Regular Article

Search for plasma biomarkers in drug-free patients with bipolar disorder and schizophrenia using metabolome analysis

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Aim: There is an urgent need for diagnostic biomarkers of bipolar disorder (BD) and schizophrenia (SZ); however, confounding effects of medication hamper biomarker discovery. In this study, we conducted metabolome analyses to identify novel plasma biomarkers in drug-free patients with BD and SZ.

Methods: We comprehensively analyzed plasma metabolites using capillary electrophoresis time-of-flight mass spectrometry in patients with SZ (n = 17), BD (n = 6), and major depressive disorder (n = 9) who had not received psychotropics for at least 2 weeks, and in matched healthy controls (n = 19). The results were compared with previous reports, or verified in an independent sample set using an alternative analytical approach.

Results: Lower creatine level and higher 2-hydroxybutyric acid level were observed in SZ than in controls (uncorrected $P = 0.016$ and 0.043, respectively), whereas they were unaltered in a previously reported dataset. Citrulline was nominally significantly decreased in BD compared to controls (uncorrected $P = 0.043$); however, this finding was not replicated in an independent sample set of medicated patients with BD. N-methyl-norsalsolinol, a metabolite of dopamine, was suggested as a candidate biomarker of BD; however, it was not detected by the other analytical method. Levels of betaine, a previously reported candidate biomarker of schizophrenia, were unchanged in the current dataset.

Conclusion: Our preliminary findings suggest that the effect of confounding factors, such as duration of illness and medication, should be carefully controlled when searching for plasma biomarkers. Further studies are required to establish robust biomarkers for these disorders.

Key words: biomarker, bipolar disorder, capillary electrophoresis time-of-flight mass spectrometry, major depressive disorder, schizophrenia.

A DEQUATE DIAGNOSIS AND treatment are essential to improve symptomatic and functional outcomes in psychiatric disorders.1,2 However, diagnosis of psychiatric disorders is currently only based on the clinical assessment of symptoms during interviews and misdiagnosis cannot be avoided,3 worsening the prognosis.4 Therefore, biomarkers are needed. Candidate biomarkers have already been reported, yet studies continue to lack reproducibility and selectivity.5,6

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Recently, metabolomics has emerged as a potential method for identifying biomarkers because the individual metabolic state can potentially reflect both genomic and environmental factors. Candidate biomarkers have been reported for schizophrenia (SZ), major depressive disorder (MDD), and bipolar disorder (BD) using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). In the previous reports, however, many patients took medication and thus possible effects of medication cannot be excluded.

The present study aimed to explore candidate biomarkers in plasma samples of drug-free patients with BD or SZ using CE-TOFMS. Candidate markers were either compared with a previous study (SZ) or verified in an independent sample set (BD).

**METHODS**

**Participants**

The first discovery set of samples (six samples from patients with BD, nine with MDD, 17 with SZ, and 19 healthy controls) was obtained from the National Center of Neurology and Psychiatry (NCNP) Biobank (project number NCNPBB-0002). Patients and healthy controls were recruited at the NCNP Hospital, or through advertisements on the website and in free local magazines. The patients had not taken any antipsychotics or antidepressants for at least two weeks. For the second set of samples, participants were recruited from the outpatient unit at Osaka City University Hospital or Hannan Hospital (16 medicated patients with BD and 11 healthy controls). Healthy spouses of the patients were recruited as controls to minimize potential effects of the diet. Trained psychologists or psychiatrists conducted a structured interview with all the participants using a Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.). Diagnoses were made according to the criteria of the DSM-IV, based on the M.I.N.I. Patients with any comorbid axis I disorders, severe head trauma, substance abuse/dependence, or a prior medical history of central nervous system diseases were excluded. Depressive symptoms were assessed using the Japanese version of the 21-item Hamilton Depression Rating Scale (HAMD-21). Blood samples were obtained between 09.00 hours and 16.00 hours without controlling meals. Blood samples were withdrawn by vein puncture and immediately placed on ice. The samples were centrifuged at 2500 g and 4°C for 10 min within 30 min of blood collection, and plasma aliquots were stored frozen at −80°C until assayed.

**Ethics statement**

All participants provided their written informed consent after the study procedures had been fully explained. Patients’ records/information were anonymized and de-identified prior to analysis. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committees of the participating institutes: the Ethics Committee of Hannan Hospital, the Research Ethics Committee of Osaka City University, Wako First Research Ethics Committee of RIKEN, and the Ethics Committee of NCNP.

**Metabolome profiling of human plasma samples**

Metabolome analysis was performed using CE-TOFMS, on a subset of the first set of plasma samples, at Human Metabolome Technology Inc. (Tsuruoka, Japan). The experimental procedure is provided in File S1.

**Determination of plasma levels of N-methyl-norsalsolinol**

Plasma levels of N-methyl-norsalsolinol were analyzed using high-performance liquid chromatography-electrochemical detection (HPLC-ECD) at the Support Unit for Bio-Material Analysis in RIKEN BSI Research Resources Center (RRC). Experimental details are given in File S1.

**Determination of plasma levels of amino acids**

Amino acids were quantified using the ninhydrin method in a subset of the second sample set at the Support Unit for Bio-Material Analysis in RIKEN BSI RRC. Details are given in File S1.

**Statistical analysis**

Data are reported as means ± standard deviation (SD). Means were compared using Welch t-test, Mann–Whitney U-test, one-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test, or Kruskal–Wallis test followed by post-hoc Steel–Dwass
test. Analysis of covariance (ANCOVA), with a dependent variable of metabolite, an independent variable of diagnosis, and covariates of age and sex, was performed to investigate the effects of age and sex on citrulline, creatine, choline, cis-aconitate, and 2-hydroxybutyric acid levels. Categorical variables were compared using the χ²-test. Logistic regression model with stepwise selection method was used for multivariate analysis. Adequacy of the fitted model was tested using the Hosmer–Lemeshow test. The results for independent variables were typically reported as odds ratios (OR) with 95% confidence intervals (CI). Spearman’s rank correlation coefficient was used for correlation analysis. The threshold for statistical analyses was set at P < 0.05 for all analyses. Multiple comparisons were performed by estimating the false discovery rate using q-values. Statistical power was calculated using G-Power version 3.1.16 Statistical analyses were performed using IBM SPSS Statistics 23, Japanese version (IBM Japan, Tokyo, Japan), and ‘R’ (http://cran.r-project.org/), which is a freely available open-source software package.

RESULTS

Characteristics of the participants

Demographic and clinical data for the first and second sets of subjects are shown in Tables 1 and 2, respectively. There were no significant differences in the sex ratios and ages between the groups.

Metabolome analysis

Full datasets are included in Table S1. In total, 188 candidate peaks were detected in the first set of plasma samples. The detection limit was determined at a signal-to-noise ratio (S/N) > 3. In these 188 peaks, 58 were assigned to a specific metabolite and could be robustly quantified using a reference standard. For the remaining 130 peaks, they were assigned a specific metabolite identity based on their m/z ± 10 p.p.m. and migration time ± 0.5 min (as determined by TOFMS); however, the absolute concentrations were not determined and relative peak areas were used for comparisons. There was a possibility that a relative peak area reflected total value of metabolites that had similar m/z and migration time. We showed the data of these 130 non-identified peaks as an exploratory analysis. Therefore, we presented the 130 non-specific metabolites and 58 quantified metabolites separately. We used ANOVA and the Kruskal–Wallis test to select metabolites from 58 robustly detected metabolites and 130 candidate peaks that showed significant differences for the four sample groups (SZ, MDD, BD, and controls). From 58 metabolites with determined absolute concentrations, a nominally significant effect of diagnosis was found for five metabolites: citrulline (mean [μM] ± SD; SZ, 32.8 ± 8.6; MDD, 28.6 ± 8.0; BPD, 22.0 ± 3.5; control, 31.8 ± 7.3), creatine (SZ, 31.4 ± 14.2; MDD, 44.1 ± 17.8; BPD, 48.0 ± 14.1; control, 48.4 ± 17.6), choline (SZ, 12.6 ± 3.0; MDD, 16.8 ± 3.8; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), cis-aconitate (SZ, 6.4 ± 1.5; MDD, 17.6; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), citrulline (SZ, 32.8 ± 8.6; MDD, 28.6 ± 8.0; BPD, 22.0 ± 3.5; control, 31.8 ± 7.3), creatine (SZ, 31.4 ± 14.2; MDD, 44.1 ± 17.8; BPD, 48.0 ± 14.1; control, 48.4 ± 17.6), choline (SZ, 12.6 ± 3.0; MDD, 16.8 ± 3.8; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), cis-aconitate (SZ, 6.4 ± 1.5; MDD, 17.6; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), citrulline (SZ, 32.8 ± 8.6; MDD, 28.6 ± 8.0; BPD, 22.0 ± 3.5; control, 31.8 ± 7.3), creatine (SZ, 31.4 ± 14.2; MDD, 44.1 ± 17.8; BPD, 48.0 ± 14.1; control, 48.4 ± 17.6), choline (SZ, 12.6 ± 3.0; MDD, 16.8 ± 3.8; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), cis-aconitate (SZ, 6.4 ± 1.5; MDD, 17.6; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), citrulline (SZ, 32.8 ± 8.6; MDD, 28.6 ± 8.0; BPD, 22.0 ± 3.5; control, 31.8 ± 7.3), creatine (SZ, 31.4 ± 14.2; MDD, 44.1 ± 17.8; BPD, 48.0 ± 14.1; control, 48.4 ± 17.6), choline (SZ, 12.6 ± 3.0; MDD, 16.8 ± 3.8; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), cis-aconitate (SZ, 6.4 ± 1.5; MDD, 17.6; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), citrulline (SZ, 32.8 ± 8.6; MDD, 28.6 ± 8.0; BPD, 22.0 ± 3.5; control, 31.8 ± 7.3), creatine (SZ, 31.4 ± 14.2; MDD, 44.1 ± 17.8; BPD, 48.0 ± 14.1; control, 48.4 ± 17.6), choline (SZ, 12.6 ± 3.0; MDD, 16.8 ± 3.8; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2), cis-aconitate (SZ, 6.4 ± 1.5; MDD, 17.6; BPD, 14.5 ± 2.0; control, 15.7 ± 4.2).
7.2 ± 1.6; BPD, 5.5 ± 1.6; control, 5.9 ± 1.4), and 2-hydroxybutyric acid (SZ, 40.9 ± 16.2; MDD, 38.8 ± 11.6; BPD, 36.3 ± 16.1; control, 28.7 ± 10.0) (Fig. 1). Citrulline level was nominally significantly lower in patients with BD than in controls \( (P = 0.043) \) or patients with SZ \( (P = 0.023) \). A nominally significant decrease of creatine level \( (P = 0.016) \) and increase of 2-hydroxybutyric acid level \( (P = 0.043) \) were found in patients with SZ compared with controls. Choline level was lower in patients with SZ than in patients with MDD \( (P = 0.029) \), and cis-aconitate level was higher in patients with MDD than in controls \( (P = 0.039) \). These \( P \)-values were not corrected for multiple testing, and none of these differences was statistically significant after multiple testing correction. To examine the effects of confounding factors on the metabolites (citrulline, creatine, choline, cis-aconitate, and 2-hydroxybutyric acid), we applied ANCOVA with a dependent variable of metabolite, an independent variable of diagnosis, and covariates of age and sex. Citrulline level was affected by age, sex, and diagnosis \( (P = 0.022, 0.029, \text{ and } 0.021, \text{ respectively}) \). Creatine level was affected by age and diagnosis \( (P = 0.047 \text{ and } 0.007, \text{ respectively}) \). Choline and 2-hydroxybutyric acid levels were only affected by diagnosis \( (P = 0.034 \text{ and } 0.041, \text{ respectively}) \). No significant effect of age, sex, or diagnosis was seen for cis-aconitate level by ANCOVA.

From 130 candidate peaks, a significant effect of diagnosis on the relative peak area was found for two peaks tentatively identified as \( N \)-methyl-norsalsolinol and methionine sulfoxide (Fig. S1).

The peak corresponding to \( N \)-methyl-norsalsolinol was nominally significantly decreased in patients with BD \( (P = 0.037) \). The peak corresponding to methionine sulfoxide was nominally significantly decreased in all three diseases \( (SZ, P = 0.020; \text{MDD}, P = 0.036; \text{BPD}, P = 0.042) \). These differences were not statistically significant after multiple testing correction.

### Schizophrenia

We took a different approach when choosing metabolites to discriminate between patients with SZ and controls, using 58 metabolites whose absolute concentrations were quantified. We applied multivariate logistic regression analysis to evaluate the association between the metabolites and SZ. The significance level was set at 5%. Three metabolites, creatine \( (\chi^2 = 8.013, \text{ degrees of freedom } [d.f.] = 1, P = 0.005, \text{ OR } = 1.151, 95\% \text{CI: } 1.044–1.270) \), 2-hydroxybutyric acid \( (\chi^2 = 6.888, \text{ d.f. } = 1, P = 0.009, \text{ OR } = 0.778, 95\% \text{CI: } 0.644–0.938) \), and 2-oxoisovaleric acid \( (\chi^2 = 4.326, \text{ d.f. } = 1, P = 0.038, \text{ OR } = 1.746, 95\% \text{CI: } 1.033–2.954) \), were independently associated with SZ (Table 3). In this model, the sensitivity was 82% and specificity was 90%. The Hosmer–Lemeshow goodness-of-fit statistic (internal validation of the logistic regression model) was 4.605 with 7 d.f. \( (P = 0.71) \), indicating a good fit of the model.

In a previous study using the same technology, the absolute concentrations of betaine, creatine, and gluconic acid were significantly different between patients with first-episode schizophrenia (FESZ) \( (n = 30) \), who were mostly medicated with antipsychotics, and controls \( (n = 38) \). We therefore investigated whether these findings were replicated in the present dataset. We performed a power analysis to determine how many samples were required to replicate the previous findings with a reasonable statistical power, with an alpha value of 0.05 and \( (1 - \beta) \) of 0.8, using two-tailed unpaired \( t \)-test. For the three metabolites, the number of samples required to attain the statistical power to detect a difference was smaller than the number of samples in this study \( (n = 17 \text{ for SZ and } n = 19 \text{ for controls}) \) only for betaine \( (SZ, n = 11; \text{ controls, } n = 13) \). Therefore, we examined whether the reduction in betaine levels was replicated in our sample set. However, there were no significant differences in betaine levels.
Multivariate logistic regression analysis was performed to evaluate the association between the metabolites and BD. The significance level was set at 5%. Citrulline ($\chi^2 = 4.186$, d.f. = 1, $P = 0.004$, OR = 1.405, 95%CI: 1.014–1.946) was chosen as an independent predictor of BD (Table 4). In this model, the sensitivity was 50% and specificity was 90%. The Hosmer–Lemeshow goodness-of-fit statistic was 2.309 with 6 d.f. ($P = 0.89$), indicating a good fit of the model.

To test whether this finding was reproducible, we measured the levels of citrulline, an amino acid, using the amino acid analysis method in an
independent set of samples from medicated patients with BD and controls (Table 2). However, no significant difference in citrulline levels was detected between the two groups (full datasets are given in Table S2). Notably, a previous study\textsuperscript{17} demonstrated that the citrulline-to-arginine ratio was reduced in patients with BD in comparison with controls. Therefore, we calculated the citrulline-to-arginine ratio in samples from the first and second sets. However, the citrulline-to-arginine ratio was not significantly different between patients with BD and controls in either set (data not shown).

\textbf{Major depressive disorder}

Although the number of patients with MDD was too small, considering the heterogeneity of the disease, we performed preliminary multivariate logistic regression analysis to evaluate putative associations between metabolite levels and MDD. The significance level was set at 5%. We chose 2-hydroxybutyric acid (\( \chi^2 = 5.624, \text{d.f.} = 1, P = 0.043, \text{OR} = 0.916, 95\%\text{CI}: 0.841–0.997 \)) as an independent predictor of MDD (Table S3). The sensitivity was 33% and specificity was 84% in this model. The Hosmer–Lemeshow goodness-of-fit statistic was 3.379 with 7 d.f. (\( P = 0.85 \)), indicating a good fit of the model.

\textbf{Relation between metabolite concentration and clinical assessment}

To investigate the relation between a patient’s symptoms (Positive and Negative Symptom Scale positive, negative, and general pathology scores, Young Mania Rating Scale score, and HAMD-21 score) and the five metabolites (citrulline, creatine, choline, cis-aconitate, and 2-hydroxybutyric acid), we conducted a correlation analysis using Spearman’s rank correlation coefficients. There was no significant correlation between a patient’s symptoms and the metabolites.

\textbf{DISCUSSION}

To our knowledge, this is the first study to use CE-TOFMS to evaluate plasma metabolites in drug-free patients with SZ, BD, and MDD in comparison with controls. The individual metabolite profile is known to be affected by antipsychotic\textsuperscript{18} and antidepressant drugs.\textsuperscript{19} Thus, the fact that the effect of medication was minimized herein is an advantage of this study.
Schizophrenia

Although the previous study identified betaine as a candidate biomarker of FESZ, this finding was not replicated in the first sample set. Because no effect of medication was found in the previous study, this may not be attributable to the effect of drugs. The major difference between the present and previous studies is the duration of illness. We therefore performed correlation analysis using Spearman’s rank correlation coefficient on a combined sample set of the two studies. This analysis revealed that betaine concentration positively correlated with the logarithm of duration of illness ($\rho = 0.473, P = 0.00080$). Therefore, although betaine may be a marker of FESZ, its levels are not altered in chronic SZ.

Other candidate metabolites identified by multivariate logistic regression analysis include creatine, 2-hydroxybutyric acid, and 2-oxoisovaleric acid. Creatine is an organic acid produced from amino acids that functions in the creatine–phosphocreatine shuttles in the muscles and the brain, and is important for the transport of high-energy phosphate in these tissues where energy demand is high. In this study, creatine plasma concentration was lower in patients with SZ than in controls (Fig. 1b), in contrast to a previous study. There are several possible explanations for this discrepancy, such as the effect of drugs and duration of illness. Serum creatine kinase levels are reportedly elevated during acute psychotic episodes, however, they are normal in chronic SZ.

Elevation of creatine kinase levels likely reflects its leak from skeletal muscles. It is possible that the increase of creatine in FESZ is also associated with this leak. Reduced creatine levels in patients with SZ were reportedly detected by proton magnetic resonance spectroscopy. Thus, some patients with SZ might have a latent deficiency of creatine in the brain, which underlies SZ.

The metabolite 2-hydroxybutyric acid is an organic acid derived from \( \alpha \)-ketobutyrate. An accumulation of 2-hydroxybutyric acid may therefore result from increased \( \alpha \)-ketobutyrate levels from the conversion of cystathionine to cysteine. Thus, elevated 2-hydroxybutyric acid levels might suggest increased oxidative stress in patients with SZ and MDD.

Bipolar disorder

The only candidate metabolite identified in this study by multivariate logistic regression analysis of BD was citrulline. Metabolome analysis revealed that citrulline levels were lower in samples from patients with BD than in controls. Citrulline is an amino acid synthesized from \( L \)-arginine by NO synthase (NOS) and NOS is present in the mitochondria. It has been proposed that a decline of mitochondrial function is due to decreased levels of NO produced in mitochondria. It has been reported that mitochondrial dysfunction has been suggested to play a role in BD. Because mitochondrial dysfunction has been suggested to play a role in BD, it might be a plausible candidate of biomarkers reflecting mitochondrial dysfunction in BD. In this study, citrulline levels and citrulline-to-arginine ratio were not reduced in medicated patients with BD. Because NO production is induced by lithium and valproic acid, we cannot exclude the possibility that medication affected the citrulline levels or citrulline-to-arginine ratio. Further studies in drug-free patients with BD are indispensable for the investigation of whether decreased citrulline levels could be a BD biomarker. In this study, \( \alpha \)-methyl-norsalsolinol, tentatively identified as a CE-TOFMS peak with a nominally different area in patients with BD and controls, was not detectable in plasma using HPLC-ECD. Considering that the peak assignment of \( \alpha \)-methyl-norsalsolinol was not reproducible, it would be premature to discuss a possible role of methionine sulfone, an oxidative stress marker, as a biomarker of mental disorders. This cautions that metabolite assignment during metabolome analysis should be done carefully.

This study has several limitations. First, only a few patients were enrolled in this study. Second, diet, circadian variation, and other confounding factors could have affected the plasma levels of metabolites. We did not strictly control the meals and sample collection time; thus, the observed changes could be ascribed to these confounding factors. Therefore, further studies using larger samples controlled for confounding factors will be needed.

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DISCLOSURE STATEMENT
The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS
T. Kasahara, T. Kato, and Y.K. conceived and designed the study. Y.K., T. Kasahara, T. Kato, N.M., and H.M. acquired and analyzed the data. K.H., S.Y., K.I., M.T., Y.D., K.K., and Y.K. collected human samples and drafted the manuscript. Y.K., T. Kasahara, and T. Kato wrote the paper.

REFERENCES
1. Post RM, Leverich GS, Kupka RW et al. Early-onset bipolar disorder and treatment delay are risk factors for poor outcome in adulthood. J. Clin. Psychiatry 2010; 71: 864–872.
2. Gaebel W, Zielasek J. Schizophrenia in 2020: Trends in diagnosis and therapy. Psychiatry Clin. Neurosci. 2015; 69: 661–673.
3. Altamura AC, Buoli M, Caldiroli A et al. Misdiagnosis, duration of untreated illness (DUI) and outcome in bipolar patients with psychotic symptoms: A naturalistic study. J. Affect. Disord. 2015; 182: 70–75.
4. Drancourt N, Etain B, Lajnef M et al. Duration of untreated bipolar disorder: Missed opportunities on the long road to optimal treatment. Acta Psychiatr. Scand. 2013; 127: 136–144.
5. Buoli M, Caldiroli A, Cumerlato Melter C, Serati M, de Nijs J, Altamura AC. Biological aspects and candidate biomarkers for psychotic bipolar disorder: A systematic review. Psychiatry Clin. Neurosci. 2016; 70: 227–244.
6. Kunugi H, Hori H, Ogawa S. Biochemical markers subtyping major depressive disorder. Psychiatry Clin. Neurosci. 2015; 69: 597–608.
7. Quinones MP, Kaddurah-Daour K. Metabolomics tools for identifying biomarkers for neuropsychiatric diseases. Neuropsychobiology 2009; 59: 126–134.
8. Koike S, Bundo M, Iwamoto K et al. A snapshot of plasma metabolites in first-episode schizophrenia: A capillary electrophoresis time-of-flight mass spectrometry study. Transl. Psychiatry 2014; 4: e379.
9. Ogawa S, Hattori K, Sasayama D et al. Reduced cerebrospinal fluid ethanolamine concentration in major depressive disorder. Sci. Rep. 2015; 5: 7796.

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25. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog. Neuropsychopharmacol. Biol. Psychiatry 2011; 35: 676–692.

26. Ghafourifar P, Richter C. Nitric oxide synthase activity in mitochondria. FEBS Lett. 1997; 418: 291–296.

27. Atkuri KR, Cowan TM, Kwan T et al. Inherited disorders affecting mitochondrial function are associated with glutathione deficiency and hypocitrullinemia. Proc. Natl. Acad. Sci. U. S. A. 2009; 106: 3941–3945.

28. El-Hattab AW, Emrick LT, Chanprasert S, Craigen WJ, Scaglia F. Mitochondria: Role of citrulline and arginine supplementation in MELAS syndrome. Int. J. Biochem. Cell Biol. 2014; 48: 85–91.

29. Kasahara T, Takata A, Kato TM et al. Depression-like episodes in mice harboring mtDNA deletions in paraventricular thalamus. Mol. Psychiatry 2016; 21: 39–48.

30. Feinstein DL. Potentiation of astroglial nitric oxide synthase type-2 expression by lithium chloride. J. Neurochem. 1998; 71: 883–886.

31. Cho D-H, Park J-H, Joo Lee E et al. Valproic acid increases NO production via the SH-PTP1-CDK5-eNOS-Ser(116) signaling cascade in endothelial cells and mice. Free Radic. Biol. Med. 2014; 76C: 96–106.

32. Cevallos-cevallos JM, Etxeberria E, Danyluk MD, Rodrick GE. Metabolomic analysis in food science: A review. Trends Food Sci. Technol. 2009; 20: 557–566.

33. Dallmann R, Viola AU, Tarokh L, Cajochen C, Brown SA. The human circadian metabolome. Proc. Natl. Acad. Sci. U. S. A. 2012; 109: 2625–2629.

34. Lawton KA, Berger A, Mitchell M et al. Analysis of the adult human plasma metabolome. Pharmacogenomics 2008; 9: 383–397.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

File S1. Supplemental information. Methods for high-performance liquid chromatography-electrochemical detection (HPLC-ECD), capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), and amino acid analysis and discussion for N-methyl-norsalsolinol.

Figure S1. Relative peak areas that showed significant differences. Names of metabolites tentatively assigned to each peak are shown. Bars indicate the mean concentration and SD in each group. (a) $P$-value is based on Kruskal–Wallis test followed by post-hoc Steel–Dwass test. (b) $P$-values are based on one-way analysis of variance with post-hoc Tukey test. BD, bipolar disorder; MDD, major depressive disorder; SZ, schizophrenia.

Table S1. Full datasets for metabolome analysis (capillary electrophoresis time-of-flight mass spectrometry [CE-TOFMS]).

Table S2. Full datasets for amino acid analysis.

Table S3. Candidate biomarker in plasma samples of major depressive disorder patients by logistic regression.