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Evidence for perturbed metabolic patterns in bipolar disorder subjects associated with lithium responsiveness

Manjunath B Joshi[a], Supriya Pai[a], Aswath Balakrishnan[a], Manoj Bhat[a], Ananthpadmanabha Kotambail[a], PSVN Sharma[b], Kapaettu Satyamoorthy[a,*]

[a] School of Life Sciences, Manipal Academy of Higher Education, Manipal, India
[b] Department of Psychiatry, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India

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ABSTRACT

Bipolar disorder (BD) is multifactorial mood disorder characterized by alternating episodes of hyperactive mania and severe depression. Lithium is one of the most preferred drug used as mood stabilizer in treating BD. In this study, we examined the changes in plasma metabolome in BD subjects in the context of lithium responsiveness. Plasma samples from clinically defined, age and gender matched unrelated healthy controls and BD subjects (lithium responders and non-responders) were obtained and processed in positive and negative mode using untargeted liquid chromatography/mass spectrometry analysis. We identified significant alterations in plasma levels of dopamine along with its precursors (tyrosine and phenylalanine), branched chain amino acid such as valine and excitatory neurotransmitter glutamate between healthy control and BD subjects. Lipid molecules such as, eicosenoic acid and retinyl ester also showed distinguished patterns between control and BD individuals. Lithium responsiveness was markedly associated with significant differences in proline, L-gamma-glutamyl-isoleucine, dopamine, palmitic acid methyl ester, cholesterol sulfate, androsterone sulfate and 9S,12S,13S-triHOME levels. Altered metabolites enriched with key biochemical pathways associated with neuropsychiatry disorders. We hypothesize that BD pathogenesis and lithium responsiveness is associated with impaired homeostasis of amino acid and lipid metabolism.

1. Introduction

Severe mood disorder such as Bipolar disorder (BD) affects about 1% of the world population and is characterized by alternating episodes of hyperactive mania and severe depression with several cognitive and behavioral abnormalities. BD is recognized as one of the leading cause of human disability worldwide (Grande et al., 2016). As a complex polygenic disorder, the etiology of BD has been proposed to be influenced by combination of genetic, epigenetic and environmental factors (Young and Wang, 2007). Studies have demonstrated that BD tend to be clustered in families and highly concordant among twins, indicating the disorder is primarily genetic in origin (Craddock and Forty, 2006).

Lithium has the longest history of its therapeutic use in BD and continues to be the primary drug choice for acute and maintenance treatment of the disorder. Lithium exhibits efficient anti-manic and antidepressant, long-term prophylactic and anti-suicidal effects (Grof and Müller-Oerlinghausen, 2009). However, many treated individuals continue to remain refractory to treatment or exhibit clinically significant residual symptoms. A complete non-response or partial response has been observed in more than half of the BD patients treated with lithium (Abou-Saleh, 1993). Family history and the clinical presentation of BD symptoms have indicated a strong link between genetic factors and lithium response (Alda et al., 2005) suggesting genetic association to lithium response.

Systematic identification and quantitation of small molecules (< 1500 Da) using high throughput analysis has been extensively explored to understand disease mechanism, novel therapeutic targets and drug response (Psychogios et al., 2011). Recently, metabolomics approach has revealed new prospects in diagnostics of debilitating conditions including neuropsychiatric disorders (Sethi and Brietzke, 2016). Pharmacometabolomic approach was employed to understand antipsychotic treatment in schizophrenia using a lipidomic platform and investigated lipid changes after 2–3 weeks of olanzapine, risperidone and aripiprazole treatment (Kaddurah-Daouk and Weinshilboum, 2015). Prior to therapy, authors found baseline increase in phosphatidylethanolamine and phosphatidylcholine levels and upon treatment phosphatidylethanolamine decreased to baseline levels. Metabolomics analysis with respect to ketamine and esketamine response

* Corresponding author at: School of Life Sciences, Planetarium Complex Manipal Academy of Higher Education, Manipal 576104, India
E-mail address: ksatyamoorthy@manipal.edu (K. Satyamoorthy).

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in refractory major depressive disorder revealed significant changes in plasma levels of metabolites related to tryptophan metabolism and urea cycle (Rotroff et al., 2016). Further, authors observed alterations in glutamine and circulating phospholipids correlated to decrease in depression severity (Rotroff et al., 2016). Sussulini et al. demonstrated influence of drugs including lithium on serum metabolome in BD subjects and observed perturbations in lipids (acetate, choline and myo-inositol) and amino acids (glutamate and glutamine) (Sussulini et al., 2009). High resolution mass spectrometry analysis of serum showed significant differences in 121 lipids between control and bipolar disorder and phosphatidylinositols were most altered molecules (Rebeiro et al., 2017).

Research strategies to decipher pathogenesis and lithium response in BD based on genetic approach such as linkage analysis, candidate gene(s) and GWAS studies rely on germline changes, and onset and progression of the sporadic disease are unable to explain the mechanisms. However, small molecules such as amino acids, lipids and their derivatives can cross the blood-brain barrier enabling reflection of diseased state systemically and hence metabolite profiling of serum has been used as a powerful tool to understand mechanisms of diseases specific to tissues. In the present study, we examined a LC-MS based high throughput non-targeted serum metabolomics approach to identify alterations in metabolites that could discriminate euthymic (state of remission) BD subjects from healthy controls and among responders and non-responders to lithium maintenance therapy.

2. Methods

2.1. Study subjects and diagnostic assessments

Our study group consisted of 36 unrelated male BD patients and 18 age and gender matched healthy control subjects who were recruited from the Kasturba hospital, Manipal Academy of Higher Education, Manipal. The study protocol was approved by the institutional ethics committee, Kasturba hospital, Manipal, in accordance with the declaration of Helsinki and Indian Council of Medical Research (ICMR), Government of India, ethical guidelines. Written informed consent for the study was obtained from all the subjects.

The diagnosis of primary symptoms in BD was performed based on the criteria described in the diagnostic and statistical manual of mental disorders, 4th edition text revision (DSM-IV TR). The diagnosis was made by psychiatrists, considering all available data, including mental health examination, case records from hospital outpatient departments and face-to-face interview. Study subjects were selected based on BD as primary disorder, causing the most significant distress or dysfunction and thus providing the primary cause to seek treatment. All patients diagnosed with BD belonged to type I category. The exclusion criteria included other disorders that could be related to neuropsychological impairment such as significant physical or neurological illness, head injury, substance abuse or dependence and mental retardation. Patients with comorbid diagnosis of other Axis-I disorders were excluded.

2.1.1. Assessment of lithium response

The response to lithium maintenance therapy was ascertained retrospectively by the psychiatrists based on patient's clinical symptoms during long-term lithium therapy. Patients were evaluated weekly, or more, during active phases of the illness and every three months during euthymia. If patients presented a major depressive or a manic episode after a euthymic period of at least 6 months, they were recognized as having a new recurrence and received additional care (hospitalization, change of medications when needed) and treatment according to the judgment of their clinician. Subclinical episodes such as minor depressive symptoms, which did not require hospitalization were not defined as new recurrences. All patients received standard clinical management intervention and monitoring of the course of the illness. No formal cognitive, behavioral or other psychotherapy and ECT was administered. Two years was considered a minimum duration of follow-up to determine the degree of clinical improvement after lithium treatment. Therapeutic adherence to lithium was determined by estimating the plasma lithium levels, in the clinical biochemistry laboratory associated with Kasturba hospital. Plasma lithium levels were measured at least once in three-month period and the lithium levels of 0.6–1.2 mmol/L was considered therapeutically relevant. The response to lithium therapy was ascertained retrospectively by using a categorical definition as described earlier (Michelon et al., 2006; Tharoor et al., 2013) and is stated below.

2.1.2. Responders

Patients on lithium maintenance therapy for at least two years with compliance indicated by a stable plasma lithium level within the range of 0.6–1.2 mmol/L, without recurrence of any impairing mood episodes were considered as good responders. Cases showing a recurrence of mild symptoms, promptly controlled by adjusting the lithium dose or with short courses of benzodiazepines or antipsychotics but no other mood stabilizing drug or continuous administration of antipsychotics were included in this group.

2.1.3. Non-responders

Non-response was defined as the recurrence of mania or severe depressive episodes requiring hospitalization despite compliant on lithium (stable plasma lithium level 0.6–1.2 mmol/L). These patients were treated with other mood stabilizers such as valproate or carbamazepine (alone or in combination with lithium), antipsychotic drugs or combination therapy with multiple agents. BD patients who have discontinued lithium therapy (within 2 years) due to severe toxicity or intervening contraindications were excluded. Patients who were non-compliant (fluctuating plasma lithium levels falling below 0.6 mmol/L), inadequate clinical information or lack of follow-up data to evaluate the effectiveness of lithium were also excluded.

2.1.4. Healthy control subjects

Control subjects from the same ethnic region were healthy male volunteers matched, age and ethnicity with no psychiatric diagnosis and no self-reported positive family history for psychiatric disorders in their first-degree relatives. Subjects with any other chronic medical conditions and who are on regular medications were not included as control subjects.

All BD subjects recruited in this study were euthymic and were maintained on their stable dose of respective pharmacological treatment for at least one week prior to the enrollment in the study. Assessments included the data collection of a panel of clinical features (described in Table 1 and Supplementary Table 1). Upon informed consent, 5 ml of venous blood sample was collected in fasting condition. Plasma was separated and stored at −80 °C.

2.2. Chemicals and reagents

Mass spectrometry grade chemicals such as methanol and acetonitrile (RCI Labscan, Bangkok, Thailand), formic acid (Fluka, Buchs, Switzerland) and Water purified in a Milli-Q system (Millipore, São Paulo, Brazil) were used for experiments.

2.3. Sample preparation

Plasma metabolites were extracted using cold methanol from frozen samples. One volume of plasma was mixed with two volumes of ice cold methanol, followed by centrifugation at 12,000 rpm for 15 min. The extract obtained was dried under vacuum and residue was reconstituted in 30 µl of water: acetonitrile (95:5) containing 0.1% formic acid. For quality control and to assess reproducibility of the data, plasma samples (20 µl) from five individuals in each study group were pooled and metabolites were extracted as described above. Samples were run in

Table 1
Demographics and clinical characterization of Bipolar Disorder Patients.

Clinical features	Bipolar subjects (n = 36) Non responders (n = 18)	Responders (n = 18)	Healthy subjects (n = 18)
Age (mean ± SD)	42.5 ± 14.38	46 ± 15.41	31.4 ± 9.31
Age of Onset (mean ± SD)	24.9 ± 8.98	28.6 ± 12.7	NA
Number of Illness Episodes (mean ± SD)	3.39 ± 2.63	3.44 ± 2.59	NA
Suicide attempts %	16.6	11.1	NA
Psychotic symptoms %	55.5	27.7	NA
Co-morbid conditions (%)			
A) Thyroid abnormalities	5.5	16.6	NA
B) Asthma	11	–	
C) Hypertension	11	22	
Family history %	50	50	NA
Environmental risk factors %	5.5	5.5	NA

NA: Not applicable

untargeted MS and MS/MS mode. Details of data acquisition and processing are provided as supplementary information.

2.4. Statistical analysis

Abundance values of provisionally identified metabolites from control, responder and non-responder groups were transformed to log2 values used for creating circular representation using CIRCOS. Metabolites were subjected to statistical analysis such as unpaired t test for comparing control and bipolar groups and Kruskal–Wallis ANOVA for multiple groups (control, responders and non-responders) followed by Tukey's multiple comparison test. Pearson's correlation analysis of metabolites present in all groups was performed using R Program package corrplot. Metabolite enrichment analysis of significant molecules were performed using Metabolomics Pathway Analysis (MetPA) using MetaboAnalyst software 3.0.

3. Results

3.1. Global plasma metabolomic pattern of normal and BD subjects

LC-MS analysis of plasma samples analyzed in triplicates revealed a total of 3845 and 2489 spectral features in positive and negative mode respectively. In positive mode, 1526, 1201, 1118 and in negative mode 906, 871, 712 metabolite peaks were obtained in control, responders and non-responders respectively. Upon aligning the features with respect to m/z values and retention time along with isotope abundance, spacing and further annotation, we found common and unique features which are represented in Venn diagram (Supplementary Fig. 1a). Partial least square discriminant analysis (PLSDA) using spectral profiles derived from both positive and negative mode showed significant discrimination of clinically defined subgroups of 98.07% (Supplementary Fig. 1b) which allowed us to perform further statistical analysis. Subsequently, data was narrowed down to metabolites present in at least in 50% of the samples in each group and subjected to identification of metabolites using mass within tolerance of 15 ppm in METLIN and HMDB databases. We identified a total of 701 compounds attributing to various functional groups such as xenobiotics (48%), lipids (33.3%), amino acids and derivatives (8.4%), sugars (2.1%), nucleotides (3.4%) and energy molecules (1.4%), organic compounds (2.7%) and cofactors and vitamins (0.71%) (Supplementary Fig. 1c). Further Log2 median values of identified metabolites (excluding xenobiotics) were used to plot a circos illustration to overview global metabolome status across control, lithium responders and non-responders groups (Fig. 1). As evident from circos representation which showed discrete patterns of distributions of metabolites across the BD groups suggesting metabolic differences. Considering abundance and presence of metabolites in more than 75% of samples in each group among annotated small molecules, 48 metabolites were found to be significantly modulated. In order to validate, we subjected these metabolites to ms/ms analysis to identify fragmentation patterns (Supplementary Fig. 3) using pooled plasma samples (Supplementary Table S2 and S3). Univariate analysis (T tests and ANOVA) revealed statistically significant alterations in 22 amino acids along with their derivatives and 11 lipids in BD subjects.

3.2. BD subjects showed significant alterations in amino acids and their derivatives associated with lithium response

Among various groups of biomolecules, amino acids and their derivatives showed predominant significant modulations in BD subjects (Figs. 2A and 3A; Supplementary Table S4 & S5). Compared to control subjects, branched chain amino acids such as valine ($p < 0.0001$) and leucine ($p < 0.05$) were significantly dropped down in BD subjects. Dopamine and its precursor amino acids such as tyrosine and phenylalanine showed decreased levels in plasma of BD subjects. Upon stratification of BD subjects, phenylalanine, tyrosine and dopamine were significantly reduced in lithium non-responders with respect to controls and remained unaltered in responders. Interestingly, only dopamine but not phenylalanine and tyrosine levels showed distinct differences between responders and non-responders ($p < 0.05$). We observed reduced glutamate, proline and lysine levels in BD subjects and proline levels showed distinct separation between responders and non-responders, where levels of proline in lithium responders were lower than non-responders ($p < 0.05$). Adrenaline, creatinine, pyroglutamic acid, isoleucyl proline, tryptophan and kyneurenic acid levels although detected in abundance in all groups, remained unaltered. Signatures of methionine, taurocyamine and hippuric acid were not found in responder group whereas arginine levels were within detection limit in non-responders. Protein breakdown product, L-gamma-glutamyl-L-isoleucine was reduced in BD group. Modified amino acid, Alpha-N-Phenylacetyl-L-glutamine did not show any modulation between control and BD and interestingly non-responders showed significantly higher levels ($p < 0.001$) when compared to responders. Pentosidine levels, although did not vary between control and BD subjects, lithium non-responders showed low levels when compared to responders.

3.3. Plasma lipid levels showed significant modulation in BD subjects and its subgroups

Lipid patterns in BD subjects and its sub-groups showed significant changes when compared to controls. Out of 701 identified compounds in plasma, 33.1% represented lipids and 11 compounds showed alterations among the group (Figs. 2B and 3B; Table S4 & S5). Signatures of fatty acids such as palmitic acid methyl ester, eicosenoic acid and 17-hydroxylinolenic acid were abundant and modulated. Although palmitic acid did not show any change between control and BD subjects, non-responders showed low levels ($p < 0.01$) which were significantly distinct from responders ($p < 0.05$). Eicosenoic acid levels remained

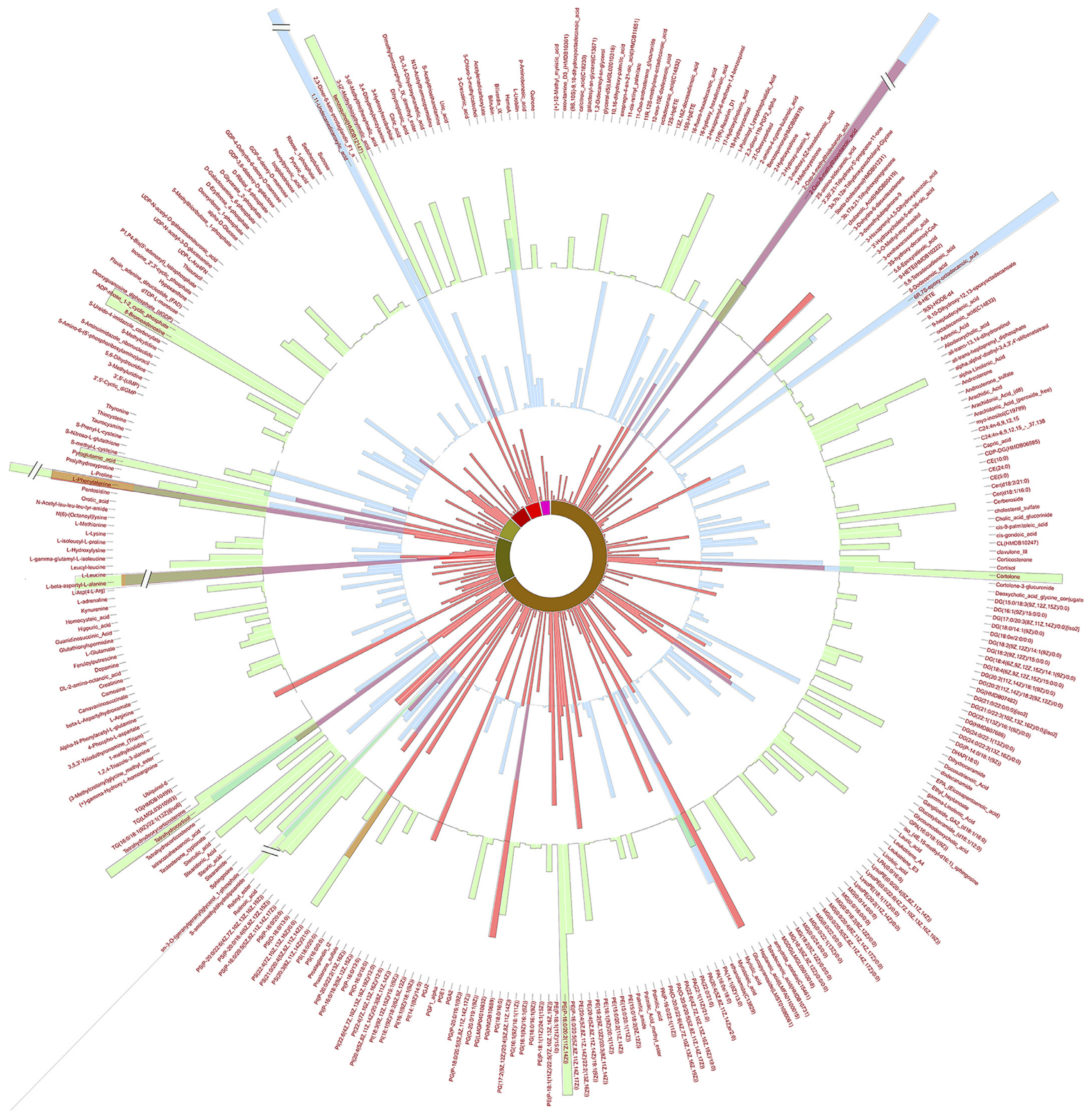

Fig. 1. An overview of plasma metabolome pattern in control and BD subgroups. Circos diagram illustrates comparison of log2 transformed abundance values of 337 identified metabolites (excluding xenobiotics) detected in control (Inner-pink), non-responder (Middle-blue) and responder (Outer-green) groups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

unaltered in BD and however, levels were reduced in responders. 17-hydroxylenolenic acid levels were significantly increased in non-responding group compared to control and responders. Steroids and their derivatives such as tetrahydrocortisol, cholesterol sulfate, androsterone sulfate and 3-beta-hydroxycholest-5-en-26-oic acid did not show changes in BD to that of control. However, upon stratification cholesterol sulfate was elevated and androsterone sulfate and 3-beta-hydroxycholest-5-en-26-oic acid were decreased in non-responders, suggesting distinct changes due to lithium responsiveness. Retinyl ester levels were overall reduced in BD and did not show changes in subgroups. On the other hand, glycerolipids such as phosphatidylserine (18:0/22:6(4Z,7Z,10Z,13Z,13Z,16Z,19Z)) and Diglyceride DG (P-14:0/18:1(9Z) were uniform in control and BD group but were reduced in non-responders.

3.4. BD subjects in association with lithium response showed altered correlation between the metabolites and influenced by biochemical pathways

Subsequently metabolites which were present in all the samples in all three subgroups were subjected to Pearson's correlation analysis. We

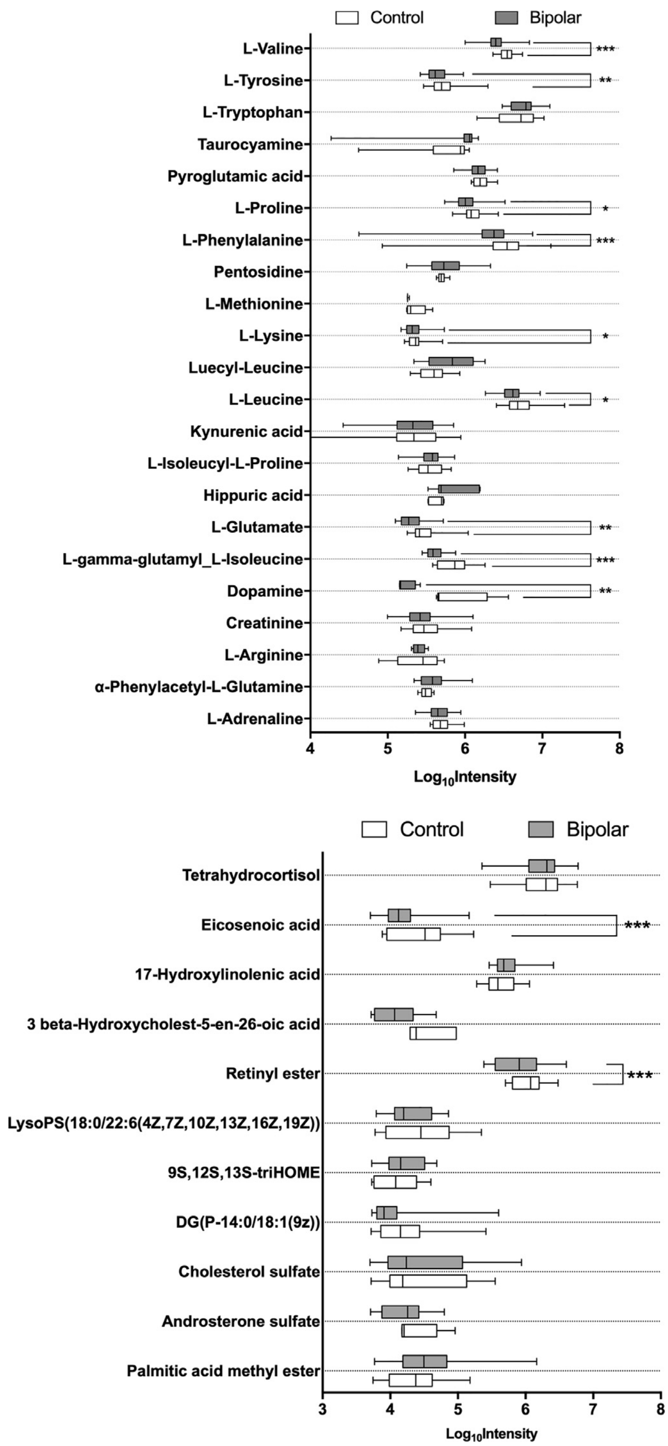

Fig. 2. Differentially altered plasma metabolites in BD subjects. Metabolite intensities of most abundant and significantly altered amino acids (A) and lipids (B) detected in plasma of control (n = 18) and BD (n = 36) of were log10 transformed and represented as Box-Whiskers plots. Statistically significant changes in metabolite intensity between the control and BD group are represented by asterisk (***$p < 0.001$, **$p < 0.01$, $p < 0.05$).

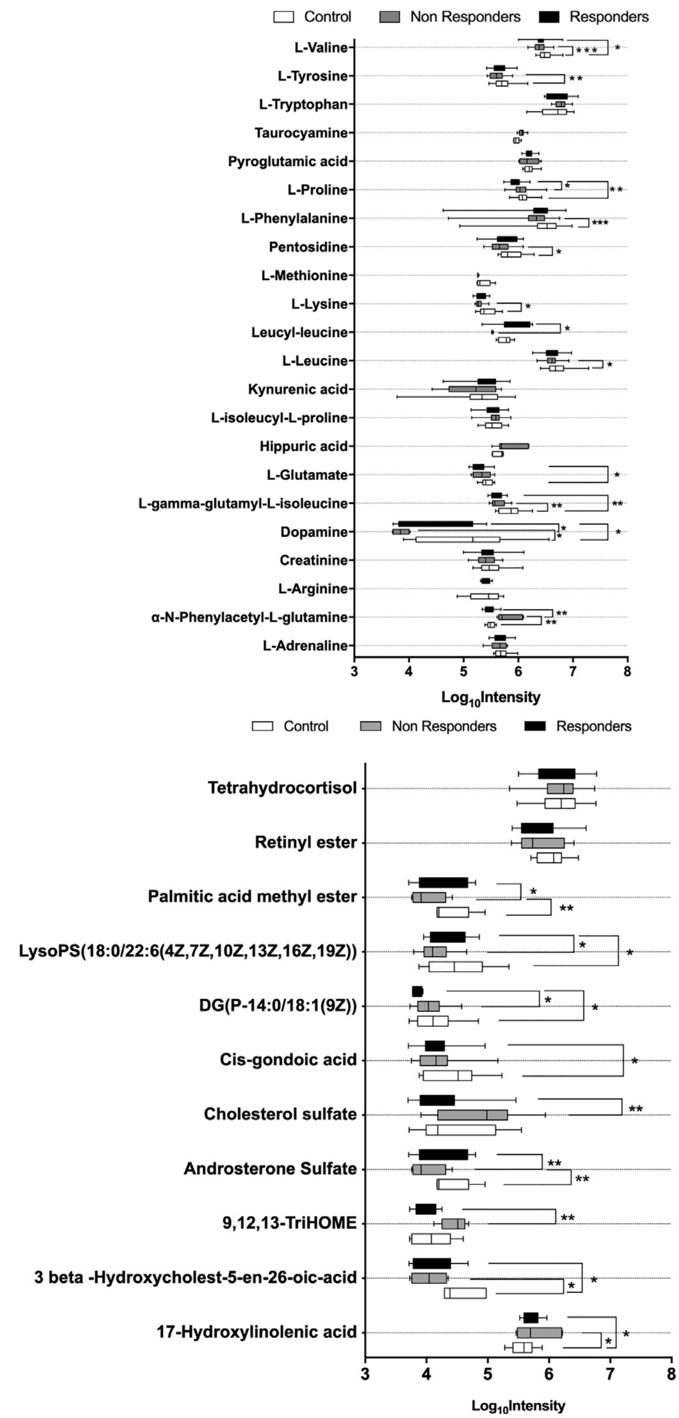

Fig. 3. Comparison of plasma metabolite levels in control and BD group. Metabolite intensities of amino acids (A) and lipids (B) in control (n = 18), responders (n = 18) and non-responders (n = 18) were log10 transformed are represented as Box-Whiskers plot. Statistically significant changes in metabolite intensity among the control, responders and non-responders are represented by asterisk (***$p < 0.001$, **$p < 0.01$, $p < 0.05$).

observed significant correlation changes among different metabolite levels in BD subjects compared to control and also between responder and non-responder groups (Fig. 4). In control group valine and phenylalanine levels (R^2 = 0.86515599; p = 0.00058) and, tryptophan and creatinine (R^2 = 0.8146413; p = 0.0023) showed significant positive correlation and however, negative correlation was observed in BD group, where valine-phenylalanine and tryptophan- creatinine was reduced to R^2 = −0.0301981 and R^2 = −0.2206573 respectively. In control group valine (R^2 = −0.7440048, p = 0.14936838) and proline (R^2 = −0.8736916, p = 0.053) to kynurenic acid showed negative correlation which was disturbed in BD group where in values for valine to kynurenic acid was R^2 = −0.029168, p = 0.952 and proline to kynurenic acid was R^2 = 0.14263 respectively. Similarly, we found altered correlations between responders and non-responders. In non-responders group we observed valine (R^2 = −0.0279168, p = 0.95262),

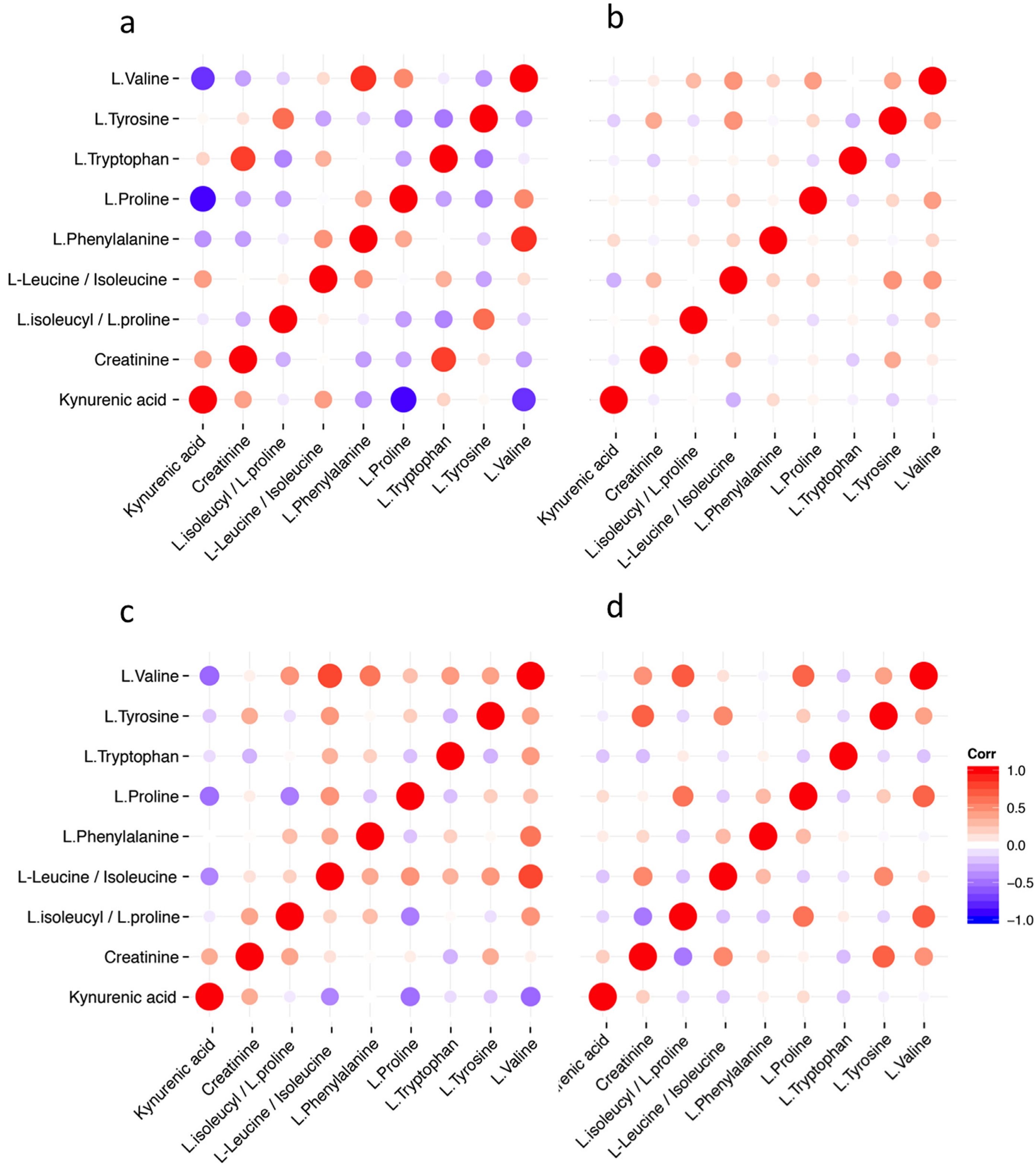

Fig. 4. Pearson's correlation analysis shows altered correlation between metabolites present in all samples in (a) control (b) BD (c) lithium responders and (d) lithium non-responders.

proline ($R^2 = -0.014263849$, $p = 0.736158$) and leucine ($R^2 = -0.191951$, $p = 0.5139$) showed negative correlation with kynurenic acid which was lost in responders. Interestingly, a tight correlation between tyrosine and creatinine ($R^2 = 0.5567$; $p = 0.019$) and valine and iso-leucyl L-proline ($R^2 = 0.461$; $p = 0.013$) was observed in responder group but was absent in non-responders.

Further, to explore influence of selected metabolites present in all samples in each group and to identify plausible biochemical pathways affected by pathogenesis of BD and also associated with lithium response, MetPA (Metabolic pathway analysis) was performed. As shown in Supplementary Fig. 2, reconstruction of pathways using these metabolites indicated possible dysregulation of 11 biochemical pathways

(−Log (p) > 2) and prominent among these pathways were aminoacyl tRNA biosynthesis (−Log (p) = 20.368), phenylalanine metabolism (−Log (p) = 5.8), phenylalanine, tyrosine and tryptophan biosynthesis (−Log (p) = 5.27), valine, leucine and isoleucine biosynthesis (−Log (p) = 5.2), arginine and proline metabolism (−Log (p) = 3.9) and fatty acid metabolism (−Log (p) = 4.2). Subsequently, we performed enrichment analysis using Metaboanalyst, based on disease associated metabolite sets obtained from blood from the databases. Interestingly, we found that significantly modulated metabolites enriched which were described in neuropsychological diseases such as acute seizures (p = 0.00031), refractory localization – related epilepsy (p = 0.000456), different seizure disorder (p = 0.0014), dopamine beta-hydroxylase deficiency (p = 0.017) and schizophrenia (p = 0.014).

4. Discussion

Lithium is one of the widely used mood stabilizer for prophylactic treatment of bipolar disorder as it decreases recurrent episodes and hence preferred as first line of maintenance therapy. Lithium response varies significantly among individuals and has been noted that approximately only 35% of patients respond to monotherapy (Gershon et al., 2009). Plasma levels of lithium in both responder and non-responder groups in our study population ranged between 0.6–1.2 mmol/L as also have been reported in other studies (Miller and Bauer, 2014). Lithium exerts pleotropic effects influencing multiple organ system and its responsiveness is attributed as pathognomonic of BD (Sproule, 2002). In this study, LC-MS based untargeted metabolomics approach was explored to elucidate plasma metabolic patterns of clinically characterized BD subjects in the context of lithium responsiveness. Our analysis revealed a distinct metabolite fingerprint between a) control and BD and b) lithium responders and non-responders based on changes in amino acids along with their derivatives and lipids.

The majority of affected metabolites in BD and subgroups were amino acids and derivatives. Glutamate is one of the primary excitatory neurotransmitter in brain and several studies have reported perturbed glutamate metabolism in psychiatry disorders such as schizophrenia and BD (Kim et al., 2017). We observed glutamate and its associated derivatives and peptides such as a-phenylacetyl-glutamine, pyroglutamic acid and L-gamma glutamyl-isoleucine were significantly modulated in our study group. Metabolomic analysis of human post-mortem brain tissue of BD patients using NMR platform, showed concordant modulation in brain glutamate, creatinine and myo-inositol levels (Lan et al., 2009). However, we did not find any alterations in creatinine among the participants and myo-inositol was not detected. N-acetylglutamic acid and pyroglutamic acid signatures were modulated in serum of Caucasian BD subjects and elevated in lithium treated group to that of patients treated with second generation anti-psychotic drugs (SGA) (Burghardt et al., 2015). Interestingly, GWAS study in Chinese population, *GADL1* gene coding for glutamate decarboxylase-like protein, was associated with glutamate metabolism showed significant correlation with lithium response (Chen et al., 2014). However, these findings were not reproducible in European population and also in our own study (Kotambail et al., 2015). Significant glutamate levels were also attributed in discriminating subclasses of BD by exploring proton magnetic resonance spectroscopy (Atagün et al., 2018). Tryptophan and its product kynurenine are known neurotransmitters with precise biological activities. Kynurenine was decreased in individuals with negative affectivity and social inhibition which indicated a reason for increased risk of cardiovascular disease in Type D patients (Altmaier et al., 2013). We observed changes in branched chain amino acids such as valine and leucine and similar changes were also noted in BD subjects treated with lithium in a Caucasian population (Lan et al., 2009). Further, dopamine and its precursors phenylalanine and tyrosine were lowered in BD subjects and dopamine levels significantly distinguished responders from non-responders. Post-mortem brain histological and neurochemical studies have identified certain abnormalities in BD which included GABA, serotonin and dopamine neurotransmitter dysfunctions, increased cellular stress, altered mitochondrial functions and intracellular Ca^{2+} homeostasis, decreased neuronal connectivity and plasticity (Yildiz-Yesiloglu and Ankerst, 2006). Metabolomics analysis of cerebrospinal fluid during remission state of major depressive disorder suggested an association of the disease with tryptophan, methionine and tyrosine metabolic pathways (Kaddurah-Daouk et al., 2012). Proline levels were lowered in BD subjects and showed significant changes between lithium responders and non-responders. Recent studies have indicated microdeletions of 22q harboring proline dehydrogenase (PRODH) and the catechol-o-methyltransferase (COMT) genes and their association with neuropsychiatric disorders and treating with valproic acid induced hyperprolinemia (Duarte et al., 2017).

We observed significant alterations in various lipid molecules. Out of 11 lipid molecules, eicosenoic acid and retinyl ester altered between control and BD subjects. Earlier serum metabolomics studies conducted in different populations also identified alterations in polyunsaturated fatty acids (Burghardt et al., 2015) and lysophosphatidylethanolamines and lysophosphatidylcholines (Villaseñor et al., 2014). In Chinese cohort, eicosenoic acid was found as potential biomarker of schizophrenia (Yang et al., 2013). Recently, post-mortem study showed alterations in steroid synthetic pathway in human prefrontal cortex in mood disorder patients where authors showed disturbance in synthesis of dehydroepiandrosterone (DHEA) and its sulfate metabolite DHEAS along with its association to BDNF-TrkB signaling (Qi et al., 2018).

Although several studies have investigated BD metabolomics using different tissues such as serum, CSF, post-mortem brain tissues and urine (Supplementary Table 6), precise analysis in the context of lithium monotherapy responsiveness is not sufficiently explored. Yoshimi et al. (2016a,b) conducted blood and CSF based metabolomics analysis in BD subjects in separate studies and found alterations in citric acid cycle, urea cycle and amino acid metabolism in blood and citric acid cycle in CSF (Supplementary Table 6). In same studies, authors also tested serum and CSF metabolites in rat models injected chronically with lithium (4 weeks) and metabolite profile did not reflect with that of human samples, suggesting lithium *per se* might not induce metabolic changes (Yoshimi et al., 2016a,b). NMR based metabolomics study of neuron and glial cells cultured in presence of stable isotope ^{13}C labelled glucose and lactate treated with lithium indicated increased glycolysis, modulated Kreb's cycle and led to extracellular release of alanine, citrate and glutamine (Fan et al., 2010). Metabolite differences in BD subjects treated with lithium were investigated with respect to ketamine response and analysis showed patients who responded well for ketamine showed increased levels lysophosphatidylethanolamines and lysophosphatidylcholines to that of non-responders (Villasenor et al., 2014). Burhgardt et al. compared metabolite profiles of BD subjects treated with second generation antipsychotic drugs or lithium and with schizophrenia subjects treated with SGA and analysis showed significant separation of all three groups associated with alterations of fatty acids, pyruvate and branched chain amino acids (Burghardt et al., 2015). Recent GWAS study in Swedish and UK population revealed lithium responsiveness associated with a variant in *SESD1*, a gene involved in regulation of phospholipids (Song et al., 2016).

Our analysis indicated clear differences in serum levels of proline, dopamine, phenyl-acetyl-L-glutamine, palmitic acid methyl ester, PS (18:0/22:6(4Z,7Z,10Z,13Z,13Z,16Z,19Z)), DG (P-14:0/18:1(9Z), cholesterol sulfate and androsterone sulfate between lithium responder and non-responder sub-groups and along with other abundant metabolites enriched with important biochemical pathways known to be deranged in neuropsychiatry disorders. Our study lacked drug naïve samples and hence did not reveal influence of lithium *per se* in both groups. On the other hand, these altered metabolites validated in large sample size might decipher unique pathways and may enable to utilize as predictive markers for assessing lithium response.

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Conflict of interest

The authors have declared no conflict of interest in the submission of this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.01.031.

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