be due to the therapy with antipsychotics (Olabi et al., 2011; Torres et al., 2016) or due the disease progression and overall clinical decline of SCZ patients (Schnack et al., 2016; Dragioti et al., 2017; Kotov et al., 2017).

The development of insulin resistance was reported in the course of treatment with a number of current employed psychotropic drugs, particularly with atypical antipsychotics (mainly olanzapine, clozapine, quetiapine, and risperidone), which have a well-established diabetogenic risk (Newcomer, 2005; Manu et al., 2013). In addition, glucose impairment was also demonstrated at the onset of several serious mental illnesses, even before medication starts (Calkin et al., 2013; Garcia-Rizo et al., 2016; Rajkumar et al., 2017). More recently, glucose-6-phosphate dehydrogenase (G6PD), which is the first and rate-limiting enzyme of the pentose phosphate pathway and especially plentiful in brain, was linked to mood and psychotic disorders and its association with mitochondrial impairment and increased generation of reactive oxygen species was suggested (Puthumana and Regenold, 2019). More importantly, a recent report also suggest that high baseline serum triglycerides and coexisting hypertension may significantly predict the hyperglycaemic progression in both BD or SCZ, while the most frequently used antipsychotics did not significantly differ in their associated hyperglycaemic progression rates (Kusumi et al., 2018).

Unfortunately, not all metabolites identified here could be correlated with mental disorders or to pathways with some significance that could be discussed herein. However, taken together, the present metabolomics findings might allow the classification of the subjects as SCZ, BD or HC, regardless of clinical status or medication use, age or gender, as shown in Table 1. In addition, blood serum metabolites were shown to come from CNS and also periphery, and they may reflect changes occurring in the CNS (Davidson et al., 1987; Konicki et al., 1991), supporting the validity of the measurements performed in the present study.

However, these results should be interpreted in light of some limitations. First, the small sample size precluded subgroups analysis, including for ethnicity influence (although self-declaration from patient was considered here). Second, all patients with SCZ and BD were taking medication, which may represent a potential confounder, although medications influence in the PCA showed only a statistically non-significant tendency of clustering (Supplementary Figs. S5 and S6). The duration of illness also has the potential to influence the present data, and the effects of both should be carefully controlled. We have chosen to include only stable patients aiming to mitigate the effect of a possible variation in severity of symptoms, but, as it is very rare to find patients with severe mental illnesses stable without medication, it became impossible to control these two major potential confounders at the same time, without sacrificing the study feasibility. In addition, only typical cases, with no diagnostic doubt, were included, and we cannot estimate if the methodology used could differentiate atypical or incomplete presentations of SCZ and BD. Next steps in this area of research will necessarily involve the inclusion of subjects with a less prototypical clinical profile, as well as the evaluation of BMI (Body Mass Index) or

genetic background influence, which could not be controlled here. Nevertheless, this study was the first to show the possibility of the use of metabolomics by ^{1}H NMR in the identification of possible biomarkers for SCZ and BD, and these limitations should be addressed in further studies.

5. Conclusions

Blood serum metabolomics by proton magnetic resonance enabled the discrimination of individuals with psychiatric disorders, including schizophrenia (SCZ) and bipolar disorder (BD). These patients were also discriminated from healthy control (HC) individuals. As SCZ and BD share clinically common features as symptoms and are both treated with antipsychotics, mainly aiming to block the dopamine pathway (Harvey et al., 2008), we believe that the biomarkers identified in this study will strongly contribute to the support of diagnoses in the near future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.09.009.

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Conflict of interest disclosure

The authors declare no competing financial interest.

References

Adkins, D.E., McClay, J.L., Vunck, S.A., Batman, A.M., Vann, R.E., Clark, S.L., Souza, R.P., Crowley, J.J., Sullivan, P.F., van den Oord, E.J., Beardsley, P.M., 2013. Behavioral metabolomics analysis identifies novel neurochemical signatures in methamphetamine sensitization. Genes Brain Behav. 12 (8), 780–791. https://doi.org/10.1111/gbb.12081.

Albaugh, V.L., Vary, T.C., Ilkayeva, O., Wenner, B.R., Maresca, K.P., Joyal, J.L., Breazeale, S., Elich, T.D., Lang, C.H., Lynch, C.J., 2012. Atypical antipsychotics rapidly and inappropriately switch peripheral fuel utilization to lipids, impairing metabolic flexibility in rodents. Schizophr. Bull. 38 (1), 153–166. https://doi.org/10.1093/schbul/sbq053.

Almeida, M.R., Fidelis, C.H., Barata, L.E., Poppi, R.J., 2013. Classification of Amazonian rosewood essential oil by Raman spectroscopy and PLS-DA with reliability estimation. Talanta 117, 305–311. https://doi.org/10.1016/j.talanta.2013.09.025.

Ballon, J.S., Pajvani, U., Freyberg, Z., Leibel, R.L., Lieberman, J.A., 2014. Molecular pathophysiology of metabolic effects of antipsychotic medications. Trends Endocrinol. Metab. 25 (11), 593–600. https://doi.org/10.1016/j.tem.2014.07.004.

Benarroch, E.E., 2008. *N*-Acetylaspartate and *N*-acetylaspartylglutamate: neurobiology and clinical significance. Neurology 70 (16), 1353–1357. https://doi.org/10.1212/01.wnl.0000311267.63292.6c.

Berrueta, L.A., Alonso-Salces, R.M., Héberger, K., 2007. Supervised pattern recognition in food analysis. J. Chromatogr. A 1158 (1–2), 196–214. https://doi.org/10.1016/j.chroma.2007.05.024.

Bertolino, A., Callicott, J.H., Elman, I., Mattay, V.S., Tedeschi, G., Frank, J.A., Breier, A., Weinberger, D.R., 1998. Regionally specific neuronal pathology in untreated patients with schizophrenia: a proton magnetic resonance spectroscopic imaging study. Biol. Psychiatry 43, 641–648.

Brady Jr., R.O., McCarthy, J.M., Prescot, A.P., Jensen, J.E., Cooper, A.J., Cohen, B.M., Renshaw, P.F., Ongür, D., 2013. Brain gamma-aminobutyric acid (GABA) abnormalities in bipolar disorder. Bipolar Disord. 15 (4), 434–439. https://doi.org/10.1111/bdi.12074.

Bressan, R.A., Chaves, A.C., Shirakawa, I., de Mari, J., 1998. Validity study of the Brazilian version of the calgary depression scale for schizophrenia. Schizophr. Res. 32 (1), 41–49.

Buoli, M., Serati, M., Caldiroli, A., Cremaschi, L., Altamura, A.C., 2017. Neurodevelopmental versus neurodegenerative model of schizophrenia and bipolar disorder: comparison with physiological brain development and aging. Psychiatr. Danub. 29 (1), 24–27.

Calkin, C.V., Gardner, D.M., Ransom, T., Alda, M., 2013. The relationship between bipolar disorder and type 2 diabetes: more than just co-morbid disorders. Ann. Med. 45 (2), 171–181. https://doi.org/10.3109/07853890.2012.687835.

Chiapponi, C., Piras, F., Piras, F., Caltagirone, C., Spalletta, G., 2016. GABA System in schizophrenia and mood disorders: a mini review on third-generation imaging studies. Front. Psychiatry 7, 61. https://doi.org/10.3389/fpsyt.2016.00061.

Correll, C.U., Ng-Mak, D.S., Stafkey-Mailey, D., Farrelly, E., Rajagopalan, K., Loebel, A., 2017. Cardiometabolic comorbidities, readmission, and costs in schizophrenia and bipolar disorder: a real-world analysis. Ann. Gen. Psychiatr. 16, 9. https://doi.org/10.1186/s12991-017-0133-7.

Davidson, M., Losonczy, M.F., Mohs, R.C., Lesser, J.C., Powchik, P., Freed, L.B., Davis, B.M., Mykytyn, V.V., Davis, K.L., 1987. Effects of debrisoquin and haloperidol on plasma homovanillic acid concentration in schizophrenic patients. Neuropsychopharmacology 1, 17–23.

Demjaha, A., MacCabe, J.H., Murray, R.M., 2012. How genes and environmental factors determine the different neurodevelopmental trajectories of schizophrenia and bipolar disorder. Schizophr. Bull. 38 (2), 209–214. https://doi.org/10.1093/schbul/sbr100.

Dragioti, E., Wiklund, T., Siamouli, M., Moutou, K., Fountoulakis, K.N., 2017. Could PANSS be a useful tool in the determining of the stages of schizophrenia? A clinically operational approach. J. Psychiatr. Res. 86, 66–72. https://doi.org/10.1016/j.jpsychires.2016.11.013.

Ende, G., Braus, D.F., Walter, S., Weber-Fahr, W., Soher, B., Maudsley, A.A., Henn, F.A., 2000. Effects of age, medication, and illness duration on the N-acetylaspartate signal of the anterior cingulate region in schizophrenia. Schizophr. Res. 41, 389–395.

Esan, O., Osunbote, C., Oladele, O., Fakunle, S., Ehindero, C., Fountoulakis, K.N., 2017. Bipolar I disorder in remission vs. schizophrenia in remission: is there a difference in burden? Compr. Psychiatry 72, 130–135. https://doi.org/10.1016/j.comppsych.2016.10.009.

Forstner, A.J., Hecker, J., Hofmann, A., et al., 2017. Identification of shared risk loci and pathways for bipolar disorder and schizophrenia. PLoS One 12 (2), e0171595. https://doi.org/10.1371/journal.pone.0171595.

Freyberg, Z., Aslanoglou, D., Shah, R., Ballon, J.S., 2017. Intrinsic and antipsychotic drug-induced metabolic dysfunction in schizophrenia. Front. Neurosci. 11, 432. https://doi.org/10.3389/fnins.2017.00432.

Fukuzako, H., Takeuchi, K., Hokazono, Y., Fukuzako, T., Yamada, K., Hashiguchi, T., Obo, Y., Ueyama, K., Takigawa, M., Fujimoto, T., 1995. Proton magnetic resonance spectroscopy of the left medial temporal and frontal lobes in chronic schizophrenia: preliminary report. Psychiatry Res. Neuroimaging 61, 193–200.

Garcia-Rizo, C., Kirkpatrick, B., Fernandez-Egea, E., Oliveira, C., Bernardo, M., 2016. Abnormal glycemic homeostasis at the onset of serious mental illnesses: a common pathway. Psychoneuroendocrinology 67, 70–75. https://doi.org/10.1016/j.psyneuen.2016.02.001.

Glausier, J.R., Lewis, D.A., 2017. GABA and schizophrenia: where we stand and where we need to go. Schizophr. Res. 181, 2–3. https://doi.org/10.1016/j.schres.2017.01.050.

Grande, I., Magalhães, P.V., Chendo, I., Stertz, L., Panizutti, B., Colpo, G.D., Rosa, A.R., Gama, C.S., Kapczinski, F., Vieta, E., 2014. Staging bipolar disorder: clinical, biochemical, and functional correlates. Acta Psychiatr. Scand. 129 (6), 437–444. https://doi.org/10.1111/acps.12268.

Grover, S., Chakrabarti, S., Ghormode, D., Dutt, A., 2015. A comparative study of caregivers' perceptions of health-care needs and burden of patients with bipolar affective disorder and schizophrenia. Nord. J. Psychiatry 69 (8), 629–636. https://doi.org/10.3109/08039488.2015.1033010.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. J. Soc. Clin. Psychol. 6 (4), 278–296.

Harvey, P.D., Endicott, J.M., Loebel, A.D., 2008. The factor structure of clinical symptoms in mixed and manic episodes prior to and after antipsychotic treatment. Bipolar Disord. 10 (8), 900–906. https://doi.org/10.1111/j.1399-5618.2008.00634.x.

Hashimoto, K., 2010. Glycine transport inhibitors for the treatment of schizophrenia. Open Med. Chem. J. 4, 10–19. https://doi.org/10.2174/1874104501004010010.

He, Y., Yu, Z., Giegling, I., Hartmann, A.M., Prehn, C., Adamski, J., Kahn, R., Li, Y., Illig, T., Wang-Sattler, R., Rujescu, D., 2012. Schizophrenia shows a unique metabolomics signature in plasma. Transl. Psychiatry 2, e149. https://doi.org/10.1038/tp.2012.76.

Jani, S., Johnson, R.S., Banu, S., Shah, A., 2016. Cross-cultural bias in the diagnosis of borderline personality disorder. Bull. Menn. Clin. 80 (2), 146–165. https://doi.org/10.1521/bumc.2016.80.2.146.

Kaddurah-Daouk, R., Krishnan, K.R.R., 2009. Metabolomics: a global biochemical approach to the study of central nervous system diseases. Neuropsychopharmacology 34, 173–186. https://doi.org/10.1038/npp.2008.174.

Ketter, T.A., Wang, P.W., Becker, O.V., Nowakowska, C., Yang, Ys, 2004. Psychotic bipolar disorders: dimensionally similar to or categorically different from schizophrenia? J. Psychiatr. Res. 38 (1), 47–61.

Konicki, P.E., Owen, R.R., Litman, R.E., Pickar, D., 1991. The acute effects of central- and peripheral-acting dopamine antagonists on plasma HVA in schizophrenic patients. Life Sci. 48, 1411–1416.

Kotov, R., Fochtmann, L., Li, K., Tanenberg-Karant, M., Constantino, E.A., Rubinstein, J., Perlman, G., Velthorst, E., Fett, A.J., Carlson, G., Bromet, E.J., 2017. Declining clinical course of psychotic disorders over the two decades following first hospitalization: evidence from the Suffolk County Mental Health Project. Am. J. Psychiatry 174 (11), 1064–1074. https://doi.org/10.1176/appi.ajp.2017.16101191.

Kusumi, I., Arai, Y., Okubo, R., Honda, M., Matsuda, Y., Matsuda, Y., Tochigi, A., Takekita, Y., Yamanaka, H., Uemura, K., Ito, K., Tsuchiya, K., Yamada, J., Yoshimura, B., Mitsui, N., Matsubara, S., Segawa, T., Nishi, N., Sugawara, Y., Kako, Y., Shinkawa, I., Shinohara, K., Konishi, A., Iga, J., Hashimoto, N., Inomata, S., Tsukamoto, N., Ito,

H., Ito, Y.M., Sato, N., 2018. Predictive factors for hyperglycaemic progression in patients with schizophrenia or bipolar disorder. B J. Psych. Open 4 (6), 454–460. https://doi.org/10.1192/bjo.2018.56.
Levine, S.Z., Rabinowitz, J., 2007. Revisiting the 5 dimensions of the positive and negative syndrome scale. J. Clin. Psychopharmacol. 27 (5), 431–436. https://doi.org/10.1097/jcp/.0b013e31814cfabd.
Li, B., He, X., Jia, W., Li, H., 2017. Novel applications of metabolomics in personalized medicine: a mini-review. Molecules 22 (7), E1173. https://doi.org/10.3390/molecules22071173.
Manu, P., Correll, C.U., Wampers, M., van Winkel, R., Yu, W., Shiffeldrim, D., Kane, J.M., De Hert, M., 2013. Insulin secretion in patients receiving clozapine, olanzapine, quetiapine and risperidone. Schizophr. Res. 143, 358–362. https://doi.org/10.1016/j.schres.2012.11.016.
Marsman, A., Mandl, R.C.W., Klomp, D.W.J., Bohlken, M.M., Boer, V.O., Andreychenko, A., Cahn, W., Kahn, R.S., Luijten, P.R., Hulshoff Pol, H.E., 2014. GABA and glutamate in schizophrenia: a 7 T ^{1}H-MRS study. Neuroimage Clin. 6, 398–407. https://doi.org/10.1016/j.nicl.2014.10.005.
Mehdizadeh, M., Rezaei, O., Dolatshahi, B., 2016. Determining the effectiveness of the third person interview in the level of insight psychotic patients. Psychiatry Res. 245, 238–242. https://doi.org/10.1016/j.psychres.2016.08.001.
Mehmood, T., Liland, K.H., Snipen, L., Sæbø, S., 2012. Chemometrics and intelligent laboratory systems a review of variable selection methods in partial least squares regression. Chemometr. Intell. Lab. Syst. 118, 62–69. https://doi.org/10.1016/j.chemolab.2012.07.010.
Meiboom, S., Gill, D., 1958. Modified spin-echo method for measuring nuclear relaxation times. Rev. Sci. Instrum. 29, 688–691.
Neale, J.H., Bzdega, T., Wroblewska, B., 2000. N-Acetylaspartylglutamate: the most abundant peptide neurotransmitter in the mammalian central nervous system. J. Neurochem. 75, 443–452.
Newcomer, J.W., 2005. Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. CNS Drugs 19 (Suppl. 1), 1–93. https://doi.org/10.2165/00023210-200519001-00001.
Nudmamud, S., Reynolds, L.M., Reynolds, G.P., 2003. N-acetylaspartate and N-Acetylaspartylglutamate deficits in superior temporal cortex in schizophrenia and bipolar disorder: a postmortem study. Biol. Psychiatry 53 (12), 1138–1141.
Olabi, B., Ellison-Wright, I., McIntosh, A.M., Wood, S.J., Bullmore, E., Lawrie, S.M., 2011. Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. Biol. Psychiatry 70 (1), 88–96. https://doi.org/10.1016/j.biopsych.2011.01.032.
Orešič, M., Tang, J., Seppänen-Laakso, T., Mattila, I., Saarni, S.E., Saarni, S.I., Lönnqvist, J., Sysi-Aho, M., Hyötyläinen, T., Perälä, J., Suvisaari, J., 2011. Metabolome in schizophrenia and other psychotic disorders: a general population-based study. Genome Med. 3 (3), 19. https://doi.org/10.1186/gm233.
Pillinger, T., Beck, K., Gobjila, C., Donocik, J.G., Jauhar, S., Howes, O.D., 2017. Impaired glucose homeostasis in first-episode schizophrenia: a systematic review and meta-analysis. JAMA Psychiatr. 74 (3), 261–269. https://doi.org/10.1001/jamapsychiatry.2016.3803.
Piotto, M., Saudek, V., Sklenár, V., 1992. Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions. J. Biomol. NMR 2, 661–665.
Puthumana, J.S., Regenold, W.T., 2019. Glucose-6-phosphate dehydrogenase activity in bipolar disorder and schizophrenia: relationship to mitochondrial impairment. J. Psychiatr. Res. 112, 99–103. https://doi.org/10.1016/j.jpsychires.2019.03.004.
Quinones, M.P., Kaddurah-Daouk, R., 2009. Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. Neurobiol. Dis. 35 (2), 165–176. https://doi.org/10.1016/j.nbd.2009.02.019.
Quintero, M., Stanisic, D., Cruz, G., Pontes, J.G., Costa, T.B.B.C., Tasic, L., 2019. Metabolomic biomarkers in mental disorders: bipolar disorder and schizophrenia. In: Reviews on Biomarker Studies in Psychiatric and Neurodegenerative Disorders. Springer, Cham, pp. 271–293.
Rajkumar, A.P., Horsdal, H.T., Wimberley, T., Cohen, D., Mors, O., Børglum, A.D., Gasse, C., 2017. Endogenous and antipsychotic-related risks for diabetes mellitus in young people with schizophrenia: a Danish population-based cohort study. Am. J. Psychiatry 174 (7), 686–694. https://doi.org/10.1176/appi.ajp.2016.16040442.
Rasmus, B., Smilde, A.K., 2014. Principal component analysis. Anal. Methods 2812–2831.
Renshaw, P.F., Yurgelun-Todd, D.A., Tohen, G.S., Gruber, S., Cohen, B.M., 1995. Temporal lobe proton magnetic resonance spectroscopy of patients with first-episode psychosis. Am. J. Psychiatry 152, 444–446. https://doi.org/10.1176/ajp.152.3.444.
Savorani, F., Tomasi, G., Engelsen, S.B., 2010. icoshift: a versatile tool for the rapid alignment of 1D NMR spectra. J. Magn. Reson. 202 (2), 190–202.
Schnack, H.G., van Haren, N.E., Nieuwenhuis, M., Hulshoff Pol, H.E., Cahn, W., Kahn, R.S., 2016. Accelerated brain aging in schizophrenia: a longitudinal pattern recognition study. Am. J. Psychiatry 173 (6), 607–616. https://doi.org/10.1176/appi.ajp.2015.15070922.
Sethi, S., Pedrini, M., Rizzo, L.B., Zeni-Graiff, M., Mas, C.D., Cassinelli, A.C., Noto, M.N., Asevedo, E., Cordeiro, Q., Pontes, J.G.M., Brasil, A.J.M., Lacerda, A., Hayashi, M.A.F., Poppi, R., Tasic, L., Brietzke, E., 2017. ^{1}H-NMR, ^{1}H-NMR T2-edited, and 2D-NMR in bipolar disorder metabolic profiling. Int. J. Bipolar Disord. 5 (1), 23. https://doi.org/10.1186/s40345-017-0088-2.
Shaffer, M., Armstrong, A.J.S., Phelan, V.V., Reisdorph, N., Lozupone, C.A., 2017. Microbiome and metabolome data integration provides insight into health and disease. Transl. Res. 189, 51–64. https://doi.org/10.1016/j.trsl.2017.07.001.
Sharma, R., Venkatasubramanian, P.N., Bárány, M., Davis, J.M., 1992. Proton magnetic resonance spectroscopy of the brain in schizophrenic and affective patients. Schizophr. Res. 8 (1), 43–49.
Stein, L., Wise, C.D., 1971. Possible etiology of schizophrenia: progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science 171 (3975), 1032–1036.
Steiner, J., Bernstein, H.G., Schiltz, K., Müller, U.J., Westphal, S., Drexhage, H.A., Bogerts, B., 2014. Immune system and glucose metabolism interaction in schizophrenia: a chicken-egg dilemma. Prog. Neuropsychopharmacol. Biol. Psychiatry 48, 287–294. https://doi.org/10.1016/j.pnpbp.2012.09.016.
Stork, C., Renshaw, P.F., 2005. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. Mol. Psychiatry 10 (10), 900–919. https://doi.org/10.1038/sj.mp.4001711.
Tasic, L., Pontes, J.G., Carvalho, M.S., Cruz, G., Dal Mas, C., Sethi, S., Pedrini, M., Rizzo, L.B., Zeni-Graiff, M., Asevedo, E., Lacerda, A.L.T., Bressan, R.A., Poppi, R.J., Brietzke, E., Hayashi, M.A.F., 2017a. Metabolomics and lipidomics analyses by ^{1}H nuclear magnetic resonance of schizophrenia patient serum reveal potential peripheral biomarkers for diagnosis. Schizophr. Res. 185, 182–189. https://doi.org/10.1016/j.schres.2016.12.024.
Tasic, L., Pontes, J.G.M., de Souza, R.N., Brasil, A.J.M., Cruz, G.C.F., Asevedo, E., Dal Mas, C., Poppi, R.J., Brietzke, E., Hayashi, M.A.F., Lacerda, A.L.T., 2017b. NMR spectroscopy metabolomics applied to crack cocaine users and patients with schizophrenia: similar behavior but different molecular causes. Chem. Select 2 (10), 2927–2930. https://doi.org/10.1002/slct.201700009.
Taylor, D.L., Davies, S.E., Obrenovitch, T.P., Doheny, M.H., Patsalos, P.N., Clark, J.B., Symon, L., 1995. Investigation into the role of N-acetylaspartate in cerebral osmoregulation. J. Neurochem. 65 (1), 275–281.
Torres, U.S., Duran, F.L., Schaufelberger, M.S., Crippa, J.A., Louzã, M.R., Sallet, P.C., Kanegusuku, C.Y., Elkis, H., Gattaz, W.F., Bassitt, D.P., Zuardi, A.W., Hallak, J.E., Leite, C.C., Castro, C.C., Santos, A.C., Murray, R.M., Busatto, G.F., 2016. Patterns of regional gray matter loss at different stages of schizophrenia: a multisite, cross-sectional VBM study in first-episode and chronic illness. Neuroimage Clin. 12, 1–15. https://doi.org/10.1016/j.nicl.2016.06.002.
Ulrich, E.L., Akutsu, H., Doreleijers, J.F., Harano, Y., Ioannidis, Y.E., Lin, J., Livny, M., Mading, S., Maziuk, D., Miller, Z., Nakatani, E., Schulte, C.F., Tolmie, D.E., Kent Wenger, R., Yao, H., Markley, J.L., 2008. BioMagResBank. Nucleic Acids Res. 36, D402–D408. https://doi.org/10.1093/nar/gkm957.
van Kammen, D.P., Sternberg, D.E., Hare, T.A., Waters, R.N., Bunney Jr., W.E., 1982. CSF levels of γ-aminobutyric acid in Schizophrenia. JAMA Psychiatr. 39 (1), 91–97. https://doi.org/10.1001/archpsyc.1982.04290010065012.
van Rheenen, T.E., Lewandowski, K.E., Tan, E.J., Ospina, L.H., Ongur, D., Neill, E., Gurvich, C., Pantelis, C., Malhotra, A.K., Rossell, S.L., Burdick, K.E., 2017. Characterizing cognitive heterogeneity on the schizophrenia-bipolar disorder spectrum. Psychol. Med. 28, 1–17. https://doi.org/10.1017/S0033291717000307.
van Winkel, R., Esquivel, G., Kenis, G., Wichers, M., Collip, D., Peerbooms, O., Rutten, B., Myin-Germeys, I., Van Os, J., 2010. REVIEW: genome-wide findings in schizophrenia and the role of gene-environment interplay. CNS Neurosci. Ther. 16 (5), e185–192. https://doi.org/10.1111/j.1755-5949.2010.00155.x.
Vockley, J., Ensenauer, R., 2006. Isovaleric acidemia: new aspects of genetic and phenotypic heterogeneity. Am. J. Med. Genet. C Semin. Med. Genet. 142C (2), 95–103. https://doi.org/10.1002/ajmg.c.30089.
Volk, D.W., Sampson, A.R., Zhang, Y., Edelson, J.R., Lewis, D.A., 2016. Cortical GABA markers identify a molecular subtype of psychotic and bipolar disorders. Psychol. Med. 46 (12), 2501–2512. https://doi.org/10.1017/S0033291716001446.
Volpato, A.M., Zugno, A.I., Quevedo, J., 2013. Recent evidence and potential mechanisms underlying weight gain and insulin resistance due to atypical antipsychotics. Rev. Bras. Psiquiatr. 35 (3), 295–304. https://doi.org/10.1590/1516-4446-2012-1052.
Wishart, D.S., Tzur, D., Knox, C., et al., 2007. HMDB: the human metabolome database. Nucleic Acids Res. 35, D521–D526. https://doi.org/10.1093/nar/gkl923.
Wood, S.J., Berger, G., Velakoulis, D., Phillips, L.J., McGorry, P.D., Yung, A.R., Desmond, P., Pantelis, C., 2003. Proton magnetic resonance spectroscopy in first episode psychosis and ultra high-risk individuals. Schizophr. Bull. 29 (4), 831–843.
Xuan, J., Pan, G., Qiu, Y., Yang, L., Su, M., Liu, Y., Chen, J., Feng, G., Fang, Y., Jia, W., Xing, Q., He, L., 2011. Metabolomic profiling to identify potential serum biomarkers for schizophrenia and risperidone action. J. Proteome Res. 10 (12), 5433–5443. https://doi.org/10.1021/pr2006796.
Yang, J., Chen, T., Sun, L., Zhao, Z., Qi, X., Zhou, K., Cao, Y., Wang, X., Qiu, Y., Su, M., Zhao, A., Wang, P., Yang, P., Wu, J., Feng, G., He, L., Jia, W., Wan, C., 2013. Potential metabolite markers of schizophrenia. Mol. Psychiatry 18, 67–78. https://doi.org/10.1038/mp.2011.
Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry 133, 429–435.
Yurgelun-Todd, D.A., Renshaw, P.F., Gruber, S.A., Ed, M., Waternaux, C., Cohen, B.M., 1996. Proton magnetic resonance spectroscopy of the temporal lobes in schizophrenics and normal controls. Schizophr. Res. 19, 55–59.
Zhan, X., Long, Y., Lu, M., 2018. Exploration of variations in proteome and metabolome for predictive diagnostics and personalized treatment algorithms: innovative approach and examples for potential clinical application. J. Proteomics. 188, 30–40. https://doi.org/10.1016/j.jprot.2017.08.020.
Zhang, R., Zhang, T., Ali, A.M., Al Washih, M., 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. Comput. Struct. Biotechnol. J. 14, 106–116. https://doi.org/10.1016/j.csbj.2016.02.003.
Zhou, Y., Rosenheck, R., Mohamed, S., Ou, Y., Ning, Y., He, H., 2016. Comparison of burden among family members of patients diagnosed with schizophrenia and bipolar disorder in a large acute psychiatric hospital in China. BMC Psychiatry 16 (1), 283. https://doi.org/10.1186/s12888-016-0962-y.