JAMA Psychiatry | Brief Report

Metabolomic Biomarker Signatures for Bipolar and Unipolar Depression

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IMPORTANCE Bipolar disorder (BD) is frequently misdiagnosed as major depressive disorder (MDD) because of overlapping symptoms and the lack of objective diagnostic tools.

OBJECTIVE To identify a reproducible metabolomic biomarker signature in patient dried blood spots (DBSs) that differentiates BD from MDD during depressive episodes and assess its added value when combined with self-reported patient information.

DESIGN, SETTING, AND PARTICIPANTS This diagnostic analysis used samples and data from the Delta study, conducted in the UK between April 27, 2018, and February 6, 2020. The primary objective was to identify BD in patients with a recent (within the past 5 years) diagnosis of MDD and current depressive symptoms (Patient Health Questionnaire-9 score of 5 or more). Participants were recruited online through voluntary response sampling. The analysis was carried out between February 2022 and July 2023.

MAIN OUTCOMES AND MEASURES Patient data were collected using a purpose-built online questionnaire (n = 635 questions). DBS metabolites (n = 630) were analyzed using a targeted mass spectrometry–based platform. Mood disorder diagnoses were established using the Composite International Diagnostic Interview.

RESULTS Of 241 patients in the discovery cohort, 170 (70.5%) were female; 67 (27.8%) were subsequently diagnosed with BD and 174 (72.2%) were confirmed as having MDD; and the mean (SD) age was 28.1 (7.1) years. Of 30 participants in the validation cohort, 16 (53%) were female; 9 (30%) were diagnosed with BD and 21 (70%) with MDD; and the mean (SD) age was 25.4 (6.3) years. DBS metabolite levels were assessed in 241 patients with depressive symptoms with a recent diagnosis of MDD, of whom 67 were subsequently diagnosed with BD by the Composite International Diagnostic Interview and 174 were confirmed as having MDD. The identified 17-biomarker panel provided a mean (SD) cross-validated area under the receiver operating characteristic curve (AUROC) of 0.71 (SD, 0.12; $P < .001$), with ceramide d18:0/24:1 emerging as the strongest biomarker. Combining biomarker data with patient-reported information significantly enhanced diagnostic performance of models based on extensive demographic data, PHQ-9 scores, and the outcomes from the Mood Disorder Questionnaire. The identified biomarkers were correlated primarily with lifetime manic symptoms and were validated in a separate group of patients who received a new clinical diagnosis of MDD (n = 21) or BD (n = 9) during the study's 1-year follow-up period, with a mean (SD) AUROC of 0.73 (0.06; $P < .001$).

CONCLUSIONS AND RELEVANCE This study provides a proof of concept for developing an accessible biomarker test to facilitate the differential diagnosis of BD and MDD and highlights the potential involvement of ceramides in the pathophysiological mechanisms of mood disorders.

JAMA Psychiatry. 2024;81(1):101-106. doi:10.1001/jamapsychiatry.2023.4096
Published online October 25, 2023.

Supplemental content

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Bipolar disorder (BD) affects approximately 1% of the population,[1] yet it is frequently misdiagnosed, with nearly 40% of patients with BD initially receiving an incorrect diagnosis of major depressive disorder (MDD).[2] This can be attributed to overlapping symptoms, patients' tendency to seek medical help during depressive rather than manic episodes,[2] and the subjective nature of psychiatric evaluations of patient self-reported symptoms. Biomarker profiling offers a promising approach to overcoming these challenges and enabling earlier and more objective differential diagnosis of mood disorders.[3-5] However, studies to date have faced several limitations, such as identifying disease biomarkers in patients with established BD confounded by the effect of mood-stabilizing medication, using samples from patients with varying symptom polarities and other uncontrolled factors, and lacking independent validation cohorts. We aimed to address these limitations by identifying and validating a dried blood spot (DBS) metabolomic signature distinguishing BD from MDD in the clinically relevant context of misdiagnosed BD in patients presenting with depressive symptoms.

Key Points

Question Can bipolar disorder (BD) be distinguished from major depressive disorder (MDD) during episodes of low mood by profiling biomarkers in patient dried blood spots (DBSs)?

Findings In this diagnostic study including 241 patients, patients with depressive symptoms with misdiagnosed BD showed a distinct profile of DBS metabolites compared with patients with depressive symptoms with MDD, which correlated with lifetime manic symptoms. Incorporating biomarker measurements into diagnostic models significantly improved predictive performance in scenarios when symptom data were limited and at uncertain diagnostic thresholds.

Meaning Metabolomic profiling of patient DBS samples has the potential to improve the differential diagnosis of mood disorders in clinically relevant scenarios.

Methods

Extended methods are provided in the eMethods in Supplement 1. The study was approved by the University of Cambridge Human Biology Research Ethics Committee. Written informed consent was obtained from all participants. This study followed the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) reporting guideline.[6]

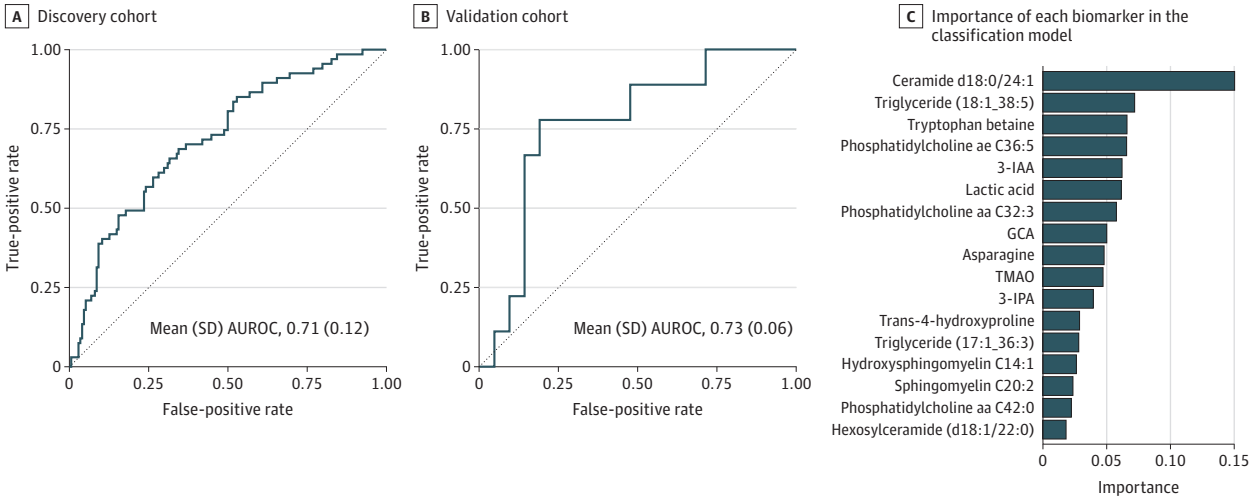
Study Participants

Samples and data analyzed were collected as part of the Delta study carried out in the UK between April 2018 and February 2020.[7] The study aimed to identify BD in patients with a recent (within the past 5 years) diagnosis of MDD and current depressive symptoms, using blood biomarker and digital questionnaire data.[7,8] Eligibility criteria included age between 18 and 45 years, UK residency, at least mild depressive symptoms (Patient Health Questionnaire-9 [PHQ-9][9] score of 5 or greater), not pregnant or breastfeeding, and not suicidal. Participants were recruited online.

Procedures

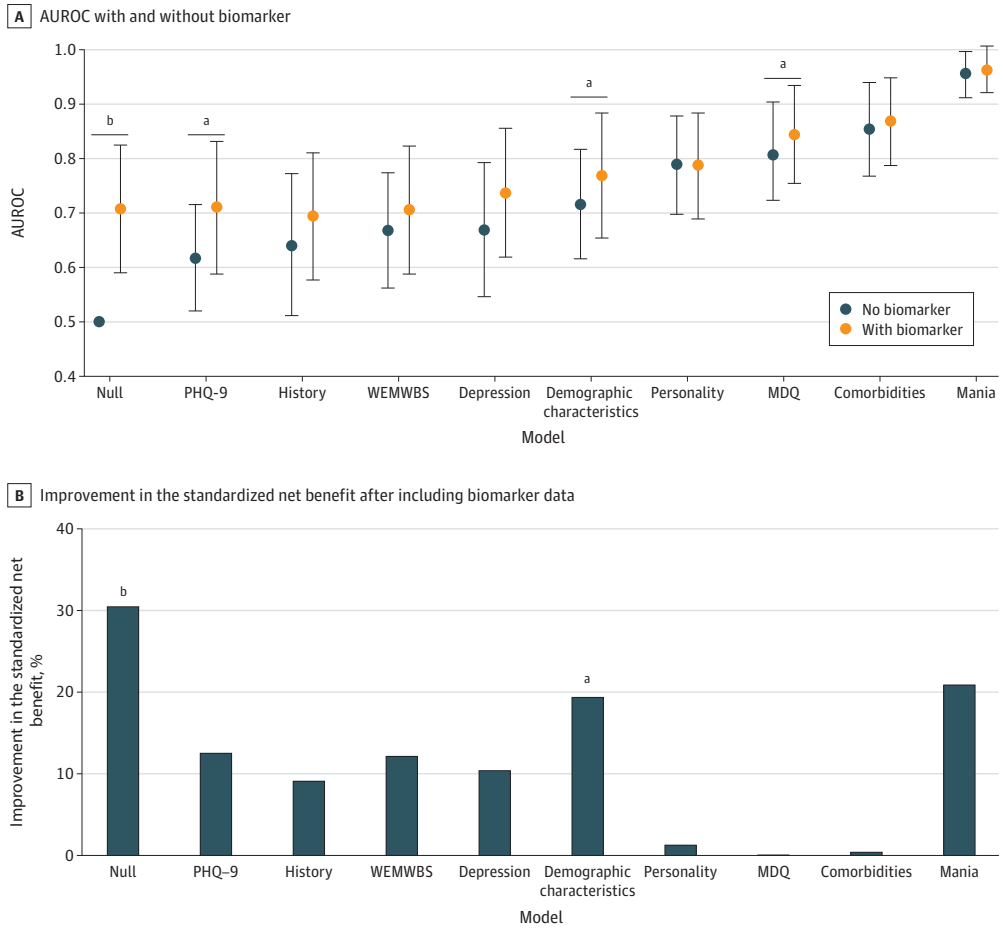
Enrolled participants were asked to complete a purpose-built online questionnaire comprising 635 adaptive questions,

Figure 1. Dried Blood Spot Metabolomic Signature Distinguishing Bipolar Disorder From Major Depressive Disorder in Patients With Current Depressive Symptoms



A, Area under the receiver operating characteristic curve (AUROC) indicating diagnostic performance of the identified biomarker panel in the discovery cohort (67 patients with misdiagnosed BD and 174 with MDD). B, AUROC in the validation cohort (9 patients with BD and 21 with MDD clinically diagnosed with a mood disorder during the 1-year follow-up period). C, Relative importance of individual biomarkers in the classification model. Values represent means obtained from the cross-validated models. GCA indicates glycocholic acid; 3-IAA, indoleacetic acid; 3-IPA, indolepropionic acid; TMAO, trimethylamine N-oxide.

Figure 2. Added Diagnostic Value of Biomarkers



A, Comparison of the area under the receiver operating characteristic curve (AUROC) for diagnostic models incorporating patient self-reported end points with and without biomarker data. Error bars indicate SDs. B, Improvement in the standardized net benefit of the diagnostic models after including biomarker data. MDQ indicates Mood Disorder Questionnaire; PHQ-9, Patient Health Questionnaire–9; WEMWBS, Warwick-Edinburgh Mental Well-Being Scale.

[a] $P < .05$.

[b] $P < .001$ (corrected resampled t test).

including items from the Mood Disorder Questionnaire (MDQ)[10] and the Warwick-Edinburgh Mental Well-Being Scale (WEMWBS).[11] Ethnicity was self-reported and included the following categories: Asian or British Asian, Black or British Black, mixed ethnicity, White, other/not listed, and prefer not to say. Eligible participants were provided with a DBS collection kit by post and asked to return a fasting blood sample. Samples were analyzed for 630 metabolites using a targeted mass spectrometry-based metabolomic platform (MxP Quant 500; Biocrates Life Sciences).

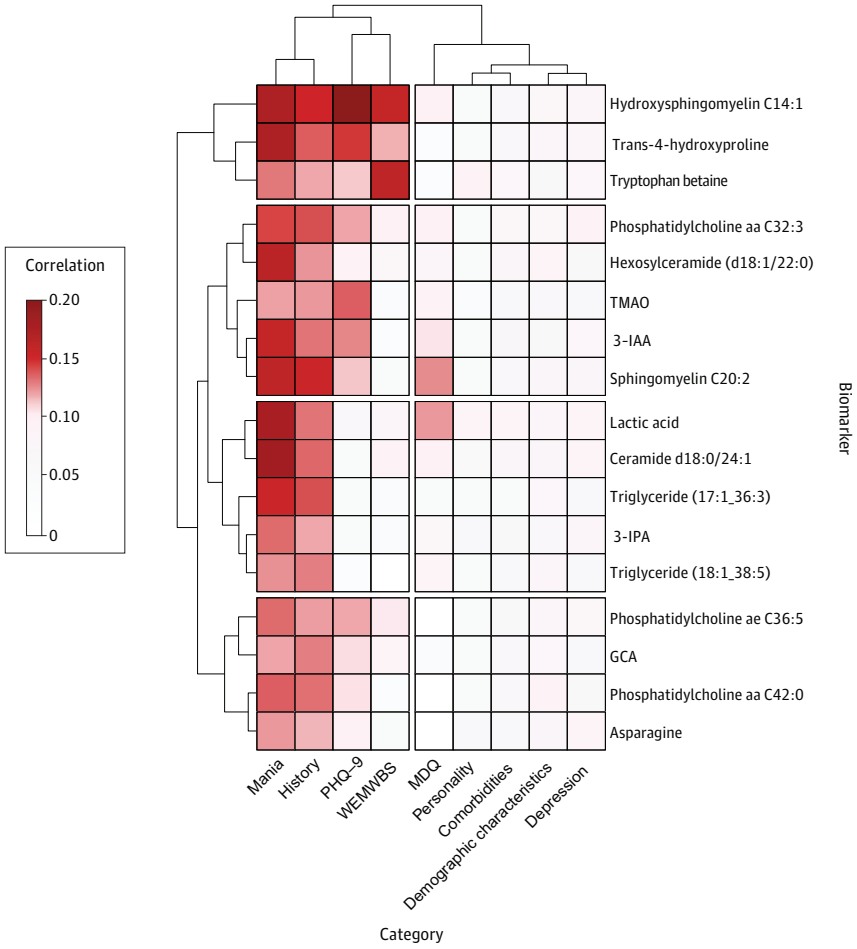
Outcomes

Mood disorder diagnoses were established by telephone using the World Health Organization World Mental Health Composite International Diagnostic Interview (CIDI) version 3.0[12] through voluntary response sampling. Follow-up data regarding changes in diagnosis were collected after 6 and 12 months. The discovery cohort consisted of participants with current depressive symptoms who had been diagnosed with MDD within the prior 5 years, whose diagnosis was either confirmed as MDD or altered to BD I by the CIDI. The validation cohort included participants experiencing depressive symptoms at the time of sample collection and professionally diagnosed with either BD or MDD during the study's 1-year follow-up period.

Statistical Analysis

Data analysis was conducted in R version 4.2.2 (The R Foundation). Diagnostic models were developed using extreme gradient boosting.[13] Model performance was evaluated using cross-validated area under the receiver operating characteristic curve (AUROC). Estimated clinical benefit was assessed using decision curve analysis.[14] Diagnostic thresholds were determined using the Youden index. P values were obtained from corrected resampled t test. Correlations were evaluated using Pearson correlation. $P < .05$ was considered significant. The corrected resampled t test was 1-tailed, testing the hypothesis that the performance of the model including biomarkers is higher than the performance

Figure 3. Association of Biomarkers With Psychopathology



Mean absolute Pearson correlation coefficients between biomarker measurements and patient self-reported end points, calculated by averaging the correlations with individual items within each symptom category. GCA indicates glycocholic acid; 3-IAA, indoleacetic acid; 3-IPA, indolepropionic acid; TMAO, trimethylamine N-oxide.

of the model without biomarkers. Pearson correlation was 2-tailed.

Results

Study Overview

Of 241 patients in the discovery cohort, 170 (70.5%) were female; 67 (27.8%) were subsequently diagnosed with BD and 174 (72.2%) were confirmed as having MDD; and the mean (SD) age was 28.1 (7.1) years (eTable 1 in Supplement 1). In the discovery cohort, patients identified with the following ethnicity categories: less than 4 Asian or British Asian (1.7%), 7 mixed ethnicity (2.9%), 172 White (71.4%), less than 4 other ethnicity (1.7%), and 58 did not report ethnicity (24.1%). In the validation cohort, patients identified with the following ethnicity categories: less than 4 Black or British Black (13%), 28 White (93%), and less than 4 did not report ethnicity (13%). Of 30 participants in the validation cohort, 16 (53%) were female; 9 (30%) were diagnosed with BD and 21 (70%) with MDD; and the mean (SD) age was 25.4 (6.3) years (eTable 2 in Supplement 1). Analyzed data comprised 290 DBS metabolite readouts representing 19 biochemical classes (eTable 3 in Supplement 1) and 992 digital questionnaire features divided into 9 categories: demographic characteristics (111 features, including age, sex, body mass index, smoking, and physical comorbidities), psychiatric history (264 features, including psychiatric comorbidities and medication), manic symptoms (142 features), depressive symptoms (199 features), comorbid psychiatric symptoms (154 features), personality traits (119 features), and single-outcome items from the MDQ, PHQ-9, and WEMWBS. Because of the questionnaire's adaptive character, 74 385 of 308 962 data points (24.1%) were missing not at random.

Differential Biomarker Signature of BD and MDD

The biomarker-based model showed a mean (SD) cross-validated AUROC of 0.71 (0.12; $P < .001$) in differentiating BD from MDD in the discovery cohort and 0.73 (0.06; $P < .001$) in the validation cohort (**Figure 1**A and B; eTable 4 in Supplement 1). The final model consisted of 17 biomarkers, among which ceramide d18:0/24:1 was the most important (Figure 1C; eTable 5 and eFigures 1, 2, and 3 in Supplement 1). Likewise, the most relevant analyte class was ceramides (eFigure 4 in Supplement 1).

Added Predictive Value of Biomarkers

Combining biomarker readouts with patient-reported data led to significant improvements in the performance of diagnostic models based on demographic information, PHQ-9 scores, and MDQ outcomes (mean [SD] change in AUROC: demographic information, 0.05 [0.08]; $P = .03$; PHQ-9, 0.09 [0.14]; $P = .03$; MDQ, 0.03 [0.05]; $P = .03$) (**Figure 2**A; eTable 6 in Supplement 1). The relative contribution of biomarkers to individual models varied from 8.8% to 99.9% (eFigure 5 in Supplement 1), with biomarker importance largely consistent across models (eFigure 6 in Supplement 1).

Clinical Utility of Biomarkers

Decision curve analysis showed that at relevant diagnostic thresholds, the inclusion of biomarkers may result in the additional identification of up to 30% of patients with BD, accounting for false-positives (Figure 2B; eTable 7 and eFigure 7 in Supplement 1).

Correlation With Psychopathology

The identified biomarkers correlated primarily with lifetime manic symptoms and psychiatric history (**Figure 3**; eTable 8 in Supplement 1). Detailed correlation with select clinicodemographic variables is shown in eFigure 8 in Supplement 1.

Discussion

Our results suggest that incorporating biomarker measurements into diagnostic models based on self-reported patient data enhances their ability to distinguish misdiagnosed BD from MDD during episodes of low mood and may lead to clinically relevant improvements. The added value of biomarkers was particularly evident in scenarios where data on psychiatric symptoms were unavailable and at intermediate diagnostic thresholds, suggesting that biomarker tests may especially benefit patients who do not report their symptoms and whose diagnoses are uncertain. Consistently, the biomarkers were correlated primarily with manic symptoms, potentially representing their surrogate markers. The results also point to the involvement of sphingolipids, specifically ceramides, in the distinct pathomechanisms of mood disorders. This may signify intrinsic immunometabolic or signaling differences between BD and MDD[15] or the body's adaptive responses to these conditions.

Strengths and Limitations

The analysis benefited from the extensively characterized, clinically relevant patient population of symptomatic help-seekers, a minimally invasive sample collection method, and a standardized metabolomic platform. Limitations include missing data on potential confounding factors, such as diet and blood pressure, a predominantly White patient population of internet users previously diagnosed with MDD who are not representative of all patients with BD, the reliance on information self-reported by participants, and the small validation cohort.

Conclusions

This diagnostic study identified a reproducible metabolomic biomarker signature in patients' DBSs that effectively differentiated misdiagnosed BD from MDD during episodes of low mood and enhanced predictive value of diagnostic models based on self-reported patient information. Our results emphasize the potential of blood biomarkers to successfully complement psychometric assessments for mood disorders and improve disease biological understanding.

ARTICLE INFORMATION

Accepted for Publication: August 18, 2023.

Published Online: October 25, 2023. doi:10.1001/jamapsychiatry.2023.4096

Author Contributions: Drs Tomasik and Bahn had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Tomasik, Harrison, Olmert, Barton-Owen, Han, Cooper, Farrag, Friend, Cowell, Bahn.
Acquisition, analysis, or interpretation of data: Tomasik, Harrison, Rustogi, Olmert, Barton-Owen, Eljasz, Bell, Bahn.
Drafting of the manuscript: Tomasik, Harrison, Bahn.
Critical review of the manuscript for important intellectual content: All authors.
Statistical analysis: Tomasik.
Obtained funding: Cowell, Bahn.
Administrative, technical, or material support: Rustogi, Olmert, Eljasz, Farrag, Bell, Cowell, Bahn.
Supervision: Tomasik, Cowell, Bahn.

Conflict of Interest Disclosures: Dr Tomasik reported having a patent pending for dried blood spot biomarkers for bipolar disorder and may benefit financially from patents arising from this work. Mr Olmert reported receiving licensing fees from the University of Cambridge for data produced in this study. Mr Barton-Owen reported being employed by and having unvested options in Psyomics during the conduct of the study. Mr Eljasz reported receiving personal fees from the University of Cambridge during the conduct of the study. Ms Farrag reported having financial interests in Psyomics. Ms Bell reported receiving grants from Innovate UK during the conduct of the study and being a shareholder in Psyomics. Mr Cowell reported receiving grants from Innovate UK during the conduct of the study and being a shareholder in Psyomics. Dr Bahn reported receiving grants from Stanley Medical Research Institute and Psyomics during the conduct of the study; being a founder and shareholder in Psyomics; being Director of Psynova Neurotech outside the submitted work; having a patent pending for dried blood spot biomarkers for bipolar disorder and may benefit financially from patents arising from this work; and receiving payments from the University of Cambridge for licensing of data from the Delta study. No other disclosures were reported.

Funding/Support: This work was supported by grant 07R-1888 from the Stanley Medical Research Institute and Psyomics.

Role of the Funder/Sponsor: Psyomics was involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and review and approval of the manuscript but had no role in the preparation of the manuscript or decision to submit the manuscript for publication. The Stanley Medical Research Institute had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 2.

Additional Contributions: We thank all participants of the Delta Study for their generous contributions to making this work possible. We also thank Sharmelee Thiahulan, MSc (Psyomics, Cambridge, United Kingdom), Mark Agius, MD (Bedford East Community Mental Health Team, Bedford, United Kingdom), Neil Hunt, MD (National Health Service, Cambridge, United Kingdom), Denise Sonntag, PhD (Biocrates Life Sciences, Innsbruck, Austria), Alice Limonciel, PhD (Biocrates

Life Sciences), Jiri Benacek, PgDip (University of Cambridge, Cambridge, United Kingdom), the Composite International Diagnostic Interview interviewers (Psyomics), dried blood spot kit packers (Psyomics), and all members of the Delta Study Service User Advisory Group for their valuable input in designing and conducting the study. Drs Agius and Hunt received compensation. All other contributors received no compensation for their contribution.

REFERENCES

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1204-1222. doi:10.1016/S0140-6736(20)30925-9

2. Hirschfeld RMA, Lewis L, Vornik LA. Perceptions and impact of bipolar disorder: how far have we really come? results of the National Depressive and Manic-Depressive Association 2000 survey of individuals with bipolar disorder. *J Clin Psychiatry*. 2003;64(2):161-174. doi:10.4088/JCP.v64n0209

3. Brunkhorst-Kanaan N, Klatt-Schreiner K, Hackel J, et al. Targeted lipidomics reveal derangement of ceramides in major depression and bipolar disorder. *Metabolism*. 2019;95:65-76. doi:10.1016/j.metabol.2019.04.002

4. Tkachev A, Stekolshchikova E, Vanyushkina A, et al. Lipid alteration signature in the blood plasma of individuals with schizophrenia, depression, and bipolar disorder. *JAMA Psychiatry*. 2023;80(3):250-259. doi:10.1001/jamapsychiatry.2022.4350

5. de Kluiver H, Jansen R, Penninx BWJH, Giltay EJ, Schoevers RA, Milaneschi Y. Metabolomics signatures of depression: the role of symptom profiles. *Transl Psychiatry*. 2023;13(1):198. doi:10.1038/s41398-023-02484-5

6. Collins GS, Reitsma JB, Altman DG, Moons KGM. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMJ*. 2015;350:g7594. doi:10.1136/bmj.g7594

7. Olmert T, Cooper JD, Han SYS, et al. A combined digital and biomarker diagnostic aid for mood disorders (the Delta trial): protocol for an observational study. *JMIR Res Protoc*. 2020;9(8):e18453. doi:10.2196/18453

8. Tomasik J, Han SYS, Barton-Owen G, et al. A machine learning algorithm to differentiate bipolar disorder from major depressive disorder using an online mental health questionnaire and blood biomarker data. *Transl Psychiatry*. 2021;11(1):41. doi:10.1038/s41398-020-01181-x

9. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606-613. doi:10.1046/j.1525-1497.2001.016009606.x

10. Hirschfeld RMA, Williams JBW, Spitzer RL, et al. Development and validation of a screening instrument for bipolar spectrum disorder: the Mood Disorder Questionnaire. *Am J Psychiatry*. 2000;157(11):1873-1875. doi:10.1176/appi.ajp.157.11.1873

11. Tennant R, Hiller L, Fishwick R, et al. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): development and UK validation. *Health Qual Life Outcomes*. 2007;5:63. doi:10.1186/1477-7525-5-63

12. Kessler RC, Ustün TB. The World Mental Health (WMH) survey initiative version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI). *Int J Methods Psychiatr Res*. 2004;13(2):93-121. doi:10.1002/mpr.168

13. Chen T, Guestrin C. XGBoost: a scalable tree boosting system. *ArXiv*. 2016;1603.02754. doi:10.1145/2939672.2939785

14. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making*. 2006;26(6):565-574. doi:10.1177/0272989X06295361

15. Bernal-Vega S, García-Juárez M, Camacho-Morales A. Contribution of ceramides metabolism in psychiatric disorders. *J Neurochem*. 2023;164(6):708-724. doi:10.1111/jnc.15759