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Blood metabolomics analysis identifies abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism in bipolar disorder

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ABSTRACT

Background: Bipolar disorder (BD) is a severe and debilitating psychiatric disorder. However, the precise biological basis remains unknown, hampering the search for novel biomarkers. We performed a metabolomics analysis to discover novel peripheral biomarkers for BD.
Methods: We quantified serum levels of 116 metabolites in mood-stabilized male BD patients (n = 54) and age-matched male healthy controls (n = 39).
Results: After multivariate logistic regression, serum levels of pyruvate, *N*-acetylglutamic acid, α-ketoglutarate, and arginine were significantly higher in BD patients than in healthy controls. Conversely, serum levels of β-alanine, and serine were significantly lower in BD patients than in healthy controls. Chronic (4-weeks) administration of lithium or valproic acid to adult male rats did not alter serum levels of pyruvate, *N*-acetylglutamic acid, β-alanine, serine, or arginine, but lithium administration significantly increased serum levels of α-ketoglutarate.
Conclusions: The metabolomics analysis demonstrated altered serum levels of pyruvate, *N*-acetylglutamic acid, β-alanine, serine, and arginine in BD patients.
General significance: The present findings suggest that abnormalities in the citric acid cycle, urea cycle, and amino acid metabolism play a role in the pathogenesis of BD.

1. Introduction

Bipolar disorder (BD) is a chronic mental illness characterized by recurrent episodes of depression, mania, and hypomania. Worldwide, the combined lifetime prevalence of BD I and II is estimated between 1 and 3% [1]. People with BD are more prone to seek care when they are depressed than when experiencing mania or hypomania. The condition might therefore mistakenly be diagnosed as major depression and BD patients are frequently treated with antidepressants in primary care [2]. This is a problem because monotherapy with antidepressants increase the risk of switch to mania in BD [3] and might sometimes worsen symptoms [4,5]. Treatment guidelines therefore recommend antidepressants only as adjunct therapy to mood stabilizers [6,7]. Misdiagnosis will thus prevent BD patients from receiving the correct medication, and risk worsening the outcome. Biomarkers for aiding the diagnosis of BD are therefore warranted.

Although the precise pathogenesis of BD is not well understood, accumulating evidence suggests that inflammation [8–10], mitochondrial dysfunction, and oxidative stress [11–19] play a role in the pathogenesis of BD. In addition, brain-derived neurotrophic factor (BDNF) and its precursor proBDNF, have been suggested as peripheral biomarkers for BD [17,20–25]. Recently, we also reported that serum levels of glutamine, glycine, and D-serine were significantly higher in BD patients than in healthy controls, whereas serum levels of L-serine were significantly lower in BD patients than in healthy controls [26]. However, we did not find alterations in these amino acids in cerebrospinal fluid (CSF) from the same BD patients [26]. The discrepancy between blood and CSF highlights the importance to measure a potential biomarker in

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both blood and CSF. Peripheral biomarkers are desirable since it is easy to collect blood samples.

Metabolomics is the profiling of small molecule metabolites and provides the potential to characterize specific metabolic phenotypes associated with a disease. Metabolomics has the advantage over other "omics" techniques in that it directly samples the metabolic changes in an organism and integrates information from changes at the gene, transcript, and protein level, as well as posttranslational modifications [27–29]. Metabolomics analysis with capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) [30] has been used in the discovery of biomarkers for psychiatric disorders, including BD [31], schizophrenia [32], and autism spectrum disorder (ASD) [33]. We recently found that CSF levels of isocitrate were significantly higher in BD patients than in healthy controls, and that the expression of isocitrate dehydrogenase (IDH3A) mRNA and protein were significantly lower in postmortem brain tissue from BD patients than in control samples [31]. These findings suggested that abnormal mitochondrial metabolism of isocitrate by IDH3A plays a key role in the pathogenesis of BD [31]. However, there are no corresponding metabolomics analyses of serum from BD patients and healthy controls.

Here, we performed a metabolomics analysis using CE-TOFMS of serum samples from mood-stabilized BD patients (n = 54) and age-matched healthy controls (n = 39) [31]. We selected 116 major metabolic compounds from the following pathways for metabolomics analysis: the glycolytic system, the pentose phosphate pathway, the citric acid cycle, the urea cycle, the polyamine-creatine metabolism pathway, the purine metabolism pathway, the glutathione metabolism pathway, the nicotinamide metabolism pathway, the choline metabolism pathway and several amino acid metabolism pathways (Supplemental Table 1). To examine potential effects of medication on the studied metabolites, we also performed a metabolomics analysis of serum samples from rats chronically treated with lithium (Li) or valproic acid (VPA).

2. Methods and Materials

2.1. Participants

The BD patients were recruited from the St. Göran bipolar project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden. The work-up and diagnostic assessments have been described in detail previously [26,34,35]. The key clinical assessment instrument was the Affective Disorder Evaluation (ADE), developed for the Systematic Treatment Enhancement Program of Bipolar Disorder (STEP-BD) [36]. The full diagnostic assessment was based on all available sources of information including patient interview, case records, and if possible interviews with the next of kin. To reduce inter-rater bias, a best-estimate diagnostic decision based on all information available at admission was made at a diagnostic case-conference by a consensus panel of experienced board certified psychiatrists (n = 2–5) specialized in BD.

The general criteria for inclusion were: 1) age of at least 18 years and 2) meeting the Diagnostic and Statistical Manual (DSM)-IV criteria for bipolar spectrum disorder (i.e., type I, type II, or not otherwise specified). Information regarding age, sex, number of lifetime manic/hypomanic/depressive/total episodes, duration of illness (defined as years since the first hypomanic or manic episode), body mass index (BMI), and previous psychotic episodes was collected. The severity of BD was rated using the Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF) rating scales. For ethical reasons, patients continued to take their prescribed medications at the time of serum sampling.

Population-based controls were randomly selected by Statistics Sweden (SCB) and contacted by mail. Given an expected response rate of 1:7, seven invitations were sent out per enrolled case. Fourteen percent of the invited controls responded to the invitation, and were subjected to a preliminary telephone screening by a research nurse to exclude severe mental health conditions, neurological diseases, and substance abuse. Eligible persons were scheduled for a one-day comprehensive assessment where they underwent further psychiatric interviewing by experienced clinicians using the Mini-International Neuropsychiatric Interview (M.I.N.I.) to exclude psychiatric disorders [37]. Substance abuse was screened for at the telephone interview by the nurse, in the psychiatric interview, by the Alcohol Use Disorders Identification Test (AUDIT), and by the Drug Use Disorders Identification Test (DUDIT), as well as by determining serum levels of carbohydrate-deficient transferrin (CDT) [38]. Overconsumption of alcohol as revealed by CDT or responses indicating large consumption (>8 standard drinks per time more than 2 times per week), and/or amnesia and/or loss of control more than once per month resulted in the exclusion of these individuals from the study. Other exclusion criteria were neurological conditions other than mild migraines, untreated endocrinological disorders, pregnancy, dementia, recurrent depressive disorder, and suspected severe personality disorders (based on interview and the Structured Clinical Interview for DSM (SCID-II) screen personality assessment), as well as a family history of schizophrenia or BD in first-degree relatives.

The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the latest Helsinki Protocol. All patients and controls consented orally and in writing to participate in the study. Informed consent was obtained during a euthymic period (i.e., during a time period when patients did not meet criteria for a depressive, hypomanic, or manic episode). All patients were capable of freely giving fully informed consent, as determined by the physicians who enrolled the patient. A total of 54 male BD patients and 39 male healthy controls were included (Table 1).

2.2. Serum sampling

Serum samples were collected from fasting subjects between 8:00 and 9:00 am. The samples were centrifuged on site and stored at –80 °C pending analysis at the Biobank at Karolinska Institutet, Stockholm, Sweden. An identical procedure was performed for the controls. The samples were stored at –80 °C until delivered by courier mail, frozen on dry ice, to Chiba University (Japan) for metabolomics analysis. This study was also approved by the Research Ethics Committee of the Graduate School of Medicine (Chiba University).

2.3. Metabolomics analysis of human serum samples

Metabolomics analyses of serum samples from healthy controls and BD patients were performed using the CE-TOFMS at Human Metabolome Technologies (Yamagata, Japan). The sampled volume of 50 μL was added to 450 μL methanol containing internal standards, and mixed. Then, 450 μL chloroform and 200 μL Milli-Q water was added to the mixture. The mixture was centrifuged at 2,300 ×*g* and 4 °C for 5 min. Subsequently, 800 μL of upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter at 9100 ×g and 4 °C for 120 min to remove proteins. The filtrate was centrifugally concentrated and re-suspended in 25 μL of Milli-Q water for analysis.

Cationic compounds were measured in the positive mode of CE-TOFMS (Agilent CE-TOFMS system Machine No. 3, Fused silica capillary, i.d. 50 μM × 80 cm), and anionic compounds were measured in the positive and negative modes of CE-MS/MS (Agilent CE system and Agilent 6400 TripleQuad LC/MS Machine No. QqQ01, Fused silica capillary, i.d. 50 μM × 80 cm), as reported previously [39, 40]. Peaks detected by CE-TOFMS and CE–MS/MS were extracted using automatic integration software (MasterHands, Keio University, Tsuruoka, Japan) [41] and MassHunter Quantitative Analysis B.04.00, Agilent Technologies, Santa Clara, CA, USA) in order to obtain peak information including m/z, migration time (MT), and peak area. The peaks were annotated with putative metabolites from the HMT metabolite database based on their MTs in CE and m/z values determined by TOFMS. The tolerance range for the peak annotation was configured at ±0.5 min for MT and ±10 ppm for