^{1}H-NMR-Based Serum Metabolomics of Bipolar Disorder Patients

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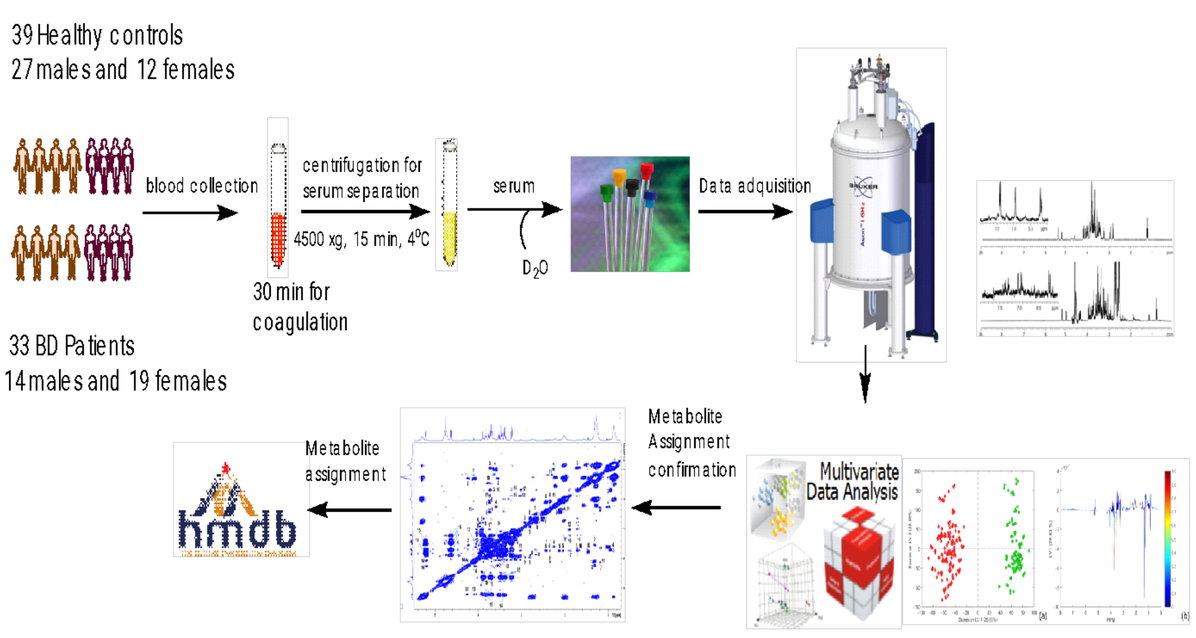
INTRODUCTION

Bipolar disorder (BD) is a mental disorder that causes alteration of mood states including mania, depression, and euthymia and it is ranked as one of the leading causes of disability and premature mortality, with a prevalence of 60 million people worldwide. Diagnosis of BD exclusively depends on the subjective recognition of symptoms without any objective methods such as a clinical test of biomarker identification, instigating misdiagnosis, inadequate treatments and deficient clinical outcomes.

OBJECTIVES

We studied a ^{1}H-NMR-based metabolomics of human blood serum of BD patients in Serbia in order to identify alterations of metabolites. Our goal was to confirm the difference between BD patients and healthy control groups based on their metabolic profiles with the intention to identify potential biomarkers for BD diagnosis and verify the possibility of their use in personalized medicine.

METHODOLOGY



RESULTS

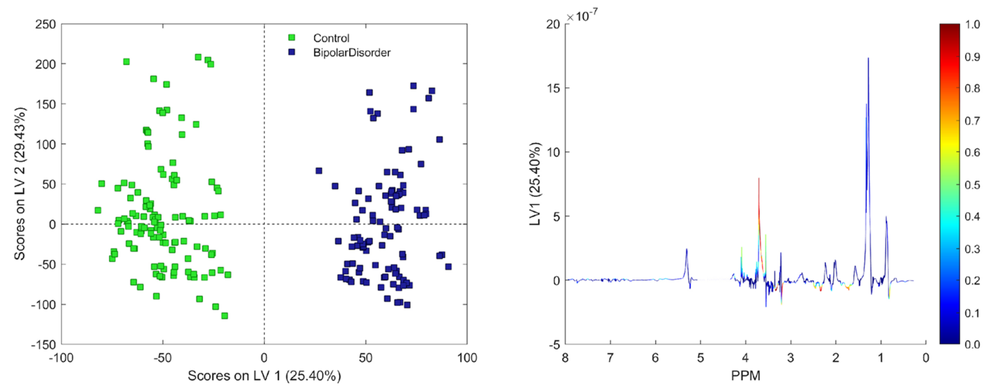


Figure 2. (a) Scores plot of the first two LV components of the OPLS-DA model using mean-centering and unit variance scaling, The BD samples are shown in dark blue and the control group samples are shown in light green. (b) Back-scale projection of loading vector LV 1 to coloring coded according to the absolute value of particular loading weighted by correlation of spectral data set and scores matrix from the OPLS-DA model.

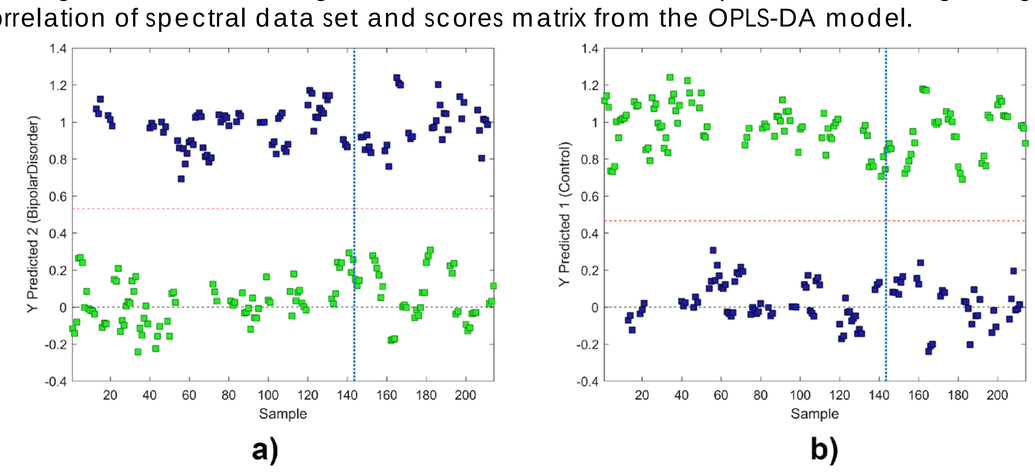


Figure 3. (a) Classification results for prediction of training (left from blue dot line) and test (right of the blue dotted line) data set for the 'BD' group of patients, and the threshold value of 0.5326. (b) Classification results for prediction of training (left from blue dot line) and test (right of the blue dotted line) data set for the 'Control' group of individuals and the threshold value of 0.4674. Auto centering and scaling were performed as data preprocessing.

RESULTS

The set of 22 potential serum BD biomarkers were identified by performing analyses of spectral 1D and 2D NMR data accomplished in TOCSY, JRES and HSQC experiments as presented with spectral data in Table 1.

Table 1. Metabolites/biomarkers identified in serum samples of the BD patients with spectral data

No	Metabolites/ Biomarkers	TOCSY Correlations (δ_H, ppm)	JRES (δ_H (ppm), Multiplicity, J(Hz))	HSQC (δ_H/δ_C(ppm))
1	Lactate/lactic acid	4.10; 1.31	CH3: 1.31, d, 6.98; CH: 4.10 q, 7.0	1.32/22.79, 4.098/71.25
2	Threonine	1.31; 3.56; 4.24	CH3: 1.32, d, overlapped with lactate; CH: 3.56 d, 5.0; CH2: 4.23 dd, 4.9, 6.6, overlapped with acylglycerol	1.34/22.54, 3.55/63.42, 4.24
3	Leucine	0.95; 1.71; 3.71	CH3: 0.94, d, 6.24; CH3: 0.95, d, 6.24	0.94/23.41, 0.95/24.72, 1.71/42.70, 3.71
4	Valine	0.98; 1.03; 2.27; 3.62	CH3: 0.97, d, 7.00; CH3: 1.03, d, 7.00; CH: 3.59 d, 4.39	0.97/19.26, 1.02/20.6, 2.27, 3.59/63.27
5	Glutamine	2.12; 2.44; 3.74	CH2: 2.12 m; CH2: 2.44 m	2.12/29.27, 2.43/33.61, 3.74/57.11
6	Glutamate/glutamic acid	2.05; 2.35; 3.75	CH2: 2.04, m and 2.11 m	2.0/29.68, 2.34/36.28, 3.74/57.11
7	Citrate/citric acid	2.51; 2.68	CH2: 2.51 d, 16.0; CH2: 2.68 d, 16.0	-
8	Aspartate/aspartic acid	2.68; 2.80; 3.88	CH2: 2.66, dd, 8.8, 17.5 and 2.80, dd 3.8, 17.4	3.80/54.56
9	Alanine	1.46; 3.77	CH3: 1.46, d, 7.26	3.76/53.21
10	3-Hydroxybutyric acid	1.19; 2.34; 4.12	CH3: 1.19 d, 6.4; CH2: 2.40, dd, 7.2, 14.4 and 2.29 dd, 6.4, 14.4	-
11	Gamma-aminobutyric acid	1.9; 3.03	CH2: 3.04, t, 7.6	-
12	Choline	3.50; 4.05	CH2: 4.05 m	4.05/58.35
13	Glucose (α + β)	3.40; 3.52; 3.7; 3.75; 5.10; 5.22	CH-4: 3.40 m; CH-2: 3.52 dd, 3.7, 9.7; CH-3: 3.70 m (overlapped); CH2-6: 3.75 dd, 5.1, 12.0 and 3.83 m; CH-5: 3.82 m; CH-1: 5.22 d, 3.9	-
14	Arginine	4.07; 4.27; 5.20	3.23 t, 6.6; 1.70, m and 1.64, m	-
15	Lysine	1.70; 1.89; 3.03; 3.74	1.91 m	-
16	2-Hydroxybutyric acid	-	CH3: 0.88, t, 7.50; CH2: 1.70, m and 1.64, m or arginine	-
17	Isoleucine	-	CH3: 0.92, t, 7.4; CH3: 0.99, d, 7.0; 3.65 d, 4.04	-
18	Serin	-	CH2: 3.97, dd, 3.8, 12.2 and 3.92, dd 5.7, 12.2; CH: 3.82 overlapped	3.95/62.94, 3.81/59.2
19	Mannose	-	CH: 3.55 t, 9.4; CH: 3.79 m; CH: 3.84 dd, 2.2, 4.0; CH: 3.95 m; CH: 5.17, d1.4	-
20	Glycerol	-	CH2: 3.64 and 3.55 m; CH: 3.70 m (overlapped)	3.63 and 3.55/65.31
21	Tyrosine	6.88; 7.18	CH: 3.96, dd, 5.0, 8.1 or phenylalanine; Ar: 6.88 and 7.18	3.95/58.78, Ar: 6.88/118.6, 7.18/133.4
22	Phenylalanine	7.30; 7.36; 7.42	Ar: 7.30 m, 7.37 m, 7.41 m	Ar: 7.31/132.01, 7.40/131.80

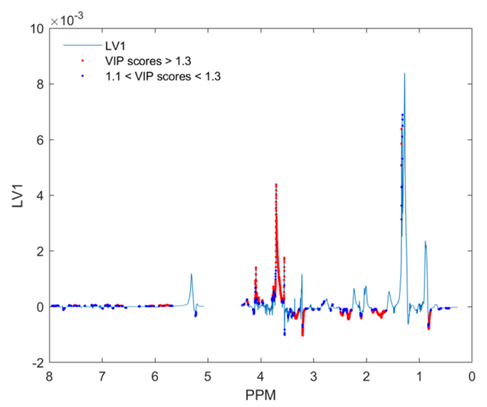


Figure 4. VIP scores presented on OPLS-DA model back-scale projection of LV 1 predicting component using autoscale centering and scaling for preprocessing.

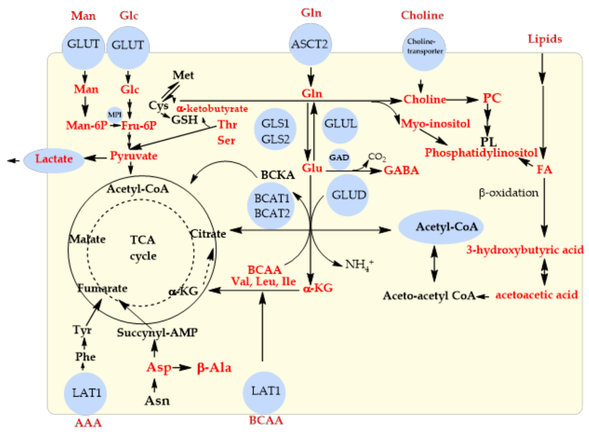


Figure 5. Illustration of the main metabolic pathways reported as altered in BD.

Chemometrics

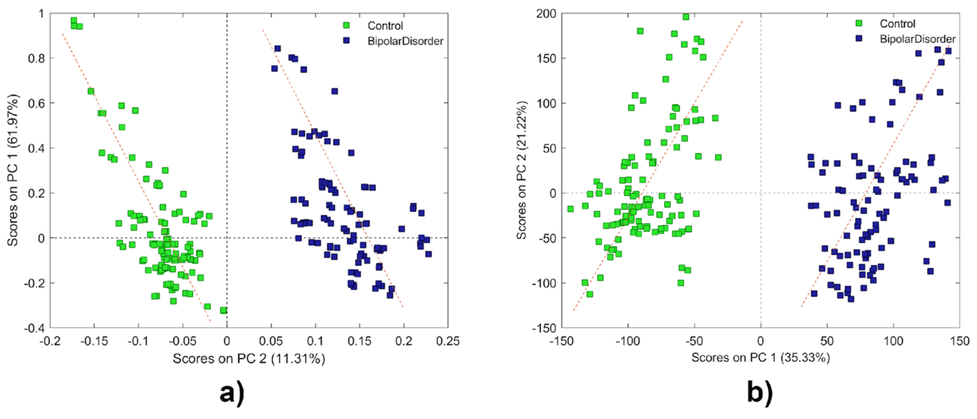
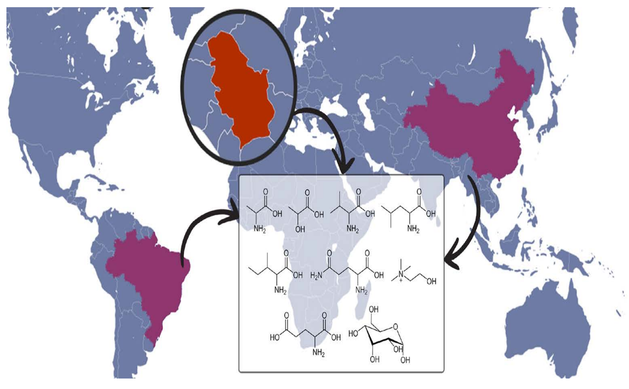


Figure 1. (a) Score plot of PCA model composed with mean centering and Pareto scaling, where dashed line denotes the direction of cluster shapes for each of the class, which is tilted slightly left in comparison to the vertical PC 1 component. (b) Score plot of PCA model composed with class centroid and centering, where dashed line denotes the direction of cluster shapes for each of the class, which is tilted slightly right in comparison to the vertical PC2 component. Both models were assembled after removing identified outliers.

CONCLUSIONS



NMR-based metabolomics study of BD patients from Serbia identified nine metabolites: lactate, alanine, valine, leucine, isoleucine, glutamine, glutamate, glucose and choline that are equal to previous reported studies in serum samples of BD patients in Brazil and China, emphasizing their crucial role in possibility of application as biomarkers for diagnosis of BD.

ACKNOWLEDGMENTS

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