RECENT ADVANCES ON THE MULTIMODAL SEARCH FOR MARKERS OF TREATMENT RESPONSE IN AFFECTIVE DISORDERS: FROM BENCH TO BEDSIDE?

EDITED BY: Frank M Schmidt, Christian Sander and Martin Walter
PUBLISHED IN: Frontiers in Psychiatry

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-230-5
DOI 10.3389/978-2-88963-230-5

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

RECENT ADVANCES ON THE MULTIMODAL SEARCH FOR MARKERS OF TREATMENT RESPONSE IN AFFECTIVE DISORDERS: FROM BENCH TO BEDSIDE?

Topic Editors:
Frank M Schmidt, University Hospital Leipzig, Germany
Christian Sander, Leipzig University, Germany
Martin Walter, University of Tübingen, Germany

Citation: Schmidt, F. M., Sander, C., Walter, M., eds. (2019). Recent Advances on the Multimodal Search for Markers of Treatment Response in Affective Disorders: From Bench to Bedside?. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-230-5

Table of Contents

05 ***Editorial: Recent Advances on the Multimodal Search for Markers of Treatment Response in Affective Disorders: From Bench to Bedside?***
Frank M. Schmidt, Christian Sander and Martin Walter

07 ***Preferences for Depression Treatment Including Internet-Based Interventions: Results From a Large Sample of Primary Care Patients***
Marie Dorow, Margrit Löbner, Alexander Pabst, Janine Stein and Steffi G. Riedel-Heller

20 ***Understanding and Predicting Antidepressant Response: Using Animal Models to Move Toward Precision Psychiatry***
David P. Herzog, Holger Beckmann, Klaus Lieb, Soojin Ryu and Marianne B. Müller

27 ***BDNF Plasma Levels and BDNF Exon IV Promoter Methylation as Predictors for Antidepressant Treatment Response***
Klaus Lieb, Nadine Dreimüller, Stefanie Wagner, Konrad Schlicht, Tanja Falter, Alexandra Neyazi, Linda Müller-Engling, Stefan Bleich, André Tadić and Helge Frieling

39 ***Serum Markers of Inflammation Mediate the Positive Association Between Neuroticism and Depression***
Frank M. Schmidt, Christian Sander, Juliane Minkwitz, Roland Mergl, Bethan Dalton, Lesca M. Holdt, Daniel Teupser, Ulrich Hegerl and Hubertus Himmerich

47 ***Cerebellar Contributions to Major Depression***
Malte S. Depping, Mike M. Schmitgen, Katharina M. Kubera and Robert C. Wolf

52 ***The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder***
Elena Bartkiene, Vesta Steibliene, Virginija Adomaitiene, Vita Lele, Darius Cernauskas, Daiva Zadeike, Dovile Klupsaite and Grazina Juodeikiene

56 ***Corrigendum: The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder***
Elena Bartkiene, Vesta Steibliene, Virginija Adomaitiene, Vita Lele, Darius Cernauskas, Daiva Zadeike, Dovile Klupsaite and Grazina Juodeikiene

57 ***Mitochondria, Microglia, and the Immune System—How are They Linked in Affective Disorders?***
Carsten Culmsee, Susanne Michels, Stefanie Scheu, Volker Arolt, Udo Dannlowski and Judith Alferink

69 ***Leveraging Machine Learning Approaches for Predicting Antidepressant Treatment Response Using Electroencephalography (EEG) and Clinical Data***
Natalia Jaworska, Sara de la Salle, Mohamed-Hamza Ibrahim, Pierre Blier and Verner Knott

85 ***Heart Rate Variability as Indicator of Clinical State in Depression***
Ralf Hartmann, Frank M. Schmidt, Christian Sander and Ulrich Hegerl

93 *Quantitative Electroencephalography in Guiding Treatment of Major Depression*
Mark J. Schiller

100 *Cytokine Research in Depression: Principles, Challenges, and Open Questions*
Hubertus Himmerich, Olivia Patsalos, Nicole Lichtblau, Mohammad A. A. Ibrahim and Bethan Dalton

116 *Sex Specific Changes in Tryptophan Breakdown Over a 6 Week Treatment Period*
Eva Z. Reininghaus, Nina Dalkner, Karin Riedrich, Dietmar Fuchs, Johanna M. Gostner and Bernd Reininghaus

125 *Is the HPA Axis as Target for Depression Outdated, or is There a New Hope?*
Andreas Menke

133 *Detection Study of Bipolar Depression Through the Application of a Model-Based Algorithm in Terms of Clinical Feature and Peripheral Biomarkers*
Yanqun Zheng, Shen He, Tianhong Zhang, Zhiguang Lin, Shenxun Shi, Yiru Fang, Kaida Jiang and Xiaohua Liu

frontiers in Psychiatry

EDITORIAL
published: 30 October 2019
doi: 10.3389/fpsyt.2019.00790

Editorial: Recent Advances on the Multimodal Search for Markers of Treatment Response in Affective Disorders: From Bench to Bedside?

Frank M. Schmidt[1], Christian Sander[1] and Martin Walter[2,3,4,5,6,7,8]*

[1] Department of Psychiatry and Psychotherapy, University of Leipzig Medical Center, Leipzig, Germany, [2] Clinical Affective Neuroimaging Laboratory, Otto von Guericke University of Magdeburg, Magdeburg, Germany, [3] Leibniz Institute for Neurobiology, Magdeburg, Germany, [4] Department of Neurology, Otto von Guericke University of Magdeburg, Magdeburg, Germany, [5] Center for Behavioral Brain Sciences, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany, [6] Max Planck Institute for Biological Cybernetics Tübingen, Tübingen, Germany, [7] Department of Psychiatry, University Hospital Tübingen, Tübingen, Germany, [8] Department of Psychiatry and Psychotherapy, University Hospital Jena, Jena, Germany

Keywords: Depression, bipolar disorder, Editorial, Neuroinflammation, therapy prediction

Editorial on the Research Topic

Recent Advances on the Multimodal Search for Markers of Treatment Response in Affective Disorders: From Bench to Bedside?

Differentiated pharmacotherapeutic and psychotherapeutic antidepressive options are available, and an increasing number of patients receive treatment pursuant to evidence-based guidelines. Nonetheless, a large proportion of depressed patients show no or only partial response to initial interventions. This could improve if treatment were individually tailored toward the most promising options. Numerous advances in research have revealed that molecular, structural, and physiological alterations can bring about affective disorders. The use of such biomarkers and individual response predictors for antidepressant and antimanic interventions would be a major step toward truly personalized medicine and away from the prevailing trial-and-error approaches. This Research Topic consolidates and highlights state-of-the-art research on target pathomechanisms and the discovery of markers that forecast the outcome of therapeutic interventions, symptom severities, and the courses of affective disorders.

In their review, Herzog et al. delineate the discrepancy between the great efforts that have been made in the search for clinically useful biological predictors of antidepressant response on the one hand, and the limited advances that have resulted on the other. The authors put forward several compelling suggestions that could help to overcome major methodological drawbacks in therapy prediction research. As such, they suggest the use of animal models, interdisciplinary collaboration, and the application of multidimensional diagnostic criteria. Work by Lieb et al. suggests that brain-derived neurotrophic factor (BDNF) could be a candidate for therapeutic response prediction in clinical routine. The authors show that remission rates for antidepressant treatments are associated with methylation of the CpG-87 site of the BDNF gene. Further, in forecasting response prediction, methylation of the CpG-87 site and an early increase in plasma BDNF levels could add considerably to the clinically established predictors for early improvement. The review by Menke focuses on the undiminished potential of the hypothalamic–pituitary–adrenal axis. He considers alterations of the hypothalamic–pituitary–adrenal axis function both as a pathogenic origin of depression and as a promising target site for future treatment options beyond the monoaminergic systems. Hartmann et al. focus on the role of the autonomous nervous system (ANS) in major depression by

OPEN ACCESS

Edited and reviewed by:
Paul Stokes,
King's College London,
United Kingdom

****Correspondence:***
Frank M. Schmidt
F.Schmidt@medizin.uni-leipzig.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *20 May 2019*
Accepted: *03 October 2019*
Published: *30 October 2019*

Citation:
Schmidt FM, Sander C and Walter M (2019) Editorial: Recent Advances on the Multimodal Search for Markers of Treatment Response in Affective Disorders: From Bench to Bedside? Front. Psychiatry 10:790. doi: 10.3389/fpsyt.2019.00790

investigating heart rate variability. Before treatment, depressed subjects showed a dysregulation of parameters indexing the parasympathetic branch of the ANS. Normalization of those parameters during the early course of therapy was associated with clinical improvement. These findings suggest that monitoring ANS activity (represented here by heart rate variability) as a state-dependent trait could improve diagnostic sensitivity and thus the therapeutic process. Depping et al. present the anatomical and functional properties of the cerebellum not only as an origin of psychomotor disturbances, but also as a participator in cognitive and self-referential processes that are relevant in affective disorders. To gather more information in this promising field, the authors propose important steps for future cerebellum-optimized analyses. The review by Himmerich et al. provides a comprehensive overview of how and where cytokines — important mediators of inflammation — are involved in depressive disorders. One noteworthy feature of the report is its emphasis on the pitfalls and yet unresolved questions that accompany cytokine research. Consequently, research results on this group of molecules remain unspecific and are vulnerable to a range of confounders. Beyond cytokine activity, the review by Culmsee et al. broadens the scope of inflammatory processes in affective disorders by outlining perturbations of mitochondrial and microglial pathways. Furthermore, the review issues compelling suggestions for novel yet unpursued targets within the immune and inflammatory system in both the treatment and early prognosis of psychiatric disorders. The investigation by Schmidt et al. further substantiates the link between inflammatory processes and the manifestation of depressive disorders. The authors describe a positive relationship between serum levels of cytokines, severity of depression, and extent of neuroticism. Moreover, proinflammatory cytokines appear to mediate the prediction of depression severity by the degree of neuroticism. These findings might encourage medical practitioners to discern whether a patient might benefit more from anti-inflammatory treatment strategies rather than conventional antidepressant therapies. The report by Reininghaus et al. reveals differences between sexes in the dynamics of tryptophan and kynurenine levels during the course of a psychiatric rehabilitation program. The findings suggest that sex may be a determinant for the relationship between tryptophan breakdown and clinical response. The report provides a basis for further research into how the tryptophan/kynurenine metabolism interacts with variations in sex-specific clinical features and how they could be considered in tailored treatment regimes. Bartkiene et al. propose a computer algorithm that registers and assesses facial expression responses to foods, a potential contactless option for predicting impending depressive episodes or for detecting them early on. Zheng et al. present an algorithm that utilizes both clinical and neurotrophic features. Their algorithm could improve the detection of bipolarity at an early stage of the disease, in turn improving the differential diagnosis of depressive episodes within a major depression or bipolar affective disorder. Schiller outlines different methods of quantitative electroencephalography (EEG) that could help in predicting antidepressant treatment response and support the choice of treatment. Beyond describing the methodological drawbacks that currently prevent these different algorithms from entering into clinical routine, he presents machine learning approaches that could potentially overcome these inconsistencies and thus come to be of interest in future clinical practice. Using a machine learning approach, Jaworska et al. identify features, prior to and at an early stage of therapy, that are early predictive of a response to a 12-week antidepressant trial in subjects with major depression. These findings show promise that combining demographic variables, scalp-level EEG power, and source-localized current density could improve predictive power in this cluster of entities and heterogeneous clinical presentation. Dorow et al. consider that the success of an antidepressant treatment strategy may strongly depend on the patients' confidence in and attitude toward the therapy. In their investigation, perceived self-efficacy and educational attainment strongly affected patients' choice of treatment. Intriguingly, secondary to psychotherapy, internet-based strategies were chosen equally as often as conventional medication.

In conclusion, the articles presented in this Frontiers Research Topic — diverse in their approaches and methods — provide a captivating overview of state-of-the-art research on pathomechanisms of affective disorders and predictors for successful treatment and give rise to the hope that some of these techniques will gain entry into clinical practice in the future.

AUTHOR CONTRIBUTIONS

FS wrote the initial draft of the Editorial. CS and MW critically revised the manuscript. All authors approved the submitted version.

ACKNOWLEDGMENTS

The authors thank Philip Horsfield for language editing of the manuscript. The authors thank all the contributors of the 14 papers for their valuable work.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 17 May 2018
doi: 10.3389/fpsyt.2018.00181

Preferences for Depression Treatment Including Internet-Based Interventions: Results From a Large Sample of Primary Care Patients

Marie Dorow, Margrit Löbner, Alexander Pabst, Janine Stein and Steffi G. Riedel-Heller*

Institute of Social Medicine, Occupational Health and Public Health, University of Leipzig, Leipzig, Germany

OPEN ACCESS

Edited by:
Martin Walter,
Universität Tübingen, Germany

Reviewed by:
Claudia Luck-Sikorski,
SRH Hochschule für Gesundheit, Germany
Georg Schomerus,
Universitätsmedizin Greifswald, Germany
Johannes Wancata,
Klinische Abteilung für Sozialpsychiatrie, Universitätsklinik für Psychiatrie und Psychotherapie, Medizinische Universität Wien, Austria

****Correspondence:***
Marie Dorow
marie.dorow@medizin.uni-leipzig.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *08 March 2018*
Accepted: *19 April 2018*
Published: *17 May 2018*

Citation:
Dorow M, Löbner M, Pabst A, Stein J and Riedel-Heller SG (2018) Preferences for Depression Treatment Including Internet-Based Interventions: Results From a Large Sample of Primary Care Patients. Front. Psychiatry 9:181. doi: 10.3389/fpsyt.2018.00181

Background: To date, little is known about treatment preferences for depression concerning new media. This study aims to (1) investigate treatment preferences for depression including internet-based interventions and (2) examine subgroup differences concerning age, gender and severity of depression as well as patient-related factors associated with treatment preferences.

Methods: Data were derived from the baseline assessment of the @ktiv-trial. Depression treatment preferences were assessed from $n = 641$ primary care patients with mild to moderate depression regarding the following treatments: medication, psychotherapy, combined treatment, alternative treatment, talking to friends and family, exercise, self-help literature, and internet-based interventions. Depression severity was specified by GPs according to ICD-10 criteria. Ordinal logistic regression models were conducted to identify associated factors of treatment preferences.

Results: Patients had a mean age of 43.9 years ($SD = 13.8$) and more than two thirds (68.6%) were female. About 43% of patients had mild depression while 57% were diagnosed with moderate depression. The majority of patients reported strong preferences for psychotherapy, talking to friends and family, and exercise. About one in five patients was very likely to consider internet-based interventions in case of depression. Younger patients expressed significantly stronger treatment preferences for psychotherapy and internet-based interventions than older patients. The most salient factors associated with treatment preferences were the patients' education and perceived self-efficacy.

Conclusions: Patients with depression report individually different treatment preferences.Our results underline the importance of shared decision-making within primary care. Future studies should investigate treatment preferences for different types of internet-based interventions.

Keywords: treatment preferences, depression, primary care, new media, iCBT, e-mental health

INTRODUCTION

Depression is a common, but often unrecognized and under-treated condition in primary care (1). According to estimates just about every 10th patient of a general practitioner (GP) seeks help due to depression in Germany (2). In line with this, Jacobi et al. (3) found a high prevalence of depressive syndromes according to ICD-10 criteria (11.3%) in a large sample of primary care patients, confirming similar prevalence rates of other studies (4, 5). Moreover, primary care is faced with a large amount of patients with sub-threshold depression. These patients do not meet the full criteria of a depressive disorder but may still be in need of help to manage their symptoms (3, 6).

Patients' preferences regarding a specific depression treatment may not only influence patients' treatment satisfaction, but may also have major implications for treatment adherence and outcome (7, 8). Thus, Gelhorn et al. conclude in their systematic literature review, that patient preferences are strongly associated with outcomes such as treatment initiation, treatment persistence, engagement and the development of therapeutic alliance. Therefore, the National Disease Management Guideline (S-3-Guideline) for unipolar depression (9) recommends a seven-step model of shared decision-making for health care providers (10, 11) and demands that the assessment and understanding of patient preferences should be considered as an indispensable step within the decision-making process.

Nevertheless, studies regarding treatment preferences for depression as well as associated factors in primary care settings are rare and so far have mainly focused on comparing psychotherapy and pharmacotherapy as preferred treatments (8, 12). This perspective leaves new treatment components like the use of new media aside. Moreover, most studies investigating treatment preferences assess the preference of one treatment toward another (13). However, especially for internet-based interventions there is growing interest in combining internet-based cognitive behavioral therapy (iCBT) with other treatments, such as face-to-face-psychotherapy, in the form of blended care (14, 15). Therefore, assessing the strength of preference for different treatment methods is important in order to offer a broad treatment range. Gaining knowledge about patients' preferences regarding various treatment options for depression including new approaches could enhance individually tailored treatment concepts and may lead to improved management of depression in primary care.

The present study therefore aims to investigate the following research questions:

(1) What are treatment preferences for depression in primary care patients?
(2) Are there subgroup differences in treatment preferences concerning gender, age and severity of depression?
(3) Which sociodemographic, work-related and illness-related variables are associated with different treatment preferences for depression in primary care patients?
(4) To what extent are internet-based interventions for depression preferred by primary care patients and which variables are associated with preferences for new media based approaches?

MATERIALS AND METHODS

Study Design and Sample

Data were derived from the @ktiv-trial (trial registration number: DRKS00005075). The trial was approved by the institutional review boards (Ethics Committees) of the University of Leipzig (reference number 222/14ff) and of the Australian National University (reference number 2013/342). The @ktiv-trial consists of a baseline assessment followed by a 6 week and a 6 month follow-up. The present study focuses on the analysis of cross-sectional data from the baseline assessment.

Patients were recruited from 112 primary care practices in Central Germany and had to fulfill the following criteria: (1) age of 18 years and above, (2) positive screening for mild to moderately severe depressive symptoms according to the 9 item version of the Patient Health Questionnaire (PHQ-9) with a total score between 5 and 19 points, (3) mild or moderate depression according to the GP's diagnosis based on ICD-10 criteria (F32.0, F32.1, F33.0, F33.1), (4) German as first language, and (5) home internet access and regular use of the Internet. Patients were excluded in case of (1) severe or persistent depression (ICD-10: F32.2, F32.3, F33.2, F33.3, F34), (2) organic mental disorders (ICD-10: F00-F09), (3) alcohol or drug dependence (F10-F16, F18, F19), (4) schizophrenia and schizoaffective disorders (F20-F29), (5) bipolar disorders (F31), (6) suicidality, (7) fatal somatic disease (e.g., final stadium of cancer), (8) current grief (due to recent loss of a beloved person), and/or (9) receiving psychotherapy at the time of recruitment.

After giving written consent $N = 647$ patients filled in a written self-report questionnaire in the primary care practice for baseline assessment. Recruitment of patients was conducted between February 1, 2014 and August 31, 2015.

Variables and Instruments

The patient self-report questionnaire comprised a wide-ranging set of structured scales and variables.

Primary Outcome

Treatment preferences were assessed using an adapted 8-item rating scale based on previous research (16, 17) with each item representing a different treatment option for depressive disorders. The treatment options were: medication, psychotherapy, combined treatment (medication and psychotherapy), alternative therapy options such as alternative practitioners, talking to friends and family, exercise, self-help literature and internet-based interventions. Patients were asked to indicate to what extent they would consider the different treatment options in case of depression on a scale ranging from 1 ("very unlikely") to 5 ("very likely").

Sociodemographic and Work-Related Variables

Sociodemographic data included the patients' age, gender, marital status (married, single, divorced, widowed) and educational level according to the new CASMIN educational classification system (18). For work-related information the patients' vocational qualification was collected.

Illness-Related Variables

As part of the recruitment process GPs were asked to specify the severity of depression, i.e., whether patients had mild or moderate depression according to ICD-10 criteria.

Other illness-related variables were collected from the patients' self-report. Patients were asked to indicate whether they had ever received treatment due to emotional stress (such as sadness, anxiousness or mental overload) before (treatment history; yes/no). Comorbid panic disorder (PD; F41.0 or F40.01) and/or generalized anxiety disorder (GAD; F41.1 or F41.9) according to ICD-10 criteria were assessed with the Patient Health Questionnaire ([PHQ-D; (19)], a validated German self-report version of the Primary Care Evaluation of Mental Disorders [PRIME-MD; (20)]). In addition, the 6-item subscale Hope and Self-efficacy with a minimum of 0 and maximum score of 24 points from the questionnaire for the assessment of Empowerment in Patients with Affective and Schizophrenic disorders [EPAS; (21)] was applied. Health-related quality of life (HRQOL) was measured using the 5-level version of the health state classifier EQ-5D-5L of the EuroQol Group (22). Sum scores of HRQOL had a possible range from 0 and 100 with higher values indicating higher HRQOL.

Statistical Analysis

Patients with missing data on age, gender or the primary outcome, i.e., patients who did not rate any item on treatment preferences, were excluded from analysis. Thus, the analytical sample consists of $n = 641$ patients. Missing data on the determinants ranged from 0.2% (marital status) to 7.8% (comorbid PD) and were replaced using multiple imputation by chained equations (23). We used the pooled estimates of 50 imputed datasets for all analyses.

Descriptive data are presented as mean with standard deviation (SD) or absolute frequencies and percentages. Subgroup analyses were performed for gender, age, and severity of depression. Age was divided into three groups (18–30, 31–50, and 51–82 years) to indicate age-related differences. Subgroup comparisons were evaluated using Wilcoxon two-sample tests or Cuzick's (24) trend test across ordered groups. In order to identify whether treatment preferences are associated with sociodemographic and illness-related variables eight ordinal logistic regression models were conducted, one for each treatment option. All continuous variables in the models were centered (mean = 0; $SD = 1$) to reduce multicollinearity. The proportionality of odds assumption was fulfilled in all ordinal regression models as suggested by approximate likelihood ratio tests of proportionality of odds (25).

All statistical analyses were performed using the Statistical Package for the Social Sciences 24.0 for Windows (SPSS Inc., Chicago, IL) or Stata 13.1 SE (Stata Corp LP, College Station, TX). Given the total number of eight models tested, all analyses are based on a more stringent level of significance with a p-value below 0.01.

RESULTS

Sample Characteristics

Table 1 shows the sociodemographic, work-related and illness-related characteristics of the sample. Study participants had a mean age of 43.9 years ($SD = 13.8$) with the following distribution of age groups: 21.5% (18–30 years), 43.8% (31–50 years) and 34.6% (51–82 years). Regarding older patients, $n = 35$ (5.5%) individuals in our sample were 65+ years of age and $n = 9$ (1.5%) were older than 75 years. More than two thirds (68.6%) of the patients were female. The majority of patients were either married (42.8%) or single (41.2%), had medium education (55.9%) and completed an apprenticeship (57.9%). 43.4% of patients had mild and 56.6% had moderate depression according to their GP. Two in three (67%) patients reported that they had been treated for emotional stress before, about one out of four patients had a comorbid panic disorder (27.0%) and 22.9% had a comorbid generalized anxiety disorder. Mean values for self-efficacy (EPAS) and HRQOL (EQ-5D-5L) were 11.9 ($SD = 4.5$) and 74.4 ($SD = 13.4$), respectively.

Treatment Preferences for Depression in Primary Care Patients

Figure 1 summarizes ranked treatment preferences for depression. The majority of patients (58%) reported that they were likely or very likely to consider psychotherapy as a treatment option for depression. Similarly, 55 and 51% were likely or very likely to consider talking to friends and family or exercise to manage their depression. Figure S1 in Supplementary Material shows more detailed information about the distributions of patients' treatment preferences.

The mean preference strengths were 3.7 ($SD = 1.4$) for psychotherapy, 3.6 ($SD = 1.4$) for talking to friends and family, 3.5 ($SD = 1.3$) for exercise, 3.1 ($SD = 1.4$) for combined treatment, 3.0 ($SD = 1.5$) for medication, 2.9 for self-help literature ($SD = 1.4$), alternative treatment ($SD = 1.4$) and internet-based interventions ($SD = 1.5$).

Subgroup Differences in Treatment Preferences for Depression Concerning Age, Gender and Severity of Depression

Table 2 presents results of the subgroup analyses concerning gender-, age-, and depression-related differences in treatment preferences. Gender-related differences were found in the treatment preferences for alternative treatment ($z = 3.36$, $p = 0.001$) and self-help literature ($z = 3.48$, $p < 0.001$) with females showing stronger preferences for these treatment options than men. Regarding age-related differences younger patients preferred psychotherapy to a greater extent than older patients ($z = -3.71$, $p < 0.001$). Patients with moderate depression showed higher preference for medication as a treatment option than patients with mild depression ($z = -2.68$, $p = 0.007$).

TABLE 1 | Sample characteristics ($n = 641$).

SOCIODEMOGRAPHIC AND WORK-RELATED VARIABLES	
Age, mean (SD)	43.9 (13.8)
Age groups	
18–30	138 (21.5)
31–50	281 (43.8)
51–82	222 (34.6)
Gender	
Female	440 (68.6)
Male	201 (31.4)
Marital status	
Married	274 (42.8)
Single	264 (41.2)
Divorced/widowed	103 (16.1)
Educational level	
High	194 (30.3)
Middle	358 (55.9)
Low	89 (13.9)
Vocational qualification	
None/other qualification	36 (5.6)
Still undergoing vocational training	25 (3.9)
Completed apprenticeship	371 (57.9)
Secondary vocational education	101 (15.8)
University degree	108 (16.9)
ILLNESS-RELATED VARIABLES	
Depression diagnosis by GP	
Mild depression	278 (43.4)
Moderate depression	363 (56.6)
Treatment history	432 (67.4)
GAD	147 (22.9)
PD	173 (27.0)
EPAS, mean (SD)	11.9 (4.5)
EQ-5D-5L, mean (SD)	74.4 (13.4)

Entries are n (%) unless indicated differently and present sample characteristics after imputation. GAD, generalized anxiety disorder according to ICD-10 (self-report); GP, general practitioner; PD, panic disorder according to ICD-10 (self-report); EPAS, 6-item subscale Hope and Self-efficacy from the questionnaire for the assessment of Empowerment in Patients with Affective and Schizophrenic disorders; EQ-5D-5L, health state classifier EQ-5D of the EuroQol Group; SD, standard deviation.

Associated Variables of Treatment Preferences for Depression

Whereas several sociodemographic and illness-related variables were associated with treatment preferences for depression, work-related variables, i.e., the patients' vocational qualification did not show significant impact on any treatment option (**Table 3**).

Sociodemographic Variables Associated With Treatment Preferences for Depression

The patients' educational level was significantly associated with treatment preferences for psychotherapy, exercise and self-help literature. Hence, patients with a higher academic education were more likely to prefer these treatment options. For example, patients with a high educational level had 2.27 (95%-CI = 1.32, 3.88) higher odds for preferring psychotherapy than patients with a low educational level, given that all other variables in the model are held constant. Gender was associated with alternative treatment and self-help literature. Thus, women had 1/0.63 = 1.59 higher odds (95%-CI = 1.15, 2.17) for expressing stronger preference toward alternative treatment and 1/0.66 = 1.52 higher odds (95%-CI = 1.11, 2.08) for preferring self-help literature. The patients' marital status emerged as a factor that was significantly associated with a higher preference for psychotherapy as singles were more likely to favor psychotherapy than married patients (OR = 1.82; 95%-CI = 1.28, 2.58).

Illness-Related Variables Associated With Treatment Preferences for Depression

Patients scoring higher on the EPAS hope and self-efficacy subscale were less likely to prefer medication (OR = 0.94; 95%-CI = 0.91, 0.97) or combined treatment (OR = 0.95; 95%-CI = 0.92, 0.98), but had higher odds for exercise (OR = 1.12; 95%-CI = 1.08, 1.16) or talking to friends and family (OR = 1.10; 95%-CI = 1.06, 1.14). Having a comorbid general anxiety disorder increased the willingness to seek alternative treatment (OR = 1.65; 95%-CI = 1.17, 2.34) and internet-based interventions (OR = 2.06; 95%-CI = 1.47, 2.91). Finally, patients who had not received treatment due to emotional stress in the past were more likely to score high on medication (OR = 1.96; 95%-CI = 1.43, 2.63) or combined treatment (OR = 1.61; 95%-CI = 1.19, 2.22) than patients who did receive help due to psychological problems before. The following illness-related factors were not significantly associated with any treatment preference: severity of depression, comorbid panic disorder and HRQOL.

Internet-Based Interventions as a Treatment Option for Depression

About 38% of the patients were likely or very likely to consider internet-based interventions in case of depression. In contrast, 42% of the patients were unlikely or very unlikely to prefer internet-based interventions for the treatment of depression. Subgroup differences were found for age, as younger patients expressed a stronger treatment preference for internet-based interventions than older patients ($z = -4.30$, $p < 0.001$). Patients with moderate depression were more likely to prefer internet-based interventions than patients with mild depression ($z = -2.13$, $p = 0.033$), even though this was not significant given the significance level of $p < 0.01$. Associated factors for internet-based interventions were the patients' age, educational level and having a comorbid anxiety disorder. Thus, younger patients were significantly more likely to express stronger acceptance toward internet-based interventions than older patients (OR = 1.01; 95%-CI = 1.01, 1.02). In addition, patients with a high educational level had 3.10 (95%-CI = 1.79, 5.34) higher odds of scoring higher on internet-based interventions than patients with a low educational level. Finally, those with a comorbid general anxiety disorder had higher preferences for internet-based interventions (OR = 2.06; 95%-CI = 1.47, 2.91).

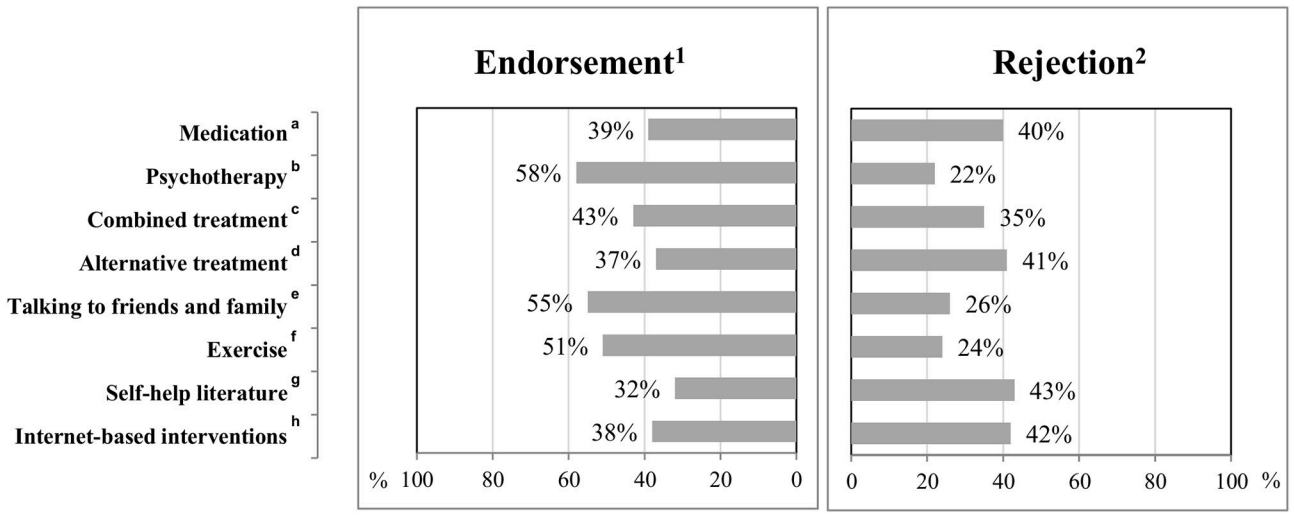

FIGURE 1 | Treatment preferences for depression in primary care. [1]Endorsement: likely or very likely; [2]Rejection: unlikely or very unlikely; [a]$n = 637$; [b]$n = 633$; [c]Combined treatment refers to medication and psychotherapy, $n = 615$; [d]Alternative treatment (e.g. alternative practitioners), $n = 623$; [e]$n = 634$; [f]$n = 638$; [g]$n = 629$; [h]$n = 631$; percentage of respondents "undecided": [a]$n = 132$ (21%); [b]$n = 125$ (20%); [c]$n = 135$ (22%); [d]$n = 141$ (23%); [e]$n = 122$ (19%); [f]$n = 159$ (25%); [g]$n = 154$ (24%); [h]$n = 127$ (20%).

DISCUSSION

Treatment Preferences for Depression in Primary Care Patients

In the present study, the strongest treatment preferences were reported for psychotherapy, talking to friends and family, and exercise. In this regard, patients expressed stronger preference for psychotherapy than for medication or combined treatment (psychotherapy and medication). Our observed mean values for psychotherapy and medication are in line with Raue et al. (26) who found a mean preference strength of 4.1 for psychotherapy and 2.9 for antidepressants using 5-point Likert scales. A large number of previous studies showed that patients often prefer psychotherapy to medication for the treatment of depression (7, 27–34). Despite the known effectiveness of antidepressants (35), low acceptance rates were reported in previous studies (36, 37) which may be explained by general reluctance or anticipated side effects caused by the drugs.

Our finding for highly favorable preferences toward other treatment options such as talking to friends and family is supported by previous research reporting that individuals prefer informal help from a confidant to formal sources of help (17, 38, 39). Existing literature indicates that positively experienced lay support may effectively help to overcome symptoms of depression (40, 41). In a qualitative study (42), $n = 417$ participants who had sought help for depression from family or friends filled in a questionnaire about advantages and disadvantages of informal support. The most frequently reported benefit was social support with emotional support being the most commonly cited type of support, followed by informational, companionship and instrumental support. On the other hand, the most salient barriers in seeking help from a confidant were seen in issues of stigma, such as stigmatizing responses or anticipated stigma, as well as inappropriate support and lack of knowledge, training and expertise.

Regarding physical exercise, a Cochrane review (43) including 35 trials found a moderate clinical effect, when comparing exercise to no treatment or a control intervention [pooled standardized mean difference (SMD) $= -0.62$]. Trials of high methodological quality indicated smaller effects, but there is ongoing research in this field providing growing evidence for the effectiveness of exercise in the treatment of depression. For example, a large effectiveness trial conducted in mild to moderately depressed primary care patients indicates that adjunctive physical exercise is more effective than treatment in primary healthcare alone (44).

Subgroup Differences in Treatment Preferences for Depression Concerning Age, Gender and Severity of Depression

We found no gender-related differences regarding treatment preferences for psychotherapy which is in contrast with many previous studies indicating that women are more likely than men to favor psychotherapy (7, 29, 30, 34, 45, 46). Houle et al. (34) point out that a possible explanation for this preference may be that women express and talk about their feelings more easily. In terms of gender differences, women in our study showed stronger preferences for alternative treatment and self-help literature which is in line with other studies (47, 48).

Our finding that younger age groups were more likely than older patients to prefer psychotherapy is supported by Boehlen et al. (49) showing that the willingness to seek help for psychological problems was lower in older age groups compared to younger study participants. Barriers to see a psychotherapist

TABLE 2 | Gender-, age-, and depression-related subgroup differences in treatment preferences for depression in primary care patients.

Treatment option	Gender-related differences						Age-related differences								Depression-related differences					
	fem (*n*)	Mean (*SD*)	Male (*n*)	Mean (*SD*)	*z*	*p*	18–30 (*n*)	Mean (*SD*)	31–50 (*n*)	Mean (*SD*)	51–82 (*n*)	Mean (*SD*)	*z*	*p*	Mild (*n*)	Mean (*SD*)	mod (*n*)	Mean (*SD*)	*z*	*p*
Medication	435	2.98 (1.47)	198	3.19 (1.44)	−1.68	0.094	137	2.99 (1.42)	276	3.01 (1.47)	220	3.14 (1.47)	1.04	0.298	276	2.87 (1.44)	357	3.18 (1.46)	−2.68	**0.007**
Psychotherapy	432	3.75 (1.34)	197	3.53 (1.38)	1.90	0.057	137	3.98 (1.21)	276	3.75 (1.29)	216	3.39 (1.47)	−3.71	**<0.001**	272	3.57 (1.37)	357	3.76 (1.34)	−1.84	0.065
Combined treatment[a]	416	3.10 (1.46)	195	3.14 (1.37)	−0.29	0.770	135	3.19 (1.42)	264	3.09 (1.40)	212	3.08 (1.49)	−0.59	0.555	265	3.03 (1.42)	346	3.18 (1.44)	−1.33	0.184
Alternative treatment[b]	425	3.07 (1.43)	195	2.66 (1.34)	3.36	**0.001**	136	3.01 (1.41)	269	3.11 (1.39)	215	2.69 (1.42)	−2.46	0.014	270	2.90 (1.43)	350	2.97 (1.41)	−0.63	0.528
Talking to friends and family	432	3.64 (1.39)	198	3.46 (1.45)	1.43	0.152	138	3.57 (1.42)	274	3.65 (1.38)	218	3.51 (1.45)	−0.39	0.698	273	3.62 (1.35)	357	3.56 (1.46)	0.19	0.852
Exercise	435	3.53 (1.31)	199	3.48 (1.36)	0.42	0.675	138	3.61 (1.28)	277	3.56 (1.29)	219	3.40 (1.40)	−1.42	0.155	275	3.51 (1.36)	359	3.52 (1.30)	−0.04	0.965
Self-help literature	430	3.01 (1.40)	196	2.59 (1.24)	3.48	**<0.001**	137	2.68 (1.31)	274	2.88 (1.34)	215	3.00 (1.43)	2.05	0.040	274	2.88 (1.33)	352	2.88 (1.40)	0.14	0.887
Internet-based interventions	431	2.89 (1.50)	197	2.86 (1.39)	0.18	0.860	137	3.16 (1.37)	278	3.02 (1.45)	213	2.52 (1.48)	−4.30	**<0.001**	275	2.74 (1.43)	353	2.99 (1.49)	−2.13	0.033

[a] *Combined treatment refers to medication and psychotherapy.*

[b] *Alternative treatment (e.g., alternative practitioners); SD, standard deviation; treatment preferences had a possible range from 1 to 5, with higher scores indicating stronger preference; gender- and depression-related differences were analyzed with Wilcoxon two-sample tests; age-related differences were calculated using Cuzicks Trend test; the level of significance was* $p < 0.01$.

for depression reported by elderly individuals were the wish to solve the emotional problems autonomously and fear of stigma (50).

Regarding severity of depression, we found that more severe depression was associated with stronger preferences for the intake of medication. This may be due to higher psychological strain in stronger affected patients. In line with this finding, Berner et al. (36) found that increasing symptom severity was associated with stronger treatment preferences for interventions that had to be initiated by a health care professional. On the other hand, the authors note that a large number of both affected and unaffected individuals do not understand depression as a treatable disorder. Even in patients who are strongly affected by depressive symptoms, almost half of them did not wish to be treated.

Associated Variables of Treatment Preferences for Depression

The patients' educational level was the most salient sociodemographic factor associated with treatment preferences for depression. Accordingly, a higher educational level was associated with stronger treatment preferences. Likewise, patients with higher education have been found to use face-to-face psychotherapy more frequently than patients with lower education (51). This may be explained by increased health literacy in patients with more formal education (52–54). Knowledge about the effectiveness of therapy options or treatment components for mental illnesses may influence patients' attitudes and result in stronger willingness to consider these options. In accordance with a previous study (34) we found that higher education was significantly associated with preference for psychotherapy but not for medication. As a possible explanation, Houle et al. (34) point out that, unlike taking antidepressants, attending psychotherapy requires a high degree of self-reflection and patience to overcome symptoms of depression, attributes which may be more pronounced in people with a higher educational level.

In accordance with subgroup analyses within the present study, female gender was associated with increased preferences for alternative therapy and self-help literature. Another sociodemographic factor influencing treatment preference was the patients' marital status. The finding that singles were more likely to express more preferences for psychotherapy than married patients may be explained by lacking emotional support from the spouse in patients without a partner, leading to a stronger wish to talk to a psychotherapist and receive professional support (55). Furthermore, Kessler et al. (56) found out that singles who had never been married before, had a higher likelihood of seeking support from a mental health specialist.

Concerning illness-related factors for treatment preferences of depression, the patients' self-efficacy, signifying the patients' beliefs to be able to change something about their situation, seems to be of particular importance. Hence, higher self-efficacy was associated with less favorable preferences toward medication and combined treatment, which involve a passive component of dealing with the disease, but with higher preferences for active

treatment options, i.e., doing sports and talking to friends and family. These findings may be associated with patient beliefs about depression etiology (12). Accordingly, previous research indicates that individuals who favor medication to psychotherapy tend to attribute their depression more to biomedical causes (12, 29, 46) and were less likely to consider pessimistic thinking as a cause for their depression (57). Therefore, patients who show high preferences for medication and low preferences for exercise or talking to a confidant may underestimate the potential of self-efficacy.

Moreover, having a treatment history of depression was associated with reduced preference for medication or combined treatment. This may be caused by negative experiences with previous intake of medication as patients with positive experience of a certain treatment method are more likely to seek the same treatment in the future (1).

Internet-Based Interventions as a Treatment Option for Depression

During the last decade e-mental health interventions have been a rapidly developing field of research. Christensen et al. (58) define e-mental health as "mental health services and information delivered or enhanced through the Internet and related technologies" (p. 17). In this regard, iCBT represents a new, innovative and effective treatment approach for mental health disorders. Systematic reviews, meta-reviews and meta-analyses point out the effectiveness and user acceptance of iCBT programs for depression (59–63). E-mental health interventions such as iCBT programs may therefore function as a clinically effective and cost-effective add-on treatment component besides the existing somato-, psycho- and psychosocial-therapy treatments within stepped care of depression (64, 65). In Germany, however, these new approaches have hardly been implemented into the German health care system due to legal barriers (66). In addition, freely available, low threshold e-mental health interventions for the adjunctive treatment of depression are still at an early stage of dissemination.

Within the present study, more than a third of the patients were likely or very likely to consider internet-based interventions in case of depression. In contrast, a slightly higher number of patients reported the opposite. Hence, internet-based interventions seem to evoke opposing reactions in primary care patients with mild to moderate depression. Nevertheless, these findings indicate that there is a considerable amount of patients showing interest in using e-mental health interventions for the treatment of depression. A previous study (67) investigating the implementation of the internet-based self-management program moodgym in inpatient psychiatric clinics found that stronger treatment preference for internet-based interventions was a significant predictor for starting the moodgym program. In view of influential sociodemographic factors, age and education were associated with preferences for internet-based interventions. Findings regarding the patients' age are in line with results from Batterham and Calear (68), who found that younger patients were more likely to prefer internet-based interventions ahead of face-to-face-therapy compared to older patients. This is most likely due to the fact that the amount of younger individuals using the internet for private purposes is higher compared to older individuals (69) and younger people use the internet more frequently than do older age groups (70). Likewise, Eichenberg et al. (71) found out that age as well as internet usage corresponded with people's willingness to use e-mental health services. Hence, older patients may be less familiar with the internet in general and may feel that they do not have sufficient computer skills to conduct an online program. In a qualitative study investigating patients with obesity and comorbid depression, Löbner et al. (72) found that according to clinical experts, the patients' age was cited as an important characteristic that needs to be considered when implementing internet-based interventions in rehabilitative care. Furthermore, Batterham and Calear (68) suggest that older individuals and also those with a lower educational level should be made more familiar with internet-based interventions. The association between higher education and stronger preferences for internet-based programs is supported by previous research (68, 71, 73, 74). People with increased education may be more aware of treatment options for depression in general, which may contribute to more positive attitudes toward internet-based treatment. Additionally, compared to patients with a high educational level, those with lower education have been found to be more likely to drop out from internet-based CBT interventions (75) or trials investigating the effectiveness and acceptability of iCBT (76).

As an illness-related factor, comorbid general anxiety disorder was found to be associated with stronger treatment preferences for internet-based interventions. A possible reason may be increased psychological stress in patients with comorbid anxiety resulting in greater perceived need for help (77). Another explanation may be that patients suffering from anxiety disorders may endorse the anonymity of online programs (78). In this regard, individuals reported that providing anonymous programs may have the effect that more people dare to seek help and that conducting an online program may be less embarrassing than face-to-face therapy (79). Klein and Cook (80) found out that individuals preferring online interventions had more stigmatizing beliefs, lower scores on extraversion and emotional stability, characteristics which may be more common in patients with comorbid anxiety.

Strengths and Limitations

Strengths of this study are the large sample, the naturalistic setting in primary care and the extended assessment of treatment preferences for depression including analyses on new media based approaches. However, this study also has some limitations. First, since we recruited primary care patients with mild or moderate depression, our findings may not apply to patients with severe depression and cannot be generalized to settings other than primary care. Treatment preferences reported by primary care patients may differ from those seen in specialized care settings. Moreover, we cannot ensure generalizability of our results for the whole population of primary care patients since the proportion of older patients in our study is not representative for this population. However, we were able to recruit a sufficient number of individuals in old age and we

TABLE 3 | Associated variables of treatment preferences for depression in primary care patients.

	Medication				**Psychotherapy**				**Combined treatment[a]**				**Alternative treatment[b]**			
Sociodemographic and work-related variables	**OR (95% CI)**	**p**	**Wald-Chi²**	**p> Chi²**	**OR (95% CI)**	**p**	**Wald-Chi²**	**p> Chi²**	**OR (95% CI)**	**p**	**Wald-Chi²**	**p> Chi²**	**OR (95% CI)**	**p**	**Wald-Chi²**	**p> Chi²**
male	1.45 (1.06, 1.98)	0.019			0.80 (0.58, 1.10)	0.167			1.12 (0.82, 1.53)	0.476			0.63 (0.46, 0.87)	**0.005**		
age	1.01 (1.01, 1.02)	0.033			1.00 (0.99, 1.01)	0.726			1.01 (1.00, 1.02)	0.027			1.00 (0.99, 1.00)	0.229		
marital status (R: married)			1.66	0.436			11.14	**0.004**			7.17	0.028			1.33	0.513
single	0.98 (0.70, 1.38)	0.907			1.82 (1.28, 2.58)	**0.001**			1.32 (0.94,1.86)	0.110			1.14 (0.81, 1.61)	0.447		
divorced/widowed	0.77 (0.51, 1.16)	0.212			1.17 (0.76, 1.80)	0.486			0.70 (0.45, 1.09)	0.117			1.26 (0.82, 1.93)	0.286		
education (R: low)			0.17	0.920			11.08	**0.004**			3.70	0.157			3.39	0.183
middle	1.09 (0.68, 1.72)	0.730			2.04 (1.29, 3.23)	**0.002**			1.52 (0.96, 2.40)	0.072			1.47 (0.93, 2.31)	0.099		
high	1.11 (0.65, 1.91)	0.696			2.27 (1.32, 3.88)	**0.003**			1.58 (0.93, 2.68)	0.093			1.59 (0.93, 2.70)	0.091		
Vocational qualification (R: none)			2.63	0.621			1.33	0.857			1.44	0.837			0.12	0.998
still undergoing training	0.93 (0.36, 2.38)	0.875			1.65 (0.62, 4.38)	0.318			1.06 (0.42, 2.70)	0.900			0.87 (0.34, 2.22)	0.774		
completed apprenticeship	0.98 (0.51, 1.88)	0.941			1.29 (0.69, 2.40)	0.425			1.14 (0.61, 2.15)	0.681			0.92 (0.48, 1.74)	0.792		
secondary vocational education	1.00 (0.48, 2.08)	0.996			1.18 (0.58, 2.41)	0.654			1.14 (0.55, 2.33)	0.729			0.94 (0.46, 1.95)	0.877		
university degree	0.67 (0.31, 1.44)	0.302			1.17 (0.56,2.48)	0.673			0.85 (0.40, 1.80)	0.672			0.95 (0.44, 2.02)	0.885		
Illness-related variables																
Moderate depression (R: mild)	1.25 (0.92, 1.68)	0.150			1.24 (0.91, 1.69)	0.179			1.05 (0.78, 1.43)	0.734			1.08 (0.79, 1.46)	0.636		
Treatment history	0.51 (0.38, 0.70)	**<0.001**			0.74 (0.54, 1.02)	0.064			0.62 (0.45, 0.84)	**0.002**			1.27 (0.93, 1.74)	0.132		
GAD	1.11 (0.79, 1.55)	0.548			1.30 (0.91, 1.85)	0.145			1.22 (0.87, 1.71)	0.250			1.65 (1.17, 2.34)	**0.005**		
PD	1.09 (0.78, 1.51)	0.628			1.32 (0.93, 1.87)	0.126			1.21 (0.87, 1.69)	0.265			1.27 (0.90, 1.79)	0.167		
EPAS	0.94 (0.91, 0.97)	**<0.001**			0.98 (0.95, 1.01)	0.177			0.95 (0.92, 0.98)	**0.002**			1.01 (0.98, 1.04)	0.493		
EQ-5D-5L	0.99 (0.98, 1.00)	0.081			1.00 (0.99, 1.01)	0.832			0.99 (0.98, 1.00)	0.102			1.01 (1.00, 1.02)	0.140		
N	633				629				611				620			
Pseudo R^2	0.03				0.03				0.03				0.02			

	Talking to friends and family				**Exercise**				**Self-help literature**				**Internet-based self-help programs**			
Sociodemographic and work-related variables	OR (95% CI)	P	Wald-Chi²	p> Chi²	OR (95% CI)	P	Wald-Chi²	p> Chi²	OR (95% CI)	p	Wald-Chi²	p> Chi²	OR (95% CI)	P	Wald-Chi²	p> Chi²
male	0.88 (0.64, 1.21)	0.436			0.99 (0.72, 1.36)	0.955			0.66 (0.48, 0.90)	**0.009**			1.13 (0.83, 1.55)	0.444		
age	1.00 (0.99, 1.01)	0.670			1.00 (0.99, 1.01)	0.834			1.00 (0.99, 1.01)	0.863			0.99 (0.98, 0.99)	**0.003**		
marital status (R: married)			2.61	0.272			1.32	0.516			3.63	0.163			0.13	0.937
single	1.33 (0.94, 1.88)	0.108			1.11 (0.79, 1.56)	0.553			1.35 (0.96, 1.90)	0.082			1.03 (0.73, 1.45)	0.882		
divorced/widowed	1.08 (0.70, 1.66)	0.731			0.85 (0.55, 1.31)	0.460			1.32 (0.87, 2.01)	0.195			1.08 (0.70, 1.68)	0.720		
education (R: low)			5.79	0.055			30.11	**<0.001**			9.68	**0.008**			16.48	**<0.001**
middle	1.69 (1.06, 2.71)	0.029			2.19 (1.37, 3.49)	**0.001**			2.06 (1.30, 3.25)	**0.002**			1.85 (1.16, 2.96)	0.010		

(Continued)

TABLE 3 | Continued

	Medication		Psychotherapy		Combined treatment[a]		Alternative treatment[b]	
high	1.84 (1.06, 3.19)	0.029	4.77 (2.73, 8.35)	**<0.001**	1.69 (0.98, 2.89)	0.058	3.10 (1.79, 5.34)	**<0.001**
Vocational qualification (R: none)		0.86 0.930		1.43 0.838		5.30 0.257		7.89 0.096
still undergoing training	1.19 (0.45, 3.19)	0.723	0.56 (0.21, 1.53)	0.260	1.70 (0.64, 4.49)	0.283	3.10 (1.21, 7.94)	0.018
completed apprenticeship	1.33 (0.69, 2.55)	0.391	0.73 (0.37, 1.44)	0.366	2.13 (1.07, 4.23)	0.031	1.50 (0.78, 2.89)	0.225
secondary vocational education	1.32 (0.63, 2.77)	0.464	0.70 (0.33, 1.50)	0.359	2.06 (0.96, 4.41)	0.063	1.41 (0.67, 2.94)	0.362
university degree	1.19 (0.55, 2.56)	0.663	0.75 (0.34, 1.66)	0.475	2.35 (1.05, 5.25)	0.037	1.07 (0.49, 2.32)	0.862
Illness-related variables								
Moderate depression (R: mild)	1.21 (0.88, 1.64)	0.237	1.21 (0.89, 1.66)	0.219	1.03 (0.76, 1.39)	0.839	1.20 (0.89, 1.63)	0.232
Treatment history	0.93 (0.67, 1.27)	0.632	1.08 (0.79, 1.49)	0.619	1.04 (0.77, 1.42)	0.786	0.96 (0.71, 1.31)	0.818
GAD	1.11 (0.77, 1.61)	0.585	1.53 (1.07, 2.18)	0.019	1.20 (0.85, 1.68)	0.302	2.06 (1.47, 2.91)	**<0.001**
PD	1.10 (0.77, 1.56)	0.603	1.34 (0.95, 1.90)	0.095	1.00 (0.71, 1.39)	0.977	1.41 (1.00, 2.00)	0.053
EPAS	1.10 (1.06, 1.14)	**<0.001**	1.12 (1.08, 1.16)	**<0.001**	1.04 (1.00, 1.07)	0.025	1.01 (0.98, 1.05)	0.458
EQ-5D-5L	1.00 (0.99, 1.01)	0.742	1.01 (1.00, 1.02)	0.253	1.00 (0.99, 1.01)	0.945	1.00 (0.99, 1.01)	0.434
N	630		634		626		628	
Pseudo R^2	0.02		0.05		0.02		0.04	

[a]*Combined treatment refers to medication and psychotherapy.*

[b]*Alternative treatment (e.g., alternative practitioners); CI, confidence interval; OR, odds ratio; R, reference category; Wald-Chi², Wald Chi² test for testing the joint significance of categorical indicators; the level of significance was p < 0.01.*

had enough power to obtain reliable results in this age range. Second, data were collected between 2014 and 2015, when internet-based interventions were hardly disseminated in public mental health care in Germany. Despite slow implementation, patients' perspectives on internet-based interventions might have changed since that time due to increasing public relations work. Third, patients were asked to indicate their treatment preferences for internet-based interventions in case of depression. However, the term internet-based interventions comprises many different aspects and may refer to guided or unguided self-management programs, mobile self-help apps, e-mail therapy, videoconference-based counseling or chat groups. In this regard, a scoping review (81) investigating public acceptability and service preferences of e-mental health services in four studies (71, 80, 82, 83) showed that most people from the general population preferred guided over unguided programs. In extension of this research, future research should investigate treatment preferences for e-mental health interventions with regard to different application types (e.g., internet- vs. mobile-based), guidance (guided vs. self-guided), costs (free availability vs. self-payment or reimbursement models) and form of communication (synchronous vs. asynchronous) in clinical populations, e.g., patients with depressive symptoms. Third, the included sociodemographic, illness-related and work-related factors yielded a low prediction of variance. Hence, we possibly missed to gather information about other variables of potential influence. These may include perceived stigma (80, 84, 85), perceived barriers to receive treatment (86) such as living in rural communities (50), vicarious experience with depression (1) and beliefs about etiology of depression (12). Future studies should take these factors into account to gain more knowledge about factors attributing to depression treatment preferences. Moreover, future studies should intensify research on reasons leading to non-preference of e-mental health interventions in primary care patients in order to address potential barriers.

CONCLUSIONS

To our knowledge, this is the first study investigating preferences for a broad range of treatment options including internet-based interventions for depression in primary care patients. Our results underline the importance of active patient involvement in order to find the perfect match between individual patient preferences and existing treatment options for depression. Since the patients' education and self-efficacy seemed to influence preferences for a variety of different treatment options, these factors may be particularly considered by GPs within the process of shared decision-making. In this regard, GPs may point out the positive effects of self-efficacy and empowerment on treatment success and recovery to their patients. To increase the patients' health literacy, patients should be informed thoroughly about the effectiveness and clinical evidence of treatment options for depression, e.g., with the help of information brochures. These should include information about internet-based interventions as patients may only have little knowledge about these new approaches.

Future research may investigate how treatment preferences for innovative treatment options such as internet-based self-management programs affect the adherence to and effectiveness of interventions based on new media.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors upon request.

AUTHOR CONTRIBUTIONS

MD, ML, and SR-H: conceptualized the paper; AP: conducted the statistical analyses; MD: wrote the paper; ML, JS, AP, and SR-H: revised it critically for important intellectual content.

ACKNOWLEDGMENTS

We would like to thank all participating patients and GPs for contributing to this study. The @ktiv trial was partly funded by the Federal Association of AOK (Project number BGAAF-0608) and published in affiliation with the German Federal Ministry of Education and Research (grant number: 01GY1613). We acknowledge support from the German Research Foundation (DFG) and Universität Leipzig within the program of Open Access Publishing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00181/full#supplementary-material

REFERENCES

1. Berkowitz SA, Bell RA, Kravitz RL, Feldman MD. Vicarious experience affects patients' treatment preferences for depression. *PLoS ONE* (2012) **7**:e31269. doi: 10.1371/journal.pone.0031269
2. Gensichen J, Huchzermeier C, Aldenhoff JB, Gerlach FM, Hinze-Selch D. Signals for the initiation of structured diagnostic procedures for depression in primary health care. A practice-relevant evaluation of international guidelines. *Z Ärztl Fortbild Qualitätssich.* (2005) **99**:57–63.
3. Jacobi F, Höfler M, Meister W, Wittchen HU. Prevalence, detection and prescribing behavior in depressive syndromes. A German federal family physician study. *Nervenarzt* (2002) **73**:651–8. doi: 10.1007/s00115-002-1299-y
4. Goldman LS, Nielsen NH, Champion HC. Awareness, diagnosis, and treatment of depression. *J Gen Intern Med.* (1999) **14**:569–80. doi: 10.1046/j.1525-1497.1999.03478.x
5. Linden M, Maier W, Achberger M, Herr R, Helmchen H, Benkert O. Psychiatric diseases and their treatment in general practice in Germany. Results of a World Health Organization (WHO) study. *Nervenarzt* (1996) **67**:205–15.
6. Pincus HA, Davis WW, McQueen LE. 'Subthreshold' mental disorders. *Br J Psychiatry* (1999) **174**:288–96.
7. Churchill R, Khaira M, Gretton V, Chilvers C, Dewey M, Duggan C et al. Treating depression in general practice: factors affecting patients' treatment preferences. *Br J Gen Pract.* (2000) **50**:905–6.

8. Gelhorn HL, Sexton CC, Classi PM. Patient preferences for treatment of major depressive disorder and the impact on health outcomes: a systematic review. *Prim Care Companion CNS Disord.* (2011) **13**:PCC.11r01161. doi: 10.4088/PCC.11r01161
9. DGPPN, BÄK, KBV, AWMF for the guideline group unipolar depression. *S3-Leitlinie/Nationale VersorgungsLeitlinie Unipolare Depression – Langfassung, 2. Auflage. Version 5* (2015). Available online at: http://www.leitlinien.de/mdb/downloads/nvl/depression/depression-2aufl-vers5-lang.pdf (Accessed Augest 10, 2017).
10. Härter M, Loh A, Spies C. *Gemeinsam entscheiden - erfolgreich behandeln. Neue Wege für Ärzte und Patienten im Gesundheitswesen*. Köln: Deutscher Ärzte-Verlag (2005).
11. Elwyn G, Edwards A, Mowle S, Wensing M, Wilkinson C, Kinnersley P et al. Measuring the involvement of patients in shared decision-making: a systematic review of instruments. *Patient Educ Couns.* (2001) **43**:5–22. doi: 10.1016/S0738-3991(00)00149-X
12. Steidtmann D, Manber R, Arnow BA, Klein DN, Markowitz JC, Rothbaum BO et al. Patient treatment preference as a predictor of response and attrition in treatment for chronic depression. *Depress Anxiety* (2012) **29**:896–905. doi: 10.1002/da.21977
13. Johansson R, Nyblom A, Carlbring P, Cuijpers P, Andersson G. Choosing between Internet-based psychodynamic versus cognitive behavioral therapy for depression: a pilot preference study. *BMC Psychiatry* (2013) **13**:268. doi: 10.1186/1471-244X-13-268
14. Schuster R, Berger T, Laireiter A-R. Computer und Psychotherapie – geht das zusammen? *Psychotherapeut* (2017) **75**:e695. doi: 10.1007/s00278-017-0214-8.
15. Kleiboer A, Smit J, Bosmans J, Ruwaard J, Andersson G, Topooco N et al. European COMPARative Effectiveness research on blended Depression treatment versus treatment-as-usual (E-COMPARED): study protocol for a randomized controlled, non-inferiority trial in eight European countries. *Trials* (2016) **17**:387. doi: 10.1186/s13063-016-1511-1
16. Luck-Sikorski C, Stein J, Heilmann K, Maier W, Kaduszkiewicz H, Scherer M et al. Treatment preferences for depression in the elderly. *Int Psychogeriatr.* (2017) **29**:389–98. doi: 10.1017/S1041610216001885
17. Angermeyer MC, Matschinger H, Riedel-Heller SG. Whom to ask for help in case of a mental disorder? Preferences of the lay public. *Soc Psychiatry Psychiatr Epidemiol.* (1999) **34**:202–10. doi: 10.1007/s001270050134
18. Brauns H, Steinmann S. Educational reform in France, West-Germany and the United Kingdom: updating the CASMIN educational classification. *ZUMA Nachrichten* (1999) **23**:7–44.
19. Löwe B, Spitzer R, Zipfel S, Herzog W. *Gesundheitsfragebogen für Patienten (PHQ D). Komplettversion und Kurzform. Testmappe mit Manual, Fragebögen, Schablonen. 2.* Auflage: Pfizer (2002).
20. Spitzer RL, Williams JB, Kroenke K, Linzer M, deGruy FV3, Hahn SR et al. Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MD 1000 study. *JAMA* (1994) **272**:1749–56. doi: 10.1001/jama.1994.03520220043029
21. Kilian R, Becker T, Schleuning G, Welschehold M, Hertle C, Matschinger H et al. *Die Entwicklung eines Standardisierten Verfahrens zur Messung von Empowerment im Prozess der psychiatrischen Behandlung von Patienten mit Schweren Psychischen Erkrankungen*. Ulm: Abschlussbericht Förderkennz. 101GX0743 (2012).
22. Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res.* (2011) **20**:1727–36. doi: 10.1007/s11136-011-9903-x
23. White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med.* (2011) **30**:377–99. doi: 10.1002/sim.4067
24. Cuzick J. A Wilcoxon-type test for trend. *Stat Med.* (1985) **4**:87–90. doi: 10.1002/sim.4780040112
25. Wolfe R, Gould W. An approximate likelihood-ratio test for ordinal response models. *Stata Tech Bull.* (1998) **7**:1–52.
26. Raue PJ, Schulberg HC, Heo M, Klimstra S, Bruce ML. Patients' depression treatment preferences and initiation, adherence, and outcome: a randomized primary care study. *Psychiatr Serv.* (2009) **60**:337–43. doi: 10.1176/ps.2009.60.3.337
27. Van Schaik DJF, Klijn AFJ, van Hout HPJ, van Marwijk HWJ, Beekman ATF, Haan M de et al. Patients' preferences in the treatment of depressive disorder in primary care. *Gen Hosp Psychiatry* (2004) **26**:184–9. doi: 10.1016/j.genhosppsych.2003.12.001
28. Löwe B, Schulz U, Gräfe K, Wilke S. Medical patients' attitudes toward emotional problems and their treatment. What do they really want? *J Gen Intern Med.* (2006) **21**:39–45. doi: 10.1111/j.1525-1497.2005.0266.x
29. Khalsa S-R, McCarthy KS, Sharpless BA, Barrett MS, Barber JP. Beliefs about the causes of depression and treatment preferences. *J Clin Psychol.* (2011) **67**:539–49. doi: 10.1002/jclp.20785
30. Dwight-Johnson M, Sherbourne CD, Liao D, Wells KB. Treatment preferences among depressed primary care patients. *J Gen Intern Med.* (2000) **15**:527–34. doi: 10.1046/j.1525-1497.2000.08035.x
31. Iacoviello BM, McCarthy KS, Barrett MS, Rynn M, Gallop R, Barber JP. Treatment preferences affect the therapeutic alliance: implications for randomized controlled trials. *J Consult Clin Psychol.* (2007) **75**:194–8. doi: 10.1037/0022-006X.75.1.194
32. Mergl R, Henkel V, Allgaier A-K, Kramer D, Hautzinger M, Kohnen R et al. Are treatment preferences relevant in response to serotonergic antidepressants and cognitive-behavioral therapy in depressed primary care patients? Results from a randomized controlled trial including a patients' choice arm. *Psychother Psychosom.* (2011) **80**:39–47. doi: 10.1159/000318772
33. Wittink MN, Cary M, Tenhave T, Baron J, Gallo JJ. Towards patient-centered care for depression: conjoint methods to tailor treatment based on preferences. *Patient* (2010) **3**:145–57. doi: 10.2165/11530660-000000000-00000
34. Houle J, Villaggi B, Beaulieu M-D, Lespérance F, Rondeau G, Lambert J. Treatment preferences in patients with first episode depression. *J Affect Disord.* (2013) **147**:94–100. doi: 10.1016/j.jad.2012.10.016
35. Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: A systematic review and network meta-analysis. *Lancet* (2018) **391**:1357–66. doi: 10.1016/S0140-6736(17)32802-7
36. Berner MM, Kriston L, Sitta P, Härter M. Treatment of depressive symptoms and attitudes towards treatment options in a representative German general population sample. *Int J Psychiatry Clin Pract.* (2008) **12**:5–10. doi: 10.1080/13651500701330783
37. Hegerl U, Althaus D, Stefanek J. Public attitudes towards treatment of depression: effects of an information campaign. *Pharmacopsychiatry* (2003) **36**:288–91. doi: 10.1055/s-2003-45115
38. Highet NJ, Hickie IB, Davenport TA. Monitoring awareness of and attitudes to depression in Australia. *Med J Aust.* (2002) **176**(Suppl. 8):63–8.
39. Gabriel A, Violata C. Depression literacy among patients and the public: a literature review. *Primary Psychiatry* (2010) **17**:55–64.
40. Zuroff DC, Blatt SJ. Vicissitudes of life after the short-term treatment of depression: roles of stress, social support, and personality. *J Soc Clin Psychol.* (2002) **21**:473–96. doi: 10.1521/jscp.21.5.473.22622
41. Nasser EH, Overholser JC. Recovery from major depression: the role of support from family, friends, and spiritual beliefs. *Acta Psychiatr Scand.* (2005) **111**:125–32. doi: 10.1111/j.1600-0447.2004.00423.x
42. Griffiths KM, Crisp DA, Barney L, Reid R. Seeking help for depression from family and friends: a qualitative analysis of perceived advantages and disadvantages. *BMC Psychiatry* (2011) **11**:196. doi: 10.1186/1471-244X-11-196
43. Cooney GM, Dwan K, Greig CA, Lawlor DA, Rimer J, Waugh FR et al. Exercise for depression. *Cochrane Database Syst Rev.* (2013) **9:**1–160. doi: 10.1002/14651858
44. Hallgren M, Kraepelien M, Öjehagen A, Lindefors N, Zeebari Z, Kaldo V et al. Physical exercise and internet-based cognitive-behavioural therapy in the treatment of depression: Randomised controlled trial. *Br J Psychiatry* (2015) **207**:227–34. doi: 10.1192/bjp.bp.114.160101
45. Givens JL, Houston TK, van Voorhees BW, Ford DE, Cooper LA. Ethnicity and preferences for depression treatment. *Gen Hosp Psychiatry* (2007) **29**:182–91. doi: 10.1016/j.genhosppsych.2006.11.002
46. Fernandez y Garcia E, Franks P, Jerant A, Bell RA, Kravitz RL. Depression treatment preferences of Hispanic individuals: exploring the influence of ethnicity, language, and explanatory models. *J Am Board Fam Med.* (2011) **24**:39–50. doi: 10.3122/jabfm.2011.01.100118

47. Rhee TG, Harris IM. Gender differences in the use of complementary and alternative medicine and their association with moderate mental distress in U.S. adults with migraines/severe headaches. *Headache* (2017) **57**:97–108. doi: 10.1111/head.12986
48. McLean S, Kapell B. She reads, he reads: gender differences and learning through self-help books. *RELA* (2015) **6**:55–72. doi: 10.3384/rela.2000-7426.rela0138
49. Boehlen FH, Herzog W, Maatouk I, Saum K-U, Brenner H, Wild B. Treatment preferences of elderly patients with mental disorders. *Z Gerontol Geriatr.* (2016) **49**:120–5. doi: 10.1007/s00391-015-0908-x
50. Kitchen KA, McKibbin CL, Wykes TL, Lee AA, Carrico CP, McConnell KA. Depression treatment among rural older adults: preferences and factors influencing future service use. *Clin Gerontol.* (2013) **36**:1–15. doi: 10.1080/07317115.2013.767872
51. Da Silva PFR, Blay SL. Prevalence and characteristics of outpatient psychotherapy use: a systematic review. *J Nerv Ment Dis.* (2010) **198**:783–9. doi: 10.1097/NMD.0b013e3181f97e1f
52. Reavley NJ, Morgan AJ, Jorm AF. Development of scales to assess mental health literacy relating to recognition of and interventions for depression, anxiety disorders and schizophrenia/psychosis. *Aust N Z J Psychiatry* (2014) **48**:61–9. doi: 10.1177/0004867413491157
53. Dunn KI, Goldney RD, Grande ED, Taylor A. Quantification and examination of depression-related mental health literacy. *J Eval Clin Pract.* (2009) **15**:650–3. doi: 10.1111/j.1365-2753.2008.01067.x
54. Kaneko Y, Motohashi Y. Male gender and low education with poor mental health literacy: a population-based study. *J Epidemiol.* (2007) **17**:114–9. doi: 10.2188/jea.17.114
55. Mackenzie CS, Gekoski WL, Knox VJ. Age, gender, and the underutilization of mental health services: the influence of help-seeking attitudes. *Aging Ment Health* (2006) **10**:574–82. doi: 10.1080/13607860600641200
56. Kessler RC, Demler O, Frank RG, Olfson M, Pincus HA, Walters EE et al. Prevalence and treatment of mental disorders, 1990 to 2003. *N Engl J Med.* (2005) **352**:2515–23. doi: 10.1056/NEJMsa043266
57. Dunlop BW, Kelley ME, Mletzko TC, Velasquez CM, Craighead WE, Mayberg HS. Depression beliefs, treatment preference, and outcomes in a randomized trial for major depressive disorder. *J Psychiatr Res.* (2012) **46**:375–81. doi: 10.1016/j.jpsychires.2011.11.003
58. Christensen H, Griffiths KM, Evans *K. e-Mental Health in Australia: Implications of the Internet and Related Technologies for Policy: ISC Disussion Paper No.3*. Canberra, ACT: Commonwealth Department of Health and Ageing (2002).
59. Karyotaki E, Riper H, Twisk J, Hoogendoorn A, Kleiboer A, Mira A et al. Efficacy of self-guided internet-based cognitive behavioral therapy in the treatment of depressive symptoms: a meta-analysis of individual participant data. *JAMA Psychiatry* (2017) **74**:351–9. doi: 10.1001/jamapsychiatry.2017.0044
60. Sikorski C, Luppa M, Kersting A, König H-H, Riedel-Heller SG. Computer-aided cognitive behavioral therapy for depression. *Psychiatr Prax.* (2011) **38**:61–8. doi: 10.1055/s-0030-1248575
61. Richards D, Richardson T. Computer-based psychological treatments for depression: a systematic review and meta-analysis. *Clin Psychol Rev.* (2012) **32**:329–42. doi: 10.1016/j.cpr.2012.02.004
62. Stein J, Röhr S, Luck T, Löbner M, Riedel-Heller SG. Indication and evidence of internationally developed online coaches as intervention for mental illness - a meta-review. *Psychiatr Prax.* (2017) **45**:7–15. doi: 10.1055/s-0043-117050
63. Rost T, Stein J, Löbner M, Kersting A, Luck-Sikorski C, Riedel-Heller SG. User acceptance of computerized cognitive behavioral therapy for depression: systematic review. *J Med Internet Res.* (2017) **19**:e309. doi: 10.2196/jmir.7662
64. Riedel-Heller SG, Gühne U, Weinmann S, Becker T. Psychosocial therapies in severe mental illness: DGPPN-S3-guideline: evidence, recommendations and challenges for mental health service research. *Psychother Psychosom Med Psychol.* (2012) **62**:425–8. doi: 10.1055/s-0032-1327676
65. Riedel-Heller SG, Gühne U, Weinmann S, Arnold K, Ay E-S, Becker T. Psychosocial interventions in severe mental illness: evidence and recommendations: psychoeducation, social skill training and exercise. *Nervenarzt* (2012) **83**:847–54. doi: 10.1007/s00115-011-3471-8
66. Klein JP, Gerlinger G, Knaevelsrud C, Bohus M, Meisenzahl E, Kersting A et al. Internetbasierte interventionen in der behandlung psychischer storungen: uberblick, qualitatskriterien, perspektiven. *Nervenarzt* (2016) **87**:1185–93. doi: 10.1007/s00115-016-0217-7
67. Dorow M, Stein J, Förster F, Löbner M, Franz M, Günther R et al. Implementation of the internet-based self-management program "moodgym" in patients with depressive disorders in inpatient clinical settings - patient and expert perspectives. *Psychiatr Prax.* (2017). doi: 10.1055/s-0043-117049. [Epub ahead of print].
68. Batterham PJ, Calear AL. Preferences for internet-based mental health interventions in an adult online sample: findings from an online community survey. *JMIR Ment Health* (2017) **4**:e26. doi: 10.2196/mental.7722
69. Statistisches Bundesamt. *IT-Nutzung* (2015). Available online at: https://www.destatis.de/DE/ZahlenFakten/GesellschaftStaat/EinkommenKonsumLebensbedingungen/ITNutzung/ITNutzung.html. (Accessed June 28, 2016).
70. Kruse RL, Koopman RJ, Wakefield BJ, Wakefield DS, Keplinger LE, Canfield SM et al. Internet use by primary care patients: where is the digital divide? *Fam Med.* (2012) **44**:342–7.
71. Eichenberg C, Wolters C, Brähler E. The internet as a mental health advisor in Germany–results of a national survey. *PLoS ONE* (2013) **8**:e79206. doi: 10.1371/journal.pone.0079206
72. Löbner M, Stein J, Rost T, Förster F, Dorow M, Keller J et al. Innovative E-Health-ansätze für komorbide depressionen bei patienten mit adipositas: nutzungsakzeptanz aus patienten- und expertenperspektive. *Psychiatr Prax.* (2017) **44**:286–95. doi: 10.1055/s-0043-107471
73. Crisp DA, Griffiths KM. Participating in online mental health interventions: who is most likely to sign up and why? *Depress Res Treat.* (2014) **2014**:790457. doi: 10.1155/2014/790457
74. Donkin L, Hickie IB, Christensen H, Naismith SL, Neal B, Cockayne NL et al. Sampling bias in an internet treatment trial for depression. *Transl Psychiatry* (2012) **2**:e174. doi: 10.1038/tp.2012.100
75. Warmerdam L, van Straten A, Twisk J, Riper H, Cuijpers P. Internet-based treatment for adults with depressive symptoms: randomized controlled trial. *J Med Internet Res.* (2008) 10:e44. doi: 10.2196/jmir.1094
76. Høifødt RS, Lillevoll KR, Griffiths KM, Wilsgaard T, Eisemann M, Waterloo K et al. The clinical effectiveness of web-based cognitive behavioral therapy with face-to-face therapist support for depressed primary care patients: randomized controlled trial. *J Med Internet Res.* (2013) **15**:e153. doi: 10.2196/jmir.2714
77. Mojtabai R, Olfson M, Mechanic D. Perceived need and help-seeking in adults with mood, anxiety, or substance use disorders. *Arch Gen Psychiatry* (2002) **59**:77–84. doi: 10.1001/archpsyc.59.1.77
78. Halmetoja CO, Malmquist A, Carlbring P, Andersson G. Experiences of internet-delivered cognitive behavior therapy for social anxiety disorder four years later: a qualitative study. *Internet Interv.* (2014) **1**:158–63. doi: 10.1016/j.invent.2014.08.001
79. Wallin EEK, Mattsson S, Olsson EMG. The preference for internet-based psychological interventions by individuals without past or current use of mental health treatment delivered online: a survey study with mixed-methods analysis. *JMIR Ment Health* (2016) **3**:e25. doi: 10.2196/mental.5324
80. Klein B, Cook S. Preferences for e-mental health services amongst an online Australian sample. *E-J Appl Psychol.* (2010) **6**:28–39. doi: 10.7790/ejap.v6i1.184
81. Apolinario-Hagen J, Kemper J, Sturmer C. Public acceptability of E-mental health treatment services for psychological problems: a scoping review. *JMIR Ment Health* (2017) **4**:e10. doi: 10.2196/mental.6186
82. Casey LM, Joy A, Clough BA. The impact of information on attitudes toward e-mental health services. *Cyberpsychol Behav Soc Netw.* (2013) **16**:593–8. doi: 10.1089/cyber.2012.0515
83. Musiat P, Goldstone P, Tarrier N. Understanding the acceptability of e-mental health–attitudes and expectations towards computerised self-help treatments for mental health problems. *BMC Psychiatry* (2014) **14**:109. doi: 10.1186/1471-244X-14-109

84. Schomerus G, Angermeyer MC, Baumeister SE, Stolzenburg S, Link BG, Phelan JC. An online intervention using information on the mental health-mental illness continuum to reduce stigma. *Eur Psychiatry* (2016) **32**:21–7. doi: 10.1016/j.eurpsy.2015.11.006
85. Stolzenburg S, Freitag S, Evans-Lacko S, Muehlan H, Schmidt S, Schomerus G. The stigma of mental illness as a barrier to self labeling as having a mental illness. *J Nerv Ment Dis*. (2017) **205**:903–9. doi: 10.1097/NMD.0000000000000756
86. Casey LM, Wright M-A, Clough BA. Comparison of perceived barriers and treatment preferences associated with internet-based and face-to-face psychological treatment of depression. *Int J Cyber Behav Psychol Learn*. (2014) **4**:16–22. doi: 10.4018/ijcbpl.2014100102

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 22 October 2018
doi: 10.3389/fpsyt.2018.00512

Understanding and Predicting Antidepressant Response: Using Animal Models to Move Toward Precision Psychiatry

David P. Herzog[1,2], *Holger Beckmann*[2,3], *Klaus Lieb*[1,2], *Soojin Ryu*[2,3] *and Marianne B. Müller*[1,2]*

[1] Department of Psychiatry and Psychotherapy, Johannes Gutenberg University Medical Center Mainz, Mainz, Germany,
[2] Focus Program Translational Neurosciences, Johannes Gutenberg University Medical Center Mainz, Mainz, Germany,
[3] German Resilience Center, Johannes Gutenberg University Medical Center Mainz, Mainz, Germany

OPEN ACCESS

Edited by:
Frank M. Schmidt,
Universitätsklinikum Leipzig, Germany

Reviewed by:
Chiara Fabbri,
King's College London, United Kingdom
Casimiro Cabrera Abreu,
Queens University, Canada

***Correspondence:**
Marianne B. Müller
marianne.mueller@uni-mainz.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *30 July 2018*
Accepted: *28 September 2018*
Published: *22 October 2018*

Citation:
Herzog DP, Beckmann H, Lieb K, Ryu S and Müller MB (2018) Understanding and Predicting Antidepressant Response: Using Animal Models to Move Toward Precision Psychiatry. Front. Psychiatry 9:512. doi: 10.3389/fpsyt.2018.00512

There are two important gaps of knowledge in depression treatment, namely the lack of biomarkers predicting response to antidepressants and the limited knowledge of the molecular mechanisms underlying clinical improvement. However, individually tailored treatment strategies and individualized prescription are greatly needed given the huge socio-economic burden of depression, the latency until clinical improvement can be observed and the response variability to a particular compound. Still, individual patient-level antidepressant treatment outcomes are highly unpredictable. In contrast to other therapeutic areas and despite tremendous efforts during the past years, the genomics era so far has failed to provide biological or genetic predictors of clinical utility for routine use in depression treatment. Specifically, we suggest to (1) shift the focus from the group patterns to individual outcomes, (2) use dimensional classifications such as Research Domain Criteria, and (3) envision better planning and improved connections between pre-clinical and clinical studies within translational research units. In contrast to studies in patients, animal models enable both searches for peripheral biosignatures predicting treatment response and in depth-analyses of the neurobiological pathways shaping individual antidepressant response in the brain. While there is a considerable number of animal models available aiming at mimicking disease-like conditions such as those seen in depressive disorder, only a limited number of preclinical or truly translational investigations is dedicated to the issue of heterogeneity seen in response to antidepressant treatment. In this mini-review, we provide an overview on the current state of knowledge and propose a framework for successful translational studies into antidepressant treatment response.

Keywords: animal model, antidepressant, depression, non-response, response, response prediction, translational medicine

INTRODUCTION

Major depressive disorder (MDD) is the second leading cause of disease burden worldwide, thus constituting serious socio-economic threat for modern societies (1, 2). Combined epidemiological and economic data on depression in Europe revealed that it is the most costly brain disorder in Europe with the cost of depression corresponding to 1% of the total economy of Europe (2). There are different approaches and strategies to treat MDD in adults, ranging from pharmacological and psychotherapeutic interventions to transcranial magnetic stimulation, electroconvulsive therapy to deep brain stimulation (3). The "National Institute for Health and Care Excellence (NICE)" guideline recommends antidepressant pharmacotherapy as a crucial pillar in the treatment for all patients with moderate to severe depression (3).

The choice of a particular antidepressant agent for an individual patient currently is based on treatment guidelines, experience, individual medical comorbidities, but unfortunately largely based on "trial and error" (4). Despite decades of research and international efforts to collect cohorts for genetic studies, we still lack a fundamental understanding of the pathophysiology for any of the classical psychiatric disorders, including MDD. In other therapeutic areas such as diabetes or heart diseases (5) a considerable proportion of hits for disease-associated genes in genome-wide association studies (GWAS) match with the targets of already marketed drugs. Precision medicine and individualized therapy has dramatically and successfully improved both our understanding and the treatment of certain somatic diseases. For example, 5-year-survival in children with acute lymphatic leukemia increased from 10% in 1990 to 90% in 2005 (6). Unfortunately, the situation is completely different in neuroscience research, where one conspicuous observation from the genetics of depressive disorders is that none of the scores of candidates from GWAS involves the usual psychopharmacologic suspects, i.e., monoamine transporters or their receptors (4). Importantly, the genetic risk variants identified thus far cover a broad spectrum of biological processes but are enriched in neurodevelopmental or synaptic genes. Taken together, these results point to new pathways involved in pathophysiology, suggesting an entirely new biology for mental disorders and the urgent need to reconsider mental illnesses as "syndromes of disrupted neural, cognitive, and behavioral systems" (7).

But how do we move from genomic variants to better treatments? Before conceptually novel and improved treatment strategies can be envisioned, we urgently need to focus on a more precise understanding of the neurobiological mechanisms underlying mental disorders and individual patient response to pharmacotherapy by appropriate translational approaches. Currently only a limited number of preclinical or truly translational investigations is dedicated to the issue of heterogeneity seen in response to antidepressant treatment.

CURRENT CHALLENGES IN ANTIDEPRESSANT DRUG TREATMENT: TO RESPOND OR NOT TO RESPOND, THAT IS THE QUESTION

The large heterogeneity in response to antidepressant treatment (8) is a major problem in depression treatment. Although the available treatments are safe, both psychiatrists and patients have to cope with a considerable variability in antidepressant treatment outcome: 20–30% of the patients treated with antidepressant drugs fail to respond to two or more pharmacological interventions (9). There are no biomarkers available monitoring treatment response, disease state, or predicting individual response to a particular compound (10). Thus, the most effective antidepressant medication for each patient can presently only be identified through trial and error and needs several weeks to test for each given compound. If early on we could predict that a chosen medication will likely be ineffective for an individual patient, we could dramatically reduce costs and patient suffering and increase treatment efficacy. Therefore, the identification of individual factors predicting treatment response is one of the most pressing needs in depression treatment. Predictive biomarkers or biosignatures would not only allow to predict or monitor treatment response in clinical practice with marketed drugs but could—if compound-independent—also assist in the evaluation of drug actions of novel compounds at an early stage in clinical trials which are frequently marred by late attrition. This is even more important as over the last decades, encouraging preclinical evidence using animal models pointed to innovative pharmacological targets to treat MDD, such as antagonists of the corticotropin releasing hormone receptor type 1 (11) or substance P receptor antagonists (12). These compounds have entered clinical trials with high hopes for a breakthrough in depression treatment, but they have failed to show convincing results. These failures have called into question as to how well traditional animal models for depression can translate to clinical efficacy (13).

Altogether, this illustrates the urgent need to develop improved translational models to better understand the neurobiological mechanisms that underlie MDD and to more specifically assess response to antidepressant treatment. We here review recent progress and highlight some of the best leads to diversify and improve discovery end points for preclinical depression research and treatment response in nonhuman organisms.

ANIMAL EXPERIMENTAL APPROACHES TO MODEL DEPRESSION AND ANTIDEPRESSANT TREATMENT EFFICACY: INDIVIDUALITY MATTERS

Why should we use animals to model complex diseases like MDD? What could be the strengths of an animal model, and what

are its limitations? From a psychiatrist's point of view, it is difficult to agree that rodent or even species such as zebrafish could be of value to investigate a complex mental disorder characterized by a set of diverse symptoms such as MDD. The same holds true for the issue of response to psychopharmacological treatment. A large heterogeneity in the symptomatology of MDD and a close association with other comorbid psychiatric disorders in a substantial proportion of MDD patients are major drawbacks and confounding factors for clinical studies (14). The exclusive use of peripheral tissue like blood can only be of limited value in deciphering the neurobiology of depression, as the brain can only be accessed indirectly, e.g., by neuroimaging approaches (14). In addition, human post-mortem brain samples suffer from many confounding variables like variation in pH, molecule degradation, age bias, and a bias toward suicide victims (14). In contrast, animal models offer unique advantages such as high level of standardization. Working with standardized animal cohorts can help to minimize biases, to deal with larger sample sizes, e.g., when dealing with small, cost-efficient species such as zebrafish and finally, they allow unrestricted access to the organ of interest, i.e., the central nervous system (14–16).

The potency of an animal model can be described based on three key elements: construct, face, and predictive validity (17, 18). *Construct validity* is present in MDD animal models, if depressive-like behavior and associated features can be clearly and unambiguously seen and interpreted in the model (17). The criterion of *face validity* is met if the model possesses similar or comparable elements in terms of "anatomical, biochemical, neuropathological or behavioral features" between animal and human (18). *Predictive validity* focuses on the ability of an animal model to serve as a tool for pharmacological research: Antidepressant agents, which induce antidepressant-like effects in animals, should also show similar or comparable effects in humans (17, 18). Based on these criteria, the strength of an animal model system can be estimated. Behavioral aspects of MDD-related phenotypes as well as behavioral tests to address the effects of antidepressant agents have been characterized within various animal experimental approaches: to model depression-like phenotypes, a number of different strategies has been used, e.g., selective breeding or applying stress during distinct windows of vulnerability of the animal's life to induce long-lasting behavioral and neurobiological changes. For excellent and recent in-depth reviews on animal models of depression-like conditions and more recent attempts to model circuit-based symptomatic dimensions in MDD see (14, 19). Considering the plethora of different attempts to model MDD-like phenotypes in the last decades, concluding that we need to fundamentally re-think animal models for depressive disorders might sound pretentious. However, how else can we overcome the current limitations and advance the field to finally translate basic progress into better care for our patients?

In the context of antidepressant research, the majority of animal models and related publications traditionally analyze and discuss an average effect of treatment or manipulation versus the respective control condition. There is only very limited insight into the question of why so many patients do not show a response, despite the fact that antidepressants have been proven to be effective in general. Unfortunately, the enigma of heterogeneity in antidepressant response has not been systematically addressed to date although it has long being recognized as one of the critical factors hampering antidepressant drug discovery, clinical evaluation, and approval of potentially novel compounds. Therefore to pave the way for so-called precision psychiatry, we would like to propose a framework for translational studies into individualized medicine in psychiatry.

Individuality—commonly defined as the collection of divergent behavioral and physiological traits among individuals—develops when unique environmental influences act on the genome, following complex routes, to produce phenotypic diversity. Individuality is considered central to the development of several neuropsychiatric disorders. Focusing on individuality rather than average outcomes has gained more and more attention both in rodents (20) and in zebrafish (21).

Approaches to focus on heterogeneity and individuality within a cohort of mice have been quite successfully used in the context of stress research to identify putative neurobiological pathways modulating individual susceptibility and resilience: In 2007, the Nestler group published the results of a groundbreaking study, where they did not analyze the mere effect of a certain manipulation (in this case a chronic social defeat stress paradigm), but stratified each individual mouse based on its performance in a defined behavioral test of social interaction as outcome (22). Stratification of the animals based on their performance in the social interaction test allowed to focus on the differences within the experimental group, accompanied by the advantage that the two new "extreme" subgroups (above or below a certain threshold) become more homogeneous (22), which might facilitate the discovery of true candidates. In resilience research, this stratification approach has proven successful in a number of excellent publications during the last years (23–25). Aiming at the identification of the neurobiological mechanisms underlying response to antidepressant treatment, we recently established an animal experimental approach using stratification into extreme subpopulations out of a considerably large number of inbred, genetically homogeneous mice in response to antidepressant treatment [**Figure 1**, (26)]. In addition to the significant average group effect between antidepressant and vehicle-treated groups, we continued to select, out of the cohort of paroxetine-treated animals, subpopulations of good, and poor responders based on their outcome in one of the major behavioral tests assessing antidepressant-like efficacy in rodents. Indeed, we were able to identify specific transcriptome signatures associated with response status in murine blood and to successfully translate and validate the findings from our animal model in a cohort of depressed patients (26). Finally, we could reveal a particular role of the glucocorticoid receptor (GR) in shaping response to antidepressants, which is even more interesting considering that those data have been generated by an animal model using a hypothesis-free approach. The putative role of the GR in modulating antidepressant-like effects had been suggested already earlier by means of hypothesis-driven basic and clinical depression research [for review: (27)], supporting the validity of our model. We believe that this was the first step toward a more in-depth and dimensional analysis of different and

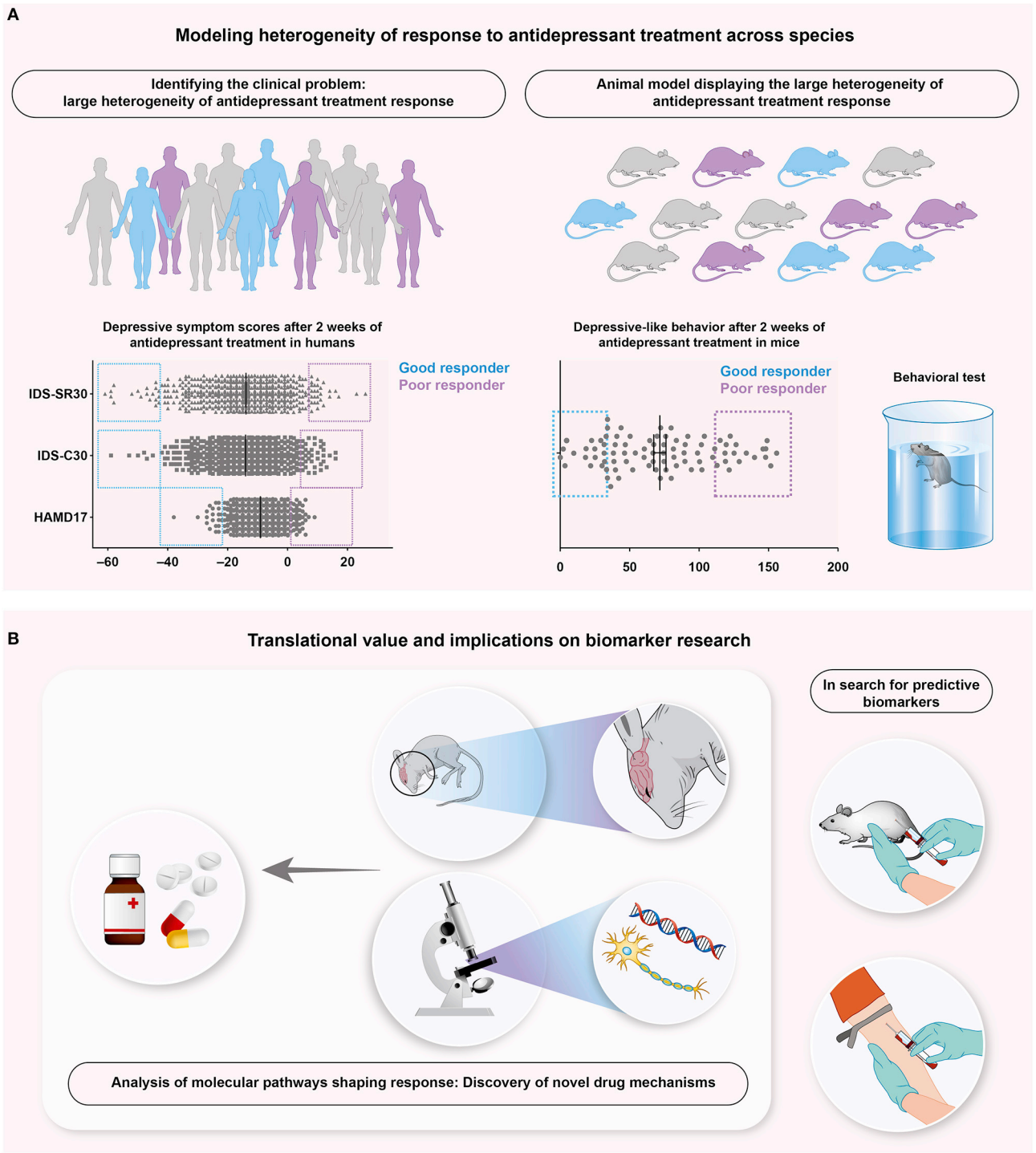

FIGURE 1 | Schematic overview illustrating an example of successful translational research focusing on antidepressant treatment response. **(A)** Treated with antidepressant agents (**A** left), human patients suffering from depression show a reduction of depressive symptomatology, assessed with several depression scores, like Hamilton Depression Rating Scale with 17 questions (HAMD17) and the Inventory of Depressive Symptoms, rated by clinicians (IDS-C30) and patients (IDS-SR30). Although overall benefit from antidepressant agents takes place, individual patients clearly differ from each other. Stratified in good (blue) and poor (purple) responders, this reveals the large heterogeneity of antidepressant treatment response as an important clinical problem. A recently published animal model (26) translates this problem into mice (**A** right), where stratification into good and poor responders of antidepressant treatment is similarly possible. Mice are stratified into responder groups based on the Forced Swim Test, a commonly used test for depressive-like behavior. **(B)** Such animal models offer the key advantages of both biomarker research and analysis of the associated, neurobiological pathways. Blood samples collected from mice and human patients can be aligned and compared in search for predictive biomarker signatures (**B** right). The accessibility of murine central nervous systems provides the possibility to search for the underlying mechanisms that shape antidepressant treatment response, ultimately leading to novel drug targets and mechanisms (**B** left).

more complex behavioral signatures of antidepressant treatment response. Future studies should implement cluster analyses of phenotypic outcomes, e.g., by automated behavioral analysis in the home cage of an animal.

To develop an approach for identifying stratification into different subpopulations out of a large number of responding animals using a low-cost animal model, we recently established an animal experimental paradigm where we analyzed the behavioral responses of a group of zebrafish subjected to stress exposure. As a vertebrate, zebrafish show high homology of the major neuromodulatory circuits involved in stress and emotional regulation. Further they exhibit behavioral phenotypes for identifying "depression-like" indices and are sensitive to different psychotropic drugs (28, 29). However, so far the studies focused on average population effect of drug treatments on behavior and have not carefully considered the heterogeneity and individuality. Our results suggest the existence of a clear stratification in the behavioral outcomes following stress exposure in zebrafish (Beckmann, Cook, and Ryu, unpublished data). Given the fact that zebrafish are cheap to maintain in large numbers and genetic manipulations of their genome are quite easy, they provide a powerful complimentary animal model to rodents for testing heterogeneity of antidepressant responses.

Thus, we propose to consider individual outcomes and meaningful stratification of the experimental group instead of average group effects in animal models of depression and response prediction to improve translation between preclinical research and clinical trials. As shown in recent examples, this strategy could contribute to increased success rate when extrapolating results from the bench to the bedside and back (26, 30).

A PLEA FOR CAREFUL TRANSLATIONAL AND TRANSDIAGNOSTIC RESEARCH IN PSYCHIATRY

It is still a long way to go for personalized medicine and clinically embedded prediction assays for mental illnesses. Current developments neither predict nor monitor disease state nor help with the antidepressant drug choice (31). Huge efforts have been undertaken in the fields of functional neuroimaging, electrophysiology, genetics and gene expression (31), immune mechanisms, neuroendocrine challenge tests such as the combined dexamethasone CRH challenge test, and polysomnography (32). However, we have to admit that despite decades of research, scientists have been unable to find any genetic or neurological evidence to support the breakdown of psychiatric disorders into the diagnostic categories such as provided in the DSM or ICD (33). So far, no cellular or genetic signatures for any mental disorder have been discovered, nor has anyone developed reliable biomarkers, blood tests, or brain scans that match perfectly with a DSM-defined mental illness. Because the focus of the field has been solely on understanding mental disorders as defined by the clusters of symptoms in the DSM, most current treatments have aimed at relieving symptoms rather than resolving the underlying pathology. For example, psychiatrists can reduce hallucinations, but they are not treating schizophrenia. They can relieve symptoms of depression, but that may not be treating the underlying disorder. To overcome these substantial and diagnosis-inherent problems, an ongoing initiative, the Research Domain Criteria (RDoC) initiative from the National Institute of Mental Health (NIMH), proposes behavioral domains, which are shared across several species and in many contexts. Using the RDoC approach, scientists are trying to better understand mental illnesses by focusing on the convergence of biology and behavior and tying different aspects of behavioral, cognitive, and emotional functions to specific brain systems. The research is organized into broad domains, namely positive valence (seeking and appreciating reward), negative valence (threat and loss), cognitive systems, social processes, and arousal and regulatory systems (34).

Focusing on domain-based inclusion criteria for human studies bridges the gap toward animal research by overcoming the artificial, highly heterogeneous, category-based DSM-5 or ICD-10 diagnostic criteria. For a recent excellent review about the integration of RDoC criteria in animal models of psychiatric disorders see (35). Traditionally, animal experimental approaches have always been focusing on core symptoms of mental disorders. Whereas some time ago, the limitation to specific core symptoms has been considered a major drawback of animal models, nowadays and in the context of RDoC, this could now turn out to be an advantage.

Initiatives like RDoC might also solve or at least reduce the reproducibility problem. In a 2006 report, Hackam and colleagues showed that from 76 top-quality animal studies, only 37% could be replicated in humans, 18% were contradicted, and 34% still remained untested in humans (36). The median time of translation from animal to human was 7 years (36). Experiments and studies are usually designed and performed separately for animals and humans, leading to different parameters, different research questions, the involvement of different experimenters, thus increasing confounding variables. These problems might be overcome by a careful and prospectively planned combination of animal and human experiments within the same project, just as proposed by Kurian and colleagues (37) in 2011. Such an approach could shift the focus toward truly translational research projects, bridging the gap between animals and humans. Recent publications with significant impact on the field have shown that this strategy could indeed serve as a template for successful approaches into complex psychiatric diseases: combining data from animal stress models with human data, the Nestler group could reveal sex-specific transcriptome differences in depression (38). Focusing on response to antidepressant treatment, we could identify response-associated transcript profiles in peripheral blood samples of mice, predicting antidepressant treatment response with an accuracy of almost 80% in a patient population (26). Those and other (39, 40) examples of translational studies are encouraging. Importantly, for any translational approach in psychiatry, the research questions originating from the daily clinical situation (i.e., Why does one patient respond to the antidepressant, whereas another does not? What are the neurobiological mechanisms underlying clinical improvement?) need to be first defined and then carefully translated into an

animal experimental approach. To tackle this challenge, a close interaction between clinician scientists from neuropsychiatry and basic researchers, which are dedicated to address clinically relevant questions, is mandatory.

In conclusion, we hope to have convinced the reader that animal models are pivotal in the effort to translate basic progress into better care. Because of practical and ethical limitations to dissecting neurobiological disease mechanisms in humans, continued progress will critically depend on our ability to emulate aspects of depressive symptomatology and treatment response in nonhuman organisms. Still, a significant challenge remains how to effectively align variables measured in animals with those assessed in human studies, i.e., in genetic studies or during the various phases of development of novel antidepressant compounds. This can only be achieved if translation is prospectively planned, allowing for the best possible match of recorded data across species. Translational psychiatry is a two-way bridge: research questions ideally emerge as a well-defined, clinically relevant problem that needs to be carefully translated into the best-possible animal experimental approach. On the other hand, preclinical research needs to inform clinical trials and diagnosis. Recent successful examples in depression research are encouraging and might serve as a template for future approaches into the neurobiology of this devastating and pervasive disorder.

AUTHOR CONTRIBUTIONS

DH and MM wrote the first draft of this paper. DH, HB, KL, SR, and MM contributed to writing and discussing the paper and approved its final version.

ACKNOWLEDGMENTS

DH is supported by the Mainz Research School of Translational Biomedicine (TransMed) with a MD-Ph.D fellowship. MM and KL are supported by the German Research Foundation (DFG) within the Collaborative Research Center 1193 (CRC1193, https://crc1193.de/) and by the Boehringer Ingelheim Foundation. HB and SR are supported by the German Research Foundation (DFG) within the Collaborative Research Center 1193 (CRC1193, https://crc1193.de/). SR is supported by the German Federal Office for Education and Research (BMBF), grant number 01GQ1404.

REFERENCES

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* (2016) 388:1545–602. doi: 10.1016/S0140-6736(16)31678-6
2. Sobocki P, Jonsson B, Angst J, Rehnberg C. Cost of depression in Europe. *J Ment Health Policy Econ.* (2006) 9:87–98.
3. NICE. *Depression in adults: recognition and management - NICE guidelines [CG90]: National Institute for Health and Care Excellence* (2018). Available online at: www.nice.org.uk/guidance/cg90 (Accessed September 24, 2018).
4. Leuchter AF, Cook IA, Hamilton SP, Narr KL, Toga A, Hunter AM, et al. Biomarkers to predict antidepressant response. *Curr Psychiatry Rep.* (2010) 12:553–62. doi: 10.1007/s11920-010-0160-4
5. Collins FS. Reengineering translational science: The time is right. *Sci Transl Med.* (2011) 3:90cm17. doi: 10.1126/scitranslmed.3002747
6. Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. *J Clin Oncol.* (2012) 30:1663–9. doi: 10.1200/JCO.2011.37.8018
7. Insel TR, Cuthbert BN. Medicine. Brain disorders? precisely. *Science* (2015) 348:499–500. doi: 10.1126/science.aab2358
8. Carter GC, Cantrell RA, Victoria Z, Haynes VS, Phillips G, Alatorre CI, et al. Comprehensive review of factors implicated in the heterogeneity of response in depression. *Depress Anxiety* (2012) 29:340–54. doi: 10.1002/da.21918
9. Johnston KM, Powell LC, Anderson IM, Szabo S, Cline S. The burden of treatment-resistant depression: A systematic review of the economic and quality of life literature. *J Affect Disord.* (2019) 242:195–210. doi: 10.1016/j.jad.2018.06.045
10. Labermaier C, Masana M, Muller MB. Biomarkers predicting antidepressant treatment response: how can we advance the field? *Dis Mark.* (2013) 35:23–31. doi: 10.1155/2013/984845
11. Griebel G, Holsboer F. Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning? *Nat Rev Drug Discov.* (2012) 11:462–78. doi: 10.1038/nrd3702
12. Herpfer I, Lieb K. Substance P receptor antagonists in psychiatry: rationale for development and therapeutic potential. *CNS Drugs* (2005) 19:275–93.
13. Belzung C. Innovative drugs to treat depression: Did animal models fail to be predictive or did clinical trials fail to detect effects? *Neuropsychopharmacology* (2014) 39:1041–51. doi: 10.1038/npp.2013.342
14. Wang Q, Timberlake MA, 2nd, Prall K, Dwivedi Y. The recent progress in animal models of depression. *Progress Neuro Psychopharmacol Biol Psychiatry* (2017) 77:99–109. doi: 10.1016/j.pnpbp.2017.04.008
15. Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature* (2008) 455:894–902. doi: 10.1038/nature07455
16. Krishnan V, Nestler EJ. Animal models of depression: Molecular perspectives. *Curr Top Behav Neurosci.* (2011) 7:121–47. doi: 10.1007/7854_2010_108
17. Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: Focus on anxiety disorders and depression. *Biol Mood Anxiety Disord.* (2011) 1:9. doi: 10.1186/2045-5380-1-9
18. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci.* (2010) 13:1161–9. doi: 10.1038/nn.2647
19. Berton O, Hahn CG, Thase ME. Are we getting closer to valid translational models for major depression? *Science* (2012) 338:75–9. doi: 10.1126/science.1222940
20. Freund J, Brandmaier AM, Lewejohann L, Kirste I, Kritzler M, Kruger A, et al. Emergence of individuality in genetically identical mice. *Science* (2013) 340:756–9. doi: 10.1126/science.1235294
21. Pantoja C, Hoagland A, Carroll EC, Karalis V, Conner A, Isacoff EY. Neuromodulatory Regulation of Behavioral Individuality in Zebrafish. *Neuron* (2016) 91:587–601. doi: 10.1016/j.neuron.2016.06.016
22. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* (2007) 131:391–404. doi: 10.1016/j.cell.2007.09.018
23. Bagot RC, Cates HM, Purushothaman I, Lorsch ZS, Walker DM, Wang J, et al. Circuit-wide transcriptional profiling reveals brain region-specific gene networks regulating depression susceptibility. *Neuron* (2016) 90:969–83. doi: 10.1016/j.neuron.2016.04.015
24. Bagot RC, Cates HM, Purushothaman I, Vialou V, Heller EA, Yieh L, et al. Ketamine and imipramine reverse transcriptional signatures of susceptibility and induce resilience-specific gene expression profiles. *Biol Psychiatry* (2017) 81:285–95. doi: 10.1016/j.biopsych.2016.06.012

25. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. *Nature* (2013) 493:532–6. doi: 10.1038/nature11713
26. Carrillo-Roa T, Labermaier C, Weber P, Herzog DP, Lareau C, Santarelli S, et al. Common genes associated with antidepressant response in mouse and man identify key role of glucocorticoid receptor sensitivity. *PLoS Biol.* (2017) 15:e2002690. doi: 10.1371/journal.pbio.2002690
27. Holsboer F, Barden N. Antidepressants and hypothalamic-pituitary-adrenocortical regulation. *Endocr Rev.* (1996) 17:187–205. doi: 10.1210/edrv-17-2-187
28. Fonseka TM, Wen XY, Foster JA, Kennedy SH. Zebrafish models of major depressive disorders. *J Neurosci Res.* (2016) 94:3–14. doi: 10.1002/jnr.23639
29. Stewart AM, Ullmann JF, Norton WH, Parker MO, Brennan CH, Gerlai R, et al. Molecular psychiatry of zebrafish. *Mol Psychiatry* (2015) 20:2–17. doi: 10.1038/mp.2014.128
30. Lopez JP, Fiori LM, Cruceanu C, Lin R, Labonte B, Cates HM, et al. MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. *Nat Commun.* (2017) 8:15497. doi: 10.1038/ncomms15497
31. Frodl T. Recent advances in predicting responses to antidepressant treatment. *F1000Res* (2017) 6:F1000. doi: 10.12688/f1000research.10300.1
32. Thase ME. Using biomarkers to predict treatment response in major depressive disorder: Evidence from past and present studies. *Dialog Clin Neurosci.* (2014) 16:539–44.
33. Casey BJ, Craddock N, Cuthbert BN, Hyman SE, Lee FS, Ressler KJ. DSM-5 and RDoC: Progress in psychiatry research? *Nature Reviews Neuroscience* (2013) 14:810–4. doi: 10.1038/nrn3621
34. NIMH. *RDoC Matrix: National Institute of Mental Health* (2018) (Accessed September 24, 2018). Available online at: https://www.nimh.nih.gov/research-priorities/rdoc/constructs/rdoc-matrix.shtml.
35. Anderzhanova E, Kirmeier T, Wotjak CT. Animal models in psychiatric research: The RDoC system as a new framework for endophenotype-oriented translational neuroscience. *Neurobiol Stress* (2017) 7:47–56. doi: 10.1016/j.ynstr.2017.03.003
36. Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. *JAMA* (2006) 296:1731–2. doi: 10.1001/jama.296.14.1731
37. Kurian SM, Le-Niculescu H, Patel SD, Bertram D, Davis J, Dike C, et al. Identification of blood biomarkers for psychosis using convergent functional genomics. *Mol Psychiatry* (2011) 16:37–58. doi: 10.1038/mp.2009.117
38. Labonte B, Engmann O, Purushothaman I, Menard C, Wang J, Tan C, et al. Sex-specific transcriptional signatures in human depression. *Nat Med.* (2017) 23:1102–11. doi: 10.1038/nm.4386
39. Kaufman J, Wymbs NF, Montalvo-Ortiz JL, Orr C, Albaugh MD, Althoff R, et al. Methylation in OTX2 and related genes, maltreatment, and depression in children. *Neuropsychopharmacology* (2018) 43:2204–11. doi: 10.1038/s41386-018-0157-y
40. Pena CJ, Kronman HG, Walker DM, Cates HM, Bagot RC, Purushothaman I, et al. Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. *Science* (2017) 356:1185–8. doi: 10.1126/science.aan4491

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 26 October 2018
doi: 10.3389/fpsyt.2018.00511

BDNF Plasma Levels and BDNF Exon IV Promoter Methylation as Predictors for Antidepressant Treatment Response

Klaus Lieb[1†], Nadine Dreimüller[1†], Stefanie Wagner[1], Konrad Schlicht[1‡], Tanja Falter[2], Alexandra Neyazi[3], Linda Müller-Engling[3], Stefan Bleich[3], André Tadić[1‡] and Helge Frieling[3†]*

[1] Department of Psychiatry and Psychotherapy, University Medical Centre, Mainz, Germany, [2] Institute of Clinical Chemistry and Laboratory Medicine, University Medical Centre, Mainz, Germany, [3] Molecular Neuroscience Laboratory, Department of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School (MHH), Hanover, Germany

OPEN ACCESS

Edited by:
Frank M. Schmidt,
Universitätsklinikum Leipzig, Germany

Reviewed by:
Marie Woelfer,
Universitätsklinikum Magdeburg, Germany
Matthew James Knight,
University of Adelaide, Australia

***Correspondence:**
Klaus Lieb
klaus.lieb@unimedizin-mainz.de

[†]These authors have contributed equally to the work

[‡]**Present Address:**
Konrad Schlicht,
Department of Neurology, University Medical Centre, Mainz, Germany
André Tadić,
Clinic for Psychiatry and Psychotherapy, Agaplesion Elisabethstift, Darmstadt, Germany

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: 30 July 2018
Accepted: 28 September 2018
Published: 26 October 2018

Citation:
Lieb K, Dreimüller N, Wagner S, Schlicht K, Falter T, Neyazi A, Müller-Engling L, Bleich S, Tadić A and Frieling H (2018) BDNF Plasma Levels and BDNF Exon IV Promoter Methylation as Predictors for Antidepressant Treatment Response. Front. Psychiatry 9:511. doi: 10.3389/fpsyt.2018.00511

Major problems of current antidepressant pharmacotherapy are insufficient response rates and difficulties in response prediction. We recently provided preliminary evidence in a small study that patients with major depressive disorder (MDD) with a hypomethylation of the CpG-87 site of the promoter IV region of the brain-derived neurotrophic factor (BDNF) gene are less likely to benefit from antidepressants. Here, we aimed at replicating this finding in a secondary analysis of 561 MDD patients (mean age 40.0 ± 11.9 years, 56% female) included into the Early Medication Change study (EMC). We measured BDNF exon IV promoter and p11 gene methylation at Baseline (BL) as well as BDNF-plasma-levels (pBDNF) at BL and day 14 and related them to treatment outcome. Although we were not able to replicate the predictor function of hypomethylation of the BDNF exon IV promoter, a subgroup of patients with severe depression (Hamilton Depression Rating Scale [HAMD-17] $\geq$ 25) ($n = 199$) and hypermethylation at CpG-87 of the BDNF exon IV promoter had significantly higher remission rates than patients without a methylation ($p = 0.032$). We also found that 421 (75%) of 561 patients showed an early improvement ($\geq$ 20% HAMD-17 reduction after 2 weeks), which was associated with a 4.24-fold increased likelihood to remit at study end compared to the 140 patients without early improvement. However, specificity of response prediction of early improvement was low (34%) and false positive rate high (66%). The combination of early improvement with a pBDNF increase between BL and day 14, however, increased the specificity of response prediction from 34 to 76%, and the combination with methylation of the CpG-87 site of the BDNF exon IV promoter from 34 to 62%. Thus, the combined markers reduced false positives rates from 66 to 24% and 38%, respectively. Methylation at other sites or p11 promoter methylation failed to increase specificity of early improvement prediction. In sum, the results add to previous findings that BDNF, BDNF promoter methylation and the

combination of clinical and biological markers may be interesting candidates for therapy response prediction which has to be confirmed in further studies.

Clinical Trial Registration: https://clinicaltrials.gov/ct2/show/NCT00974155, identifier: NCT00974155

Keywords: BDNF-promoter methylation, BDNF plasma level, MDD, early improvement, response prediction

INTRODUCTION

Antidepressant pharmacotherapy with monoaminergic drugs leads to insufficient responses in up to two-third of patients with major depressive disorder (MDD) and this is a key problem in the treatment of patients since therapy failure is normally determined only after several weeks of unsuccessful treatment (1–4). This long period until determination of treatment response asks for early clinical or biological markers to predict later treatment response in patients with MDD.

In recent years, evidence has accumulated that a combination of clinical markers with biomarkers such as blood immune markers, theta-cordance, executive test performance may improve treatment prediction (5–7). An especially promising candidate for a biological marker is brain-derived neurotrophic factor (BDNF) (7–11). Several lines of evidence have linked BDNF with both the pathophysiology of depression and the mode of action of antidepressants (12–14). Studies have shown in rodents that antidepressants including ketamine and electroconvulsive therapy (ECT) increased BDNF levels in cortex and hippocampus (15) and that BDNF protein infusion in hippocampal areas led to antidepressant-like effects (12). Furthermore, animal models of depression showed that antidepressant-like responses were dependent on BDNF/TrkB signaling (12, 16, 17). An important role of BDNF for antidepressant response was also shown in knockout studies or by pharmaceutical inhibition of BDNF which both prevented the efficacy of a variety of different therapeutic antidepressant approaches including non-pharmacological treatments such as sleep deprivation and ECT (14).

Clinical improvement and antidepressant therapy are not only related to BDNF in the brain but also to an increase of BDNF in human blood (18). Accordingly, peripheral BDNF levels can serve as a biomarker for the successful treatment of depression (10) and are relevant markers for the state of MDD (19). In a recent meta-analysis, a significant interaction between serum/plasma BDNF and antidepressant therapy was found, showing an increase of peripheral BDNF in patients treated with antidepressants (20).

Other studies demonstrated that antidepressant treatment increased central BDNF levels in animals (12) as well as peripheral BDNF levels in humans (10, 20–22). However, these finding are inconsistent, at least in humans, as other studies have shown decreases of peripheral BDNF levels during the course of treatment as well (23), no change at all (24) or differences between antidepressants (25). An especially interesting biological marker for therapy response prediction may be BDNF exon IV promoter methylation which has gained high interest in recent years, as it was shown that especially this promoter site controls BDNF expression (26). Also P11 (also known as S100A10) is an interesting candidate. It is a member of the S100 gene family that acts as an adaptor protein and is critically involved in amplification of serotonergic signaling and the regulation of gene transcription (27). In a mouse model of MDD as well as in MDD patients, P11 is down regulated and levels rise by administration of SSRIs or electroconvulsive therapy. Antidepressant effects of BDNF and ketamine have been shown to be mediated by P11 (28–30) and BDNF induces p11 by signaling using the ERK pathway (31).

In previous studies, we have used several BDNF related biomarkers to predict treatment outcome in MDD patients. We found that non-remission by antidepressant pharmacotherapy was predicted by hypomethylation of a specific CpG site (m87) in the BDNF exon IV promoter (11), and we obtained similar findings for BDNF exon I methylation and response to ECT treatment (32). In additional small studies, we described that non-response and non-remission were predicted by a non-increase of BDNF in serum (33) or plasma (7) during the first week of antidepressant treatment. This suggests that changes in peripheral BDNF during the early course of treatment may constitute or reflect a necessary prerequisite for a later treatment response.

A promising clinical marker for treatment response prediction is an early improvement of depressive symptoms, mostly defined as a $\geq$20% reduction in sum scores of depressive rating scales between baseline and day 14 (34, 35). In a recent meta-analysis including data from 14,799 patients, we showed that patients with an early improvement of depressive symptomatology after 2 weeks of antidepressant treatment had an 8-/6.5-fold increased likelihood to become responder/remitter at treatment end as compared to non-improver (36). However, although early improvement shows a high sensitivity, it has only a low specificity (true negative rate) meaning that many early improvers become later non-responders (37%) or non-remitters (67%) (36). The low specificity of the prediction of early improvement, therefore, asks for additional markers (e.g., biological markers), which could be combined with the early improvement marker to improve the specificity of prediction of treatment outcome.

In the current study, we used a large sample of patients with MDD ($n = 561$) to replicate our previous findings of a predictive role of BDNF exon IV promoter hypomethylation (11) and early peripheral BDNF changes (7, 33) for remission of MDD. As it is well-established that treatment outcome is depending on the degree of depression severity with antidepressants being

particularly efficient in patients with severe to very severe depression (37), we repeated all analyses for the subgroup of patients with at least a severe symptomatology ($N = 199$). Furthermore, we used our sample to analyze whether the combined marker of early improvement and BDNF-related markers increases the specificity of response prediction, as previous studies showed that adding of biological markers to early improvement can improve response prediction (5). Studies investigating the predictive power of combined markers found encouraging results (38). We were especially interested to see whether the following markers predict later remission or increase the specificity of therapy response prediction by early symptomatic improvement: (i) methylation status at BDNF promoter exon IV CpG-87 at baseline, as well as (ii) promoter methylation status of the multifunctional protein p11 (S100Δ10), (iii) BDNF plasma levels (pBDNF) at baseline, (iv) change of plasma BDNF levels from baseline to week 2.

MATERIALS AND METHODS

Patients

This study is a secondary scientific investigation in 561 patients with MDD who had participated between 2009 and 2014 in the "Randomized clinical trial comparing an early medication change (EMC) strategy with treatment as usual (TAU) in patients with Major Depressive Disorders (MDD)—The EMC Trial (ClinicalTrials.gov identifier n°: NCT00974155)" and who had agreed to biomarker sampling. The EMC trial was carried out in accordance with the recommendations of consort guidelines, ethics committee at the Landesärztekammer Rheinland-Pfalz. The protocol was approved by the ethics committee at the Landesärztekammer Rheinland-Pfalz, Germany. All subjects gave written informed consent in accordance with the Declaration of Helsinki. Clinical and demographical data of the 561 patients are given in **Table 1**. None of these subjects had participated in our previous pilot studies (7, 11, 39). **Table 1** also gives demographic data for the 199 patients who suffered from a severe MDD (defined as Hamilton Rating Scale for Depression; HAMD-17 $\geq$ 25) and who were analyzed separately as a subgroup. Details of the protocol have been described previously (39–42). In short, the EMC study was a multi-center, randomized, observer-blinded, controlled clinical trial investigating whether non-improver after 14 days of an antidepressant treatment with escitalopram are more likely to remit (HAMD-17 $\leq$ 7) after 8 weeks of treatment with an early medication change (EMC: immediate change to venlafaxine followed by an augmentation with lithium after non-response at day 28) compared to patients treated according to current guideline recommendations (TAU: continuing escitalopram for 2 weeks followed by venlafaxine in the case of non-response). Key inclusion criteria of the EMC trial were: (1) Major Depressive Disorder (MDD), first episode or recurrent, according to DSM-IV; (2) a HAMD-17 score of $\geq$ 18 points at screening; (3) age 18–65 and $\leq$ 60 years at the time of the first depressive episode. Minimal exclusion criteria were used to maximize generalizability. Patients with (1) a primary diagnosis of bipolar, psychotic, obsessive-compulsive, eating disorder or substance dependence (if it required inpatient detoxification); (2) female patients who were pregnant or breast-feeding; (3) patients with general medical conditions contraindicating the use of any protocol medication, or (4) a clear history of non-response or intolerance in the current MDD episode to any protocol antidepressant were excluded from the study.

TABLE 1 | Clinical and demographic characteristics of patients at treatment initiation.

Characteristic	All patients ($n = 561$)	Severe depressed patients ($n = 199$)
SOCIODEMOGRAPHIC DOMAIN		
Age—years (± SD)	40.0 ± 11.9	41.2 ± 11.2
Sex		
Female—*n* (%)	315 (56%)	118 (59%)
Male—*n* (%)	246 (44%)	81 (41%)
Ethnicity		
Caucasian—*n* (%)	546 (97%)	191 (96%)
CLINICAL DOMAIN		
Age at onset—years (± SD)	32.2 ± 12.2	32.2 ± 11.9
Course of depression		
1st episode—*n* (%)	192 (34%)	65 (33%)
recurrent—*n* (%)	369 (66%)	134 (67%)
Previous episodes—*n* (± SD)	4.1 ± 5.6	4.1 ± 6.0
Duration of index major depressive episode—weeks (± SD)	34.0 ± 59.4	31.8 ± 38.6
HAMD-17 sum score at baseline	24.1 ± 4.1	28.0 ± 2.8

HAMD, Hamilton Depression Rating Scale; SD, standard deviation; n, number.

Study Procedures

At screening visit, the diagnosis was verified by a structured interview: M.I.N.I. International Neuropsychiatric Interview (43), according to DSM-IV, Axis II Disorders by the Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II) (44). Demographic parameters (age, sex, ethnicity) and psychiatric history (number of preceding depressive episodes, length of index episode, age at onset, clinical course) were assessed relying on patients' self-reports (41, 42). The severity of depressive symptomatology was assessed weekly by the Hamilton Depression Rating Scale: HAMD-17 (45) by blinded and specially trained raters (46). Blood samples were also obtained weekly as previously described (39). All blood samples were taken between 08:00 and 12:00 h in the morning and placed within a time frame of 30 min on ice after collection. In this study, BDNF plasma levels (pBDNF) were measured at baseline, week 2, and methylation status of BDNF exon IV promoter and p11 promoter at baseline.

BDNF Exon IV Promoter and p11 Promoter Methylation Analysis

Genomic DNA was isolated from 200 μL frozen human venous blood using the NucleoMag® Blood 200 μL Kit (Macherey &

Nagel, Dueren) on a Biomek® NXP Laboratory Automation Workstation (Beckman Coulter, Brea, CA). Afterwards, 500 ng of genomic DNA were modified by sodium-bisulfite using the EpiTect® 96 Bisulfite Kit (QIAGEN).

DNA was amplified through (semi-) nested touch-down PCR. Primer sets for amplification of BDNF and p11 promoter region (Metabion GmbH, Steinkirchen, Germany) are listed in **Supplementary Table 1**. All PCRs were performed on a C1000™ Thermal Cycler (BioRad, Hercules CA, USA). Subsequently 10 µl of each PCR product were visualized on a standard 2.0% agarose gel and the remaining 40 µl were purified using Agencourt® AMPure®XP magnetic beads on a BioMek NX^P liquid handling system (Beckman Coulter) and subsequently sequenced using the reverse primer via by BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 3,500 × l Genetic Analyzer (Applied Biosystems). Sequences and electropherograms were analyzed via the specialized Epigenetic Sequencing Methylation (ESME) analysis software (47) and the percentage methylation of each CpG site within the amplified region was estimated by the ratio between peak values of Cytosine (C) and Thymine (T) (C/T).

Measurement of BDNF Plasma Levels

Whole blood was obtained in a lithium-heparin tube from baseline to day 56 in weekly intervals. After a maximum time of 30 min, whole blood was centrifuged at 1,000 × g at 4°C to separate plasma. Plasma was then pipetted into Eppendorf tubes; these were centrifuged at 10,000 × g and at 4°C. Plasma was pipetted in small Eppendorf tubes again, then kept at −80°C. Plasma BDNF concentration was assessed with a commercially available kit (Quantikine ELISA, Human free BDNF Immunoassay, R&D Systems Europe) according to the instructions of the manufacturer. All plasma samples were double-analyzed with the Human free BDNF Immunoassay; an internal control sample was continuously measured with each microtiter plate. Inter-assay coefficient of variation in our sample was 11.2% and intra-assay coefficient of variation was 6.3%.

Predictors and Outcome Parameter

As clinical predictor for remission (defined as a HAMD-17 sum score < 7 at endpoint) we used the occurrence of early improvement, defined as a ≥20% reduction in sum score of the HAMD-17 between baseline and day 14. Dropouts before day 28 were counted as non-remitters; dropouts after day 28 were counted as remitters or non-remitters according to the last HAMD-17 sum score. We furthermore assessed three sets of molecular markers and used them as predictors of remission at endpoint and combined them with the clinical marker early improvement to enhance specificity of response prediction: BDNF exon IV promoter methylation (at CpG03m87, 01m147, 02m111, 04m66, 05m58, 06m39, 07m35, 08m24, 10p18) and p11 methylation (at position 38, 44, 78, 112, 114, 128, 207, 211, 216, 244, 254, 256, 260, 314) at baseline, as well as BDNF plasma levels (at baseline and after 2 weeks) and change of BDNF plasma levels (between baseline and week 2). To assess the value of therapy response prediction by a combination of early improvement and BDNF markers, it was important to measure the biomarkers exactly at the same time, i.e., at baseline and after 2 weeks.

Statistical Analysis

Differences in the number of early improver/non-improver, and remitter/non-remitter with or without methylation at BDNF exon IV promoter were analyzed by Chi^2-tests. Correlation analyses were used to assess the effect of potential covariates like age, gender or education on pBDNF, BDNF exon IV promoter or P11 methylation. Significant covariates were included in all further analyses. Regarding BDNF exon IV promoter methylation status, the analyses focused on the dichotomous markers, due to our research question of therapy response "with" or "without" methylation. The methylation status (methylated/not methylated) could only be analyzed at 5 sites (CpG03m87, CpG04m66, CpG07m35, CpG08m24, CpG10p18), since the number of patients without a methylation was too small at the other CpG sites ($N \leq 8$).

Differences in mean P11 promoter methylation levels at baseline and mean pBDNF levels (at baseline, change from BL to day 14) were analyzed by *t*-tests for independent variables. Differences in the change of BDNF from BL to day 14 between patients with or without methylation were analyzed by *t*-test for independent variables.

Logistic regression analyses with remission as outcome and the molecular markers (BDNF exon IV promoter methylation, P11 promoter methylation, pBDNF) as predictor were used to investigate the association between molecular markers and treatment outcome.

To assess the predictive value of early improvement and the molecular markers on treatment outcome, sensitivity, specificity, positive (PPV) and negative predictive values (NPV) as well as the Odd's ratios (OR) (95%-Confidence interval) were calculated. A sensitivity or specificity lower than 45% is estimated as low, a value between 45 and 70% as moderate, 71–90% as high and a sensitivity and specificity >90% as very high (48).

To further assess a possible predicting role of a combined molecular and clinical marker of early symptomatic improvement, we analyzed a combined marker consisting of the molecular markers plus an early improvement of the depressive symptomatology after 2 weeks of treatment for prediction of remission at study end. For the molecular marker, BDNF methylation was dichotomized (methylated/not methylated). The combined marker consists of four subgroups, i.e., early improver with methylation, early improver without methylation, early non-improver with methylation and early non-improver without methylation. The different components were weighed equally and improvement was included in a single calculation. Regarding pBDNF and p11 methylation levels at baseline, a median split was used to dichotomize the markers (below median, above median). Regarding a pBDNF change from baseline to day 14, patients with a pBDNF increase were compared to those with a decrease. Differences between patients with a pBDNF increase or decrease in the number of early improver/non-improver or remitter/non-remitter were calculated by Chi^2-tests. Again, sensitivity, specificity, positive (PPV) and negative predictive values (NPV) as well as the Odd's

ratios (95%-CI) were calculated. All analyses report the whole sample first, then those with severe MDD. All analyses were done using SPSS 23.0. Significance was set at $p \leq 0.050$.

RESULTS

BDNF Markers in Patients According to Clinical Course of Treatment

BDNF Exon IV Promoter Methylation

No gender differences or effect of age in methylation status were found in our sample ($p = 0.287$). Patient groups with or without a methylation at BDNF promoter exon IV did not differ in the frequency of remitter ($p = 0.703$) after 8 weeks of antidepressant treatment at any of the investigated sites (**Figure 1A**). The logistic regression analysis with remission as dependent variable and the methylation status (dichotomic) at the investigated sites as criterion revealed no association between methylation status and treatment outcome for any site ($p = 0.084$). As shown in **Table 2A**, the methylation status at BDNF promoter exon IV predicted later remission with low to moderate sensitivity and specificity.

In the 199 patients with a severe MDD ($HAMD_{17} \geq 25$), we found that significantly more patients with methylation at CpG-87 were remitter at endpoint (CpG-87: Chi^2: 4.678, $df = 3$; $p = 0.031$, OR = 2.96) than patients without methylation (**Figure 1B**). Logistic regression analysis also revealed that the methylation status at BDNF promoter exon IV at site CpG-87 was significantly associated to remission of the depressive symptomatology ($R^2 = 4.5$, ß = 0.866; $p = 0.029$). Additionally, patients with methylation at CpG-87 had a 2.96 higher likelihood to become remitter than patients without methylation, increasing the specificity from 42% in the entire group to 74% (**Tables 2A,B**).

P11 Promoter Methylation

The p11 promoter methylation levels did not differ between early improver and non-improver ($p = 0.108$) and remitter and non-remitter ($p = 0.155$). The logistic regression analyses also showed no association between the mean p11 promoter methylation and remission at endpoint ($p = 0.055$). The p11 methylation rate predicted later remission with high to very high sensitivity, but low to very low specificity. No differences were seen in the subgroup of severely depressed patients (data shown in **Supplementary Table 2**).

BDNF Plasma Levels

Mean (±SD) pBDNF level at baseline was 62 ± 886 pg/ml (range 0.4–3,952), there were no gender differences neither at baseline ($p = 0.751$) nor at week 2 ($p = 0.656$). BDNF plasma levels at baseline did neither differ between early improver and non-improver ($p = 0.313$) nor between remitter and non-remitter ($p = 0.294$). Mean change of pBDNF between BL and week 2 was −277 ± 828 pg/ml (range −5208 to 96) and between BL and week 8 −94.62 ± 745 pg/ml (range −3,330 to 3,119). Early improver and non-improver as well as remitter and non-remitter also did not differ in the change of pBDNF between baseline and day 14 ($p = 0.117$). Additionally, patients with or without methylation at CpG-87 did not differ in the change of pBDNF between BL and day 14 in the total group (methylated: −50.8 ± 840.6; unmethylated: −61.1 ± 907.1; $p = 0.906$) as well as in severely ill patients (methylated: 1.7 ± 798.3; unmethylated: −108.0 ± 829.7; $p = 0.410$). The number of patients with pBDNF increase was also not different in remitters and non-remitters ($p = 0.922$) (**Figure 1C**) as well as in improvers and

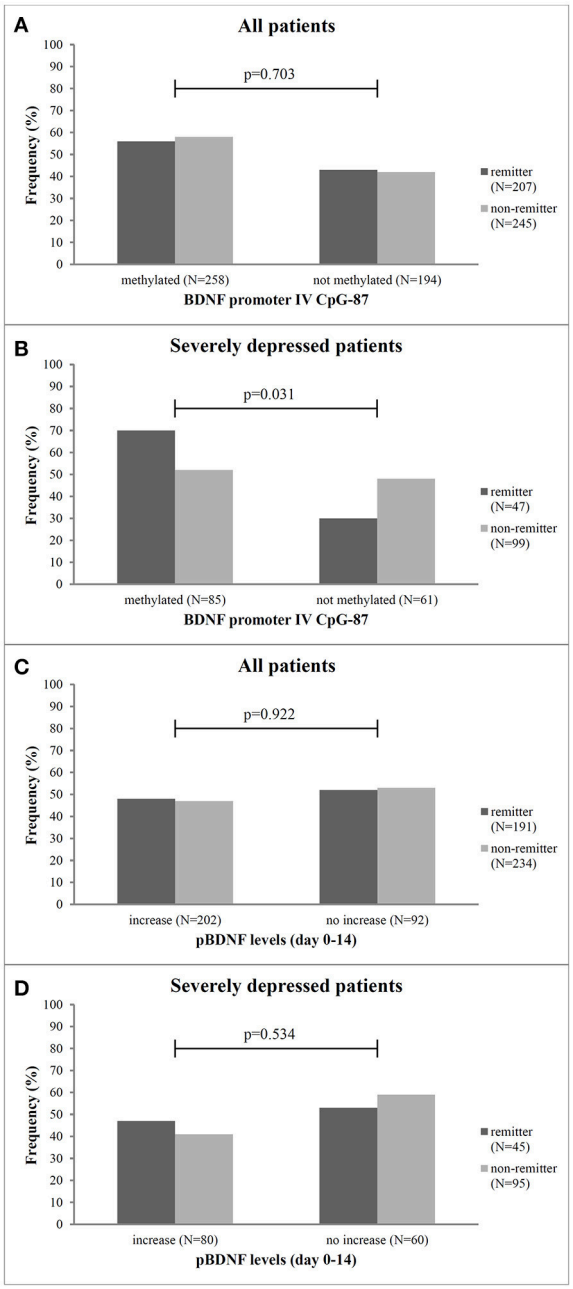

FIGURE 1 | Frequency of remitter and non-remitter in relation to methylation at CpG-87 and change of pBDNF levels between baseline and day 14 for all patients (**A,C**, respectively) and the subgroup of severely depressed patients (**B,D**, respectively). N, number, p, Chi^2-test.

TABLE 2 | Sensitivity and specificity of early improvement and its combination with BDNF markers for prediction of remission in all patients with MDD **(A)** and in the subgroup of severely depressed patients **(B)**.

(A)					
ALL patients	**Sensitivity (95%-CI)**	**Specificity (95%-CI)**	**PPV (95%-CI)**	**NPV (95%-CI)**	**Odd's ratio (95%-CI)**
OUTCOME: REMISSION AT ENDPOINT					
BDNF promoter exon IV methylation					
CpG-87	56 (52–60)	42 (38–46)	45 (41–49)	53 (49–57)	0.92 (0–2)
CpG-66	77 (73–80)	22 (0–2)	45 (41–49)	53 (49–57)	0.94 (0–2)
CpG-35	72 (68–76)	23 (20–27)	44 (40–48)	49 (45–53)	0.95 (0–2)
CpG-24	52 (48–56)	40 (36–44)	42 (38–46)	50 (46–54)	0.73 (0–2)
CpG-18	38 (34–42)	57 (53–61)	43 (39–47)	52 (48–56)	0.82 (0–2)
Combined marker early improvement plus BDNF promoter exon IV methylation					
CpG-87	50 (42–58)	62 (54–70)	54 (46–62)	58 (50–66)	1.62 (0–4)
CpG-66	97 (95–98)	12 (9–15)	52 (48–56)	78 (74–81)	3.87 (2–6)
CpG-35	71 (67–75)	25 (22–29)	50 (46–54)	44 (40–48)	0.80 (0–2)
CpG-24	46 (42–50)	51 (47–55)	47 (43–51)	50 (46–54)	0.88 (0–2)
CpG-18	37 (33–41)	57 (53–61)	49 (45–53)	46 (42–50)	0.79 (0–2)
Plasma BDNF					
Baseline	47 (43–51)	56 (52–60)	48(44–52)	55 (51–59)	1.12 (0–2)
Δ BL - day 14	48 (44–52)	53 (49–57)	46 (42–50)	56 (52–60)	1.06 (0–2)
Combined marker early improvement plus plasma BDNF					
Baseline	86 (83–89)	36 (32–40)	55 (51–59)	75 (71–79)	3.52 (2–5)
Δ BL - day 14	55 (51–59)	76 (72–80)	88 (85–91)	33 (29–37)	3.78 (2–6)
Early Improvement baseline—day 14					
	89 (86–92)	34 (30–38)	48 (44–52)	82 (79–85)	4.24 (3–6)

(B)					
Severe depressed	**Sensitivity (95%-CI)**	**Specificity (95%-CI)**	**PPV (95%-CI)**	**NPV (95%-CI)**	**Odd's ratio (95%-CI)**
OUTCOME: REMISSION AT ENDPOINT					
BDNF promoter exon IV methylation					
CpG-87	71(64–78)	74 (67–81)	40 (32–48)	77 (70–84)	2.96 (0–6)
CpG-66	33 (25–41)	30 (23–38)	29 (22–36)	34 (26–42)	0.21 (0–1)
CpG-35	67 (59–75)	24 (17–31)	29 (22–36)	61 (53–69)	0.65 (0–2)
CpG-24	33(25–41)	32 (24–40)	31 (38–46)	34 (23–39)	0.24 (0–1)
CpG-18	40 (32–48)	51 (43–59)	27 (20–34)	64 (56–72)	0.69 (0–2)
Combined marker early improvement plus BDNF promoter exon IV methylation					
CpG-87	60 (52–68)	63 (55–71)	45 (37–53)	77 (69–84)	2.63 (0–5)
CpG-66	80 (73–87)	40 (32–48)	38 (30–46)	81 (75–87)	2.64 (0–5)
CpG-35	57 (59–65)	41 (37–47)	32 (24–40)	67 (59–74	0.95 (0–3)
CpG-24	43 (35–51)	53 (45–61)	30 (26–38)	65 (57–73)	0.82 (0–2)
CpG-18	32 (24–40	64 (56–71)	29 (25–37)	66 (58–74)	0.82 (0–2)
Plasma BDNF					
Baseline	55 (47–63)	61 (53–69)	52 (44–60)	70 (63–78)	1.94 (0–4)
Δ BL - day 14	57 (49–65)	58 (49–66)	35 (27–42)	77 (70–84)	1.82 (0–4)
Combined marker early improvement plus plasma BDNF					
Baseline	72 (65–79)	32 (24–40)	48 (40–56)	64 (56–72)	1.42 (0–3)
Δ BL - day 14	44 (36–52)	72 (65–79)	44 (36–52)	71 (64–79)	1.96 (0–4)
Early Improvement baseline—day 14					
	87 (82–93)	23 (16–30)	35 (27–43)	77 (70–84)	2.07 (0–4)

95%-CI: 95% Confidence Interval; BDNF: brain derived neurotropic factor; BL, baseline; PPV, positive predictive value; NPV, negative predictive value.

non-improvers ($p = 0.335$). This was also true for the group of severely depressed patients ($p = 0.534$) (**Figure 1D**) for pBDNF increase).

The logistic regression analysis with the single markers pBDNF at baseline or the change of pBDNF from baseline to day 14 as predictors for remission as outcome showed no significant association between pBDNF and treatment outcomes. pBDNF at baseline or an early change of pBDNF from baseline to day 14 predicted remission with moderate sensitivity or specificity in all patients (**Table 2A**) and in severely depressed patients as well (**Table 2B**).

Early Improver and Non-improver and Their Relationship to Later Remission

Four hundred and twenty-one (75%) of the 561 MDD patients showed an early improvement after 2 weeks of therapy compared to 140 patients showing no early improvement (**Figure 2A**). Two hundred and two (48%) patients with an early improvement were remitter at endpoint. However, 219 (52%) early improver were non-remitter at the end of the study. Of the 140 early non-improver, 25 (18%) were remitter whereas 115 (82%) became non-remitter at day 56. Early improver had a 4.24-fold higher likelihood to become remitter than early non-improver. As shown in **Figure 2B**, 79% ($n = 157$) of the 199 patients with a severe MDD were improver after 2 weeks of treatment. 30.6% (48 patients) of the improver became remitter at the end of the study, whereas 69.4% (109 patients) became non-remitter. Of the 42 non-improver of treatment (21%), 14.3% achieved remission at the end of the study and 85.7% (36 patients) were non-remitter. Among the severe depressed patients the early improvers had a 2.07-fold likelihood to become remitter.

Combination of BDNF Markers and Early Improvement to Predict Remission at Endpoint

As shown in **Table 2A**, the combined marker of early improvement and methylation status at CpG-87 decreased the sensitivity (89–50), but increased the specificity of treatment prediction from 34 to 62%. The combined marker also led to an increase of Odd's ratios (BDNF exon IV promoter methylation as single marker OR: 0.92, combined with early improvement OR: 1.62). By combining early improvement with the methylation status at the other sites, sensitivity of prediction of remission was mostly high for the combined marker, but specificity was low, indicating that the combined marker at the other sites appears to be less predictive than the single marker "methylation status" (**Table 2A**). Patients with an early improvement and a methylated CpG-87 site at baseline more often became remitter ($Chi^2 = 22.6$, $df = 3$, OR $= 1.62$; $p = 0.001$) after 8 weeks of antidepressant treatment as compared to all other patients (**Figure 3A**). Specificity of remission prediction was improved from 34 to 76% by the combined marker of pBDNF increase plus early improvement (OR: 3.78) (**Table 2A**). Patients with an early improvement and a pBDNF increase from baseline to day 14 more often became remitter at endpoint than patients without this marker ($Chi^2 = 43.1$, $df = 3$, $p = 0.001$) (**Figure 3C**). All

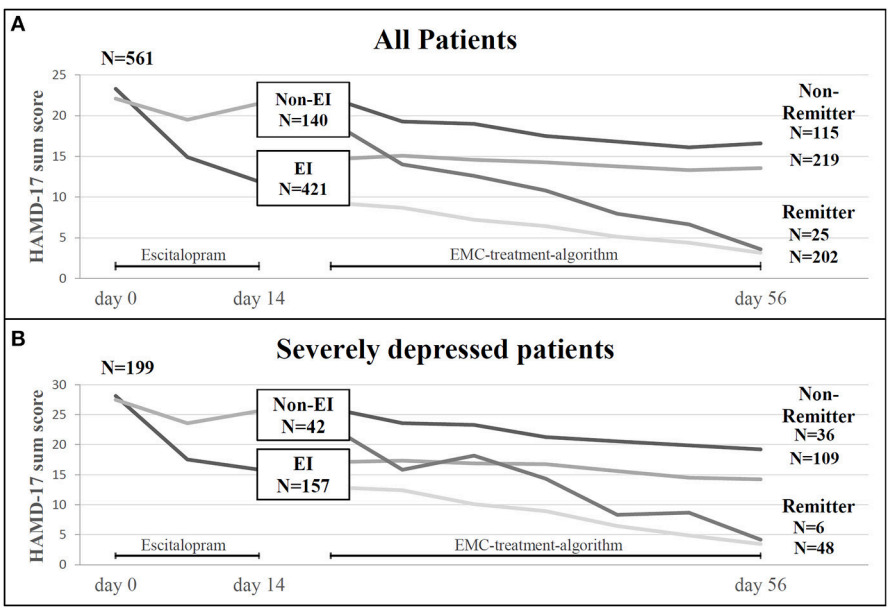

FIGURE 2 | (A) Treatment courses in 561 patients with MDD included in the study (ITT sample): Number of patients experiencing an early improvement (EI) or early non-improvement (Non-EI) after 2 weeks of antidepressant treatment and number of patients with remission or non-remission after 8 weeks of treatment in relation to EI and Non-EI-status. **(B)** Treatment courses in 199 patients with severe MDD (HAMD-17 ≥25) included in the study: Number of patients experiencing an early improvement (EI) or early non-improvement (Non-EI) after 2 weeks of antidepressant treatment and number of patients with remission or non-remission after 8 weeks of treatment in relation to EI and Non-EI-status. EI, early improvement; EMC, early medication change; HAMD-17, Hamilton Depression rating Scale; N, number.

other combined markers showed no difference in the number of remitters.

Severely depressed patients with an early improvement and a methylated CpG-87 site at baseline more often became remitter ($p = 0.02$) after 8 weeks of antidepressant treatment as compared to the other severely depressed patients (**Figure 3B**). In these patients the combined marker of early improvement and methylation status at CpG-87 decreased the sensitivity (87–60%), but increased the specificity of treatment prediction from 23 to 63% ($Chi^2 = 6.9$; $df = 3$; OR = 2.631; CI = 1.181–7.404; $p = 0.031$); resulting in a slightly lower OR as the single marker BDNF promoter methylation (OR: 2.96).

Specifity of remission prediction was improved from 23 to 72% by the combined marker of pBDNF increase plus early improvement ($Chi^2 = 8.9$; $df = 3$; OR = 1.964; CI = 0.3175–4.213; $p = 0.030$) (**Figure 3D** and **Table 2B**).

DISCUSSION

Our results partially replicate and extent our previous findings regarding a role of BDNF exon IV promoter methylation for treatment response prediction in patients with MDD (11). We found in a group of patients with severe depression that BDNF exon IV CpG-87 methylation was associated with higher remission rates, meaning that patients with a methylation at BDNF exon IV CpG-87 more often became remitter than patients without a methylation at BDNF exon IV CpG-87. However, in the total group of patients methylation status at BDNF exon IV CpG-87 was not associated with remission at endpoint. In the total EMC study sample, BDNF exon IV CpG-87 hypomethylation was only associated with later non-remission if the biological marker was used in combination with the clinical marker early improvement.

Our data are not in line with our previous pilot study, in which we had found that all MDD patients—not only the severely depressed ones—who showed a hypomethylation at the CpG-87 site of the exon IV promoter region of the BDNF gene were less likely to benefit from the therapy with antidepressants (11). Although we do not know the cause of this difference, one possible explanation might be that in the current study only escitalopram or venlafaxine were study medications whereas in our pilot study all kinds of antidepressants without any restrictions were allowed.

In line with our findings, however, are results from one small study which examined the influence of 8 weeks of antidepressant treatment with citalopram on histone H3 lysine 27 trimethylation (H3K27me3) levels at promoter-IV of the BDNF gene and BDNF expression in severely depressed patients (49). This study showed increased BDNF mRNA levels in responders and significantly reduced H3K27me3 levels (a marker for silencing genes) at BDNF exon IV promoter, which showed a negative correlation with change in depression severity. In sum, this study and our findings support the assumption that epigenetic modifications play an important role for the therapeutic action of antidepressants and may even be a prerequisite for the onset of antidepressant action (9).

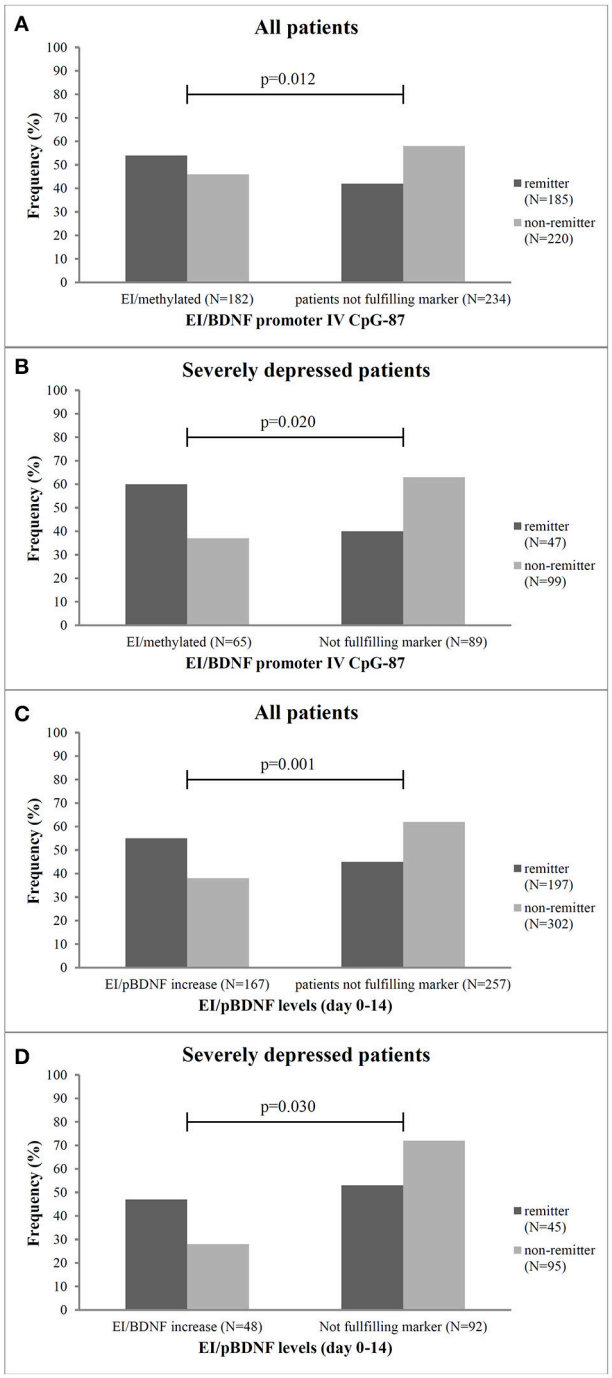

FIGURE 3 | Frequency of remitter and non-remitter in relation to the combined marker of early improvement and methylation status at the CpG-87 site and pBDNF increase from baseline to week 2 for all patients (**A,C**, respectively) and the subgroup of severely depressed patients (**B,D**, respectively). EI, early improver; N, number; p, Chi^2-test, patients not fulfilling marker, neither early improvement nor BDNF-methylation or pBDNF increase.

Contradictory to our findings are also other studies which found differences in the methylation status of the promoter in depressed individuals suffering from suicidal behaviors (50)

and which showed that the response to classical antidepressant treatment could be predicted by classifying patients into "high-" and "low-methylated" individuals, i.e., individuals with low DNA methylation at BDNF P4 showed a greater reduction in suicidal ideation (51). A possible explanation for this difference might be the investigation of different regions of the BDNF promoter and the focus on depressed patients with and without suicidality.

It is unclear why we were only able to show the predictive role of BDNF exon IV promoter methylation for antidepressant response in the subgroup of severely depressed patients. Although depression severity is one important factor associated with the response to antidepressants, with patients with more severe depression showing a higher likelihood to become responders (37), it is unclear how depression severity interacts with epigenetic modifications of the BDNF promoter to modulate treatment response. One explanation for the different response prediction patterns between severely and less severely depressed patients could lie in the fact that more severely affected patients show less placebo response rates (52) and that drug effects in those patients are "true" drug effects based on neurobiological underpinnings as described here. Fitting to this hypothesis is the observation that non-pharmacological approaches to the treatment of MDD such as transcranial direct current stimulation (tDCS) did not find associations between BDNF and treatment response (53). Further studies should investigate differences in response prediction between severely and less severely affected patients, e.g., by adding other biomarkers (e.g., inflammatory markers), epigenetic modification of genes other than BDNF (e.g., MAOA-gene) or other epigenetic alterations of BDNF (histone methylation) which were not included in the current analyses.

Although this was not a mechanistic study, our data are in line with robust neurobiological findings, connecting the response to antidepressants to the capability of the drugs to increase BDNF expression. Monoaminergic drugs can increase BDNF expression, not only via the well-described pathway of cAMP response binding protein (54), activated via 30,50-cyclic adenosine monophosphate (cAMP), but also "via phosphorylation of methyl-CpG-binding protein 2 (MeCP2) which—in its unphosphorylated form—binds to the promoter and forms a repressor complex, but dissociates from the DNA upon phosphorylation" (55). Only if the promoter is methylated, the specific MeCP2 binding can occur, meaning that only in carriers of this methylation at the CpG site of the promoter this antidepressant-induced activation of BDNF can occur. This neurobiological mechanism may explain why severely depressed patients in our study with an unmethylated BDNF promoter site were unlikely to remit with continued treatment.

Early improvement, defined as a decrease of depression severity of $\geq$20% in the first 2 weeks of treatment, is one of the best investigated and most reliable predictors for response to antidepressant treatment (35, 36). The sensitivity (true positive rate) of early improvement on remission was high in our study (i.e., 89 out of 100 remitter were early improver at day 14; sensitivity: 89%), but the specificity (true negative rate) was low (i.e., only 34 out of 100 non-remitter showed a non-improvement at day 14 or in other words 66 out of 100 early improver became non-remitter after 8 weeks of treatment and were false positives). This result is in line with previous studies (22, 23) and highlights that further markers are needed in order to improve the specificity of response prediction. By combining early improvement with the methylation status at CpG-87, we found that the specificity of response prediction increased (from 34 to 62%), i.e., only 38 out of 100 patients with an early improvement and a methylation at CpG-87 site became non-remitter at the end of the study. Thus, false positives were significantly reduced by use of the combined marker (from 66 to 38 out of 100).

In severely depressed patients we found that the combined marker of early improvement and methylation status at CpG-87 only slightly decreased the prediction of later treatment outcome (decrease in sensitivity from 74 to 63%; decrease in sensitivity from 71 to 60%). This suggests that the single molecular marker seems to be particularly relevant/useful for response prediction in severely depressed patients and that for this group of patients the combined clinical and molecular marker has no advantage. These results need to be replicated and further studies should investigate possible neurobiological underpinnings of this finding before concrete conclusions can be drawn for the clinical significance of the results.

Our results are in line with our earlier findings in a rather small sample of patients with MDD (7, 33). In 39 patients with MDD, the combination of the early improvement signal with an increase in plasma or serum BDNF between baseline and day 7 increased the specificity of response prediction up to 100%. In the present study, the specificity of response prediction raised from 34 to 76% by combining the early improvement signal with an increase of BDNF between baseline and day 14, meaning that only 24 out of 100 patients with an early improvement and pBDNF increase between baseline and day 14 became non-remitter at the end of the study. Thus, false positives were significantly reduced by the use of the combined marker from 66 to 24 out of 100). In severely depressed patients, the combined marker of pBDNF increase and early improvement increased the specificity of treatment prediction to a similar extent. Our data are in contrast to a recently published study, which showed no evidence for a better prediction of response by a combined marker of pBDNF-increase and early improvement as compared to early improvement alone, which might be due to the very small sample size of 21 depressed patients (6).

Several limitations have to be kept in mind when interpreting the results of this study. First, the study is a secondary investigation and is not powered to this research question and did not use corrections for multiple comparisons. Therefore, our results should be interpreted carefully and should be verified in larger prospective samples. Second, we did not control for a possible influence of smoking behavior, as smoking has been shown to alter BDNF levels (56, 57). A third limitation is that blood samples for BDNF measurement were taken between 08:00 and 12:00 a.m. whereas a recent study showed that there are

distinct fluctuations of pBDNF both in men and women over the day with an individual peak time unrelated to the clock time (58–60). A further limitation comprises the open delivery of treatment and the lack of a placebo control. Raters for the assessment of the efficacy outcomes, however, were blinded to group assignment and protocol medication.

The combination of clinical markers such as early improvement with molecular markers of the BDNF gene to predict treatment response is a new and innovative approach. If the predictive power of the combined markers can be replicated in further studies, this opens new avenues for the treatment of patients with MDD: A simple blood test at the initiation of antidepressant treatment and a test result within 2 weeks combined with the clinical marker of early improvement could guide physicians to change and/or optimize antidepressant therapy in patients who have a very low likelihood to respond. Further prospective and well-powered studies have to be designed to evaluate the efficacy of new treatment strategies in patients with such a very low likelihood of therapy response after 2 weeks of treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

KL, ND, SW, AT, and HF: study concept and design. KL, ND, SW, KS, TF, AN, LM-E, SB, AT, and HF: acquisition, analysis, or interpretation of the data. KL, ND, SW, AT, and HF: drafting of the manuscript.

FUNDING

The EMC trial (ClinicalTrials.gov Identifier: NCT00974155) was supported by Grant no. 01KG0906 from the German Federal Ministry for Education and Research (BMBF).

ACKNOWLEDGMENTS

We are grateful to all patients who contributed to this study and to our clinical study members for their work. This includes especially Stefan Elsner, MD (Hospital for Psychiatry and Psychotherapy, Andernach), Julia Reiff, MD (Hospital for Psychiatry and Psychotherapy, Wiesbaden), Joachim Röschke, MD, Ph.D. (Hospitals for Psychiatry and Psychotherapy, Bad Soden and Kiedrich), Ömür Baskaya, MD, Markus Lorscheider, MD, and Alexander Teml, MD (University Medical Center Mainz, Department of Psychiatry and Psychotherapy). We are also grateful to Armin Szegedi and Rolf Meinert (both formerly affiliated to University Medical Center Mainz), who participated in an early study phase of design development as well as to the members of the Data and Safety Monitoring Board (Walter Lehmacher, Institute for Medical Statistics, Informatics and Epidemiology, University of Cologne; Ulrich Hegerl, Department of Psychiatry and Psychotherapy, University of Leipzig; Ekkehard Haen, Department of Psychiatry and Psychotherapy, University of Regensburg). We thank Annette Shrestha in the Institute of Clinical Chemistry and Laboratory Medicine, University Medical Centre, Mainz, for performing assay analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00511/full#supplementary-material

REFERENCES

1. NICE: Depression in Adults: Recognition and Management: Clinical Guideline [CG90] (2009).
2. APA: Practice guideline for the treatment of patients with major depressive disorder (revision). American Psychiatric Association. *Am J Psychiatry* (2010) 157(Suppl. 4):1–45 Available online at: https://psychiatryonline.org/pb/assets/raw/sitewide/practice_guidelines/guidelines/mdd.pdf
3. Bauer M, Pfennig A, Severus E, Whybrow PC, Angst J, Moller HJ, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of unipolar depressive disorders, part 1: update 2013 on the acute and continuation treatment of unipolar depressive disorders. *World J Biol Psychiatry* (2013) 14:334–85. doi: 10.3109/15622975.2013.804195
4. Qaseem A, Barry MJ, Kansagara D, P. clinical guidelines committee of the american college of: nonpharmacologic versus pharmacologic treatment of adult patients with major depressive disorder: a clinical practice guideline from the american college of physicians. *Ann Intern Med*. (2016) 164:350–9. doi: 10.7326/M15-2570
5. Bares M, Novak T, Kopecek M, Brunovsky M, Stopkova P, Hoschl C. The effectiveness of prefrontal theta cordance and early reduction of depressive symptoms in the prediction of antidepressant treatment outcome in patients with resistant depression: analysis of naturalistic data. *Eur Arch Psychiatry Clin Neurosci*. (2015) 265:73–82. doi: 10.1007/s00406-014-0506-8
6. Bares M, Novak T, Brunovsky M, Kopecek M, Hoschl C. The comparison of effectiveness of various potential predictors of response to treatment with SSRIs in patients with depressive disorder. *J Nerv Ment Dis*. (2017) 205:618–26. doi: 10.1097/nmd.0000000000000574
7. Dreimuller N, Schlicht KF, Wagner S, Peetz D, Borysenko L, Hiemke C, et al. Early reactions of brain-derived neurotrophic factor in plasma (pBDNF) and outcome to acute antidepressant treatment in patients with major depression. *Neuropharmacology* (2012) 62:264–9. doi: 10.1016/j.neuropharm.2011.07.017
8. Fabbri C, Hosak L, Mossner R, Giegling I, Mandelli L, Bellivier F, et al. Consensus paper of the WFSBP task force on genetics: genetics, epigenetics and gene expression markers of major depressive disorder and antidepressant response. *World J Biol Psychiatry* (2017) 18:5–28. doi: 10.1080/15622975.2016.1208843
9. Lisoway AJ, Zai CC, Tiwari AK, Kennedy JL. DNA methylation and clinical response to antidepressant medication in major depressive disorder: a review and recommendations. *Neurosci Lett*. (2018) 669:14–23. doi: 10.1016/j.neulet.2016.12.071
10. Polyakova M, Stuke K, Schuemberg K, Mueller K, Schoenknecht P, Schroeter M. L. BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. *J Affect Disord*. (2015) 174:432–40. doi: 10.1016/j.jad.2014.11.044
11. Tadic A, Muller-Engling L, Schlicht KF, Kotsiari A, Dreimuller N, Kleimann A, et al. Methylation of the promoter of brain-derived neurotrophic factor exon

IV and antidepressant response in major depression. *Mol Psychiatry* (2014) 19:281–3. doi: 10.1038/mp.2013.58
12. Bjorkholm C, Monteggia LM. BDNF - a key transducer of antidepressant effects. *Neuropharmacology* (2016) 102:72–9. doi: 10.1016/j.neuropharm.2015.10.034
13. Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature* (2008) 455:894–902. doi: 10.1038/nature07455
14. van Calker D, Serchov T, Normann C, Biber K. Recent insights into antidepressant therapy: distinct pathways and potential common mechanisms in the treatment of depressive syndromes. *Neurosci Biobehav Rev*. (2018) 88:63–72. doi: 10.1016/j.neubiorev.2018.03.014
15. Lepack AE, Fuchikami M, Dwyer JM, Banasr M, Duman RS. BDNF release is required for the behavioral actions of ketamine. *Int J Neuropsychopharmacol*. (2014) 18:pyu033. doi: 10.1093/ijnp/pyu033
16. Castren E. Neurotrophins and psychiatric disorders. *Handb Exp Pharmacol*. (2014) 220:461–79. doi: 10.1007/978-3-642-45106-5_17
17. Castren E, Kojima M. Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. *Neurobiol Dis*. (2017) 97:119–26. doi: 10.1016/j.nbd.2016.07.010
18. Brunoni AR, Lopes M, Fregni F. A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*. (2008) 11:1169–80. doi: 10.1017/S1461145708009309
19. Kishi T, Yoshimura R, Ikuta T, Iwata N. Brain-Derived neurotrophic factor and major depressive disorder: evidence from meta-analyses. *Front Psychiatry* (2017). 8:308. doi: 10.3389/fpsyt.2017.00308
20. Zhou C, Zhong J, Zou B, Fang L, Chen J, Deng X, et al. Meta-analyses of comparative efficacy of antidepressant medications on peripheral BDNF concentration in patients with depression. *PLoS ONE* (2017) 12:e0172270. doi: 10.1371/journal.pone.0172270
21. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry* (2008) 64:527–32. doi: 10.1016/j.biopsych.2008.05.005
22. Molendijk ML, Spinhoven P, Polak M, Bus BA, Penninx BW, Elzinga BM. Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol Psychiatry* (2014) 19:791–800. doi: 10.1038/mp.2013.105
23. Buttenschon HN, Foldager L, Elfving B, Poulsen PH, Uher R, Mors O. Neurotrophic factors in depression in response to treatment. *J Affect Disord*. (2015) 183:287–94. doi: 10.1016/j.jad.2015.05.027
24. Chiou YJ, Huang TL. Serum brain-derived neurotrophic factors in Taiwanese patients with drug-naive first-episode major depressive disorder: effects of antidepressants. *Int J Neuropsychopharmacol*. (2017) 20:213–8. doi: 10.1093/ijnp/pyw096
25. Matrisciano F, Bonaccorso S, Ricciardi A, Scaccianoce S, Panaccione I, Wang L, et al. Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxine. *J Psychiatr Res*. (2009) 43:247–54. doi: 10.1016/j.jpsychires.2008.03.014
26. Zheleznyakova GY, Cao H, Schioth HB. BDNF DNA methylation changes as a biomarker of psychiatric disorders: literature review and open access database analysis. *Behav Brain Funct*. (2016) 12:17. doi: 10.1186/s12993-016-0101-4
27. Svenningsson P, Kim Y, Warner-Schmidt J, Oh YS, Greengard P. p11 and its role in depression and therapeutic responses to antidepressants. *Nat Rev Neurosci*. (2013) 14:673–80. doi: 10.1038/nrn3564
28. Park SW, Nhu le H, Cho HY, Seo MK, Lee CH, Ly NN, et al. p11 mediates the BDNF-protective effects in dendritic outgrowth and spine formation in B27-deprived primary hippocampal cells. *J Affect Disord*. (2016) 196:1–10. doi: 10.1016/j.jad.2016.02.010
29. Sun HL, Zhou ZQ, Zhang GF, Yang C, Wang XM, Shen JC, et al. Role of hippocampal p11 in the sustained antidepressant effect of ketamine in the chronic unpredictable mild stress model. *Transl Psychiatry* (2016) 6:e741. doi: 10.1038/tp.2016.21
30. Warner-Schmidt JL, Chen EY, Zhang X, Marshall JJ, Morozov A, Svenningsson P, et al. A role for p11 in the antidepressant action of brain-derived neurotrophic factor. *Biol Psychiatry* (2010) 68:528–35. doi: 10.1016/j.biopsych.2010.04.029
31. Ma Z, Zang T, Birnbaum SG, Wang Z, Johnson JE, Zhang CL, et al. TrkB dependent adult hippocampal progenitor differentiation mediates sustained ketamine antidepressant response. *Nat Commun*. (2017) 8:1668. doi: 10.1038/s41467-017-01709-8
32. Kleimann A, Kotsiari A, Sperling W, Groschl M, Heberlein A, Kahl KG, et al. BDNF serum levels and promoter methylation of BDNF exon I, IV and VI in depressed patients receiving electroconvulsive therapy. *J Neural Transm*. (2015) 122:925–8. doi: 10.1007/s00702-014-1336-6
33. Tadic A, Wagner S, Schlicht KF, Peetz D, Borysenko L, Dreimuller N, et al. The early non-increase of serum BDNF predicts failure of antidepressant treatment in patients with major depression: a pilot study. *Prog Neuropsychopharmacol Biol Psychiatry* (2011) 35:415–20. doi: 10.1016/j.pnpbp.2010.08.011
34. Stassen HH, Angst J, Hell D, Scharfetter C, Szegedi A. Is there a common resilience mechanism underlying antidepressant drug response? evidence from 2848 patients. *J Clin Psychiatry* (2007) 68:1195–205.
35. Szegedi A, Jansen WT, van Willigenburg AP, van der Meulen E., Stassen HH, Thase M. E. Early improvement in the first 2 weeks as a predictor of treatment outcome in patients with major depressive disorder: a meta-analysis including 6562 patients. *J Clin Psychiatry* (2009) 70:344–53. doi: 10.4088/JCP.07m03780
36. Wagner S, Engel A, Engelmann J, Herzog D, Dreimuller N, Muller MB, et al. Early improvement as a resilience signal predicting later remission to antidepressant treatment in patients with major depressive disorder: systematic review and meta-analysis. *J Psychiatr Res*. (2017) 94:96–106. doi: 10.1016/j.jpsychires.2017.07.003
37. Kirsch I, Deacon BJ, Huedo-Medina TB, Scoboria A, Moore TJ, Johnson BT. Initial severity and antidepressant benefits: a meta-analysis of data submitted to the food and drug administration. *PLoS Med*. (2008) 5:e45. doi: 10.1371/journal.pmed.0050045
38. Iniesta R, Malki K, Maier W, Rietschel M, Mors O, Hauser J, et al. Combining clinical variables to optimize prediction of antidepressant treatment outcomes. *J Psychiatr Res*. (2016) 78:94–102. doi: 10.1016/j.jpsychires.2016.03.016
39. Tadic A, Wagner S, Gorbulev S, Dahmen N, Hiemke C, Braus DFet al. Peripheral blood and neuropsychological markers for the onset of action of antidepressant drugs in patients with major depressive disorder. *BMC Psychiatry* (2011) 11:16. doi: 10.1186/1471-244x-11-16
40. Nicod J, Wagner S, Vonberg F, Bhomra A, Schlicht KF, Tadic A, et al. The amount of mitochondrial dna in blood reflects the course of a depressive episode. *Biol Psychiatry* (2016) 80:e41–e42. doi: 10.1016/j.biopsych.2015.12.019
41. Tadic A, Gorbulev S, Dahmen N, Hiemke C, Braus DF, Roschke J, et al. Rationale and design of the randomised clinical trial comparing early medication change (EMC) strategy with treatment as usual (TAU) in patients with major depressive disorder–the EMC trial. *Trials* (2010) 11:21. doi: 10.1186/1745-6215-11-21
42. Tadic A, Wachtlin D, Berger M, Braus DF, van Calker D, Dahmen N, et al. Randomized controlled study of early medication change for non-improvers to antidepressant therapy in major depression–the EMC trial. *Eur Neuropsychopharmacol*. (2016) 26:705–16. doi: 10.1016/j.euroneuro.2016.02.003
43. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* (1998) 59 (Suppl. 20):22–33; quiz: 34–57.
44. Fydrich T, Renneberg B, Schmitz B, Wittchen HU. *Strukturiertes Klinisches Interview für DSM-IV, Achse II (SKID-II)*. Göttingen: Hogrefe (1997).
45. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* (1960) 23:56–62.
46. Wagner S, Helmreich I, Lieb K, Tadic A. Standardized rater training for the hamilton depression rating scale (HAMD(1)(7)) and the inventory of depressive symptoms (IDS(C30)). *Psychopathology* (2011) 44:68–70. doi: 10.1159/000318162
47. Lewin J, Schmitt AO, Adorjan P, Hildmann T, Piepenbrock C. Quantitative DNA methylation analysis based on four-dye trace data from direct sequencing of PCR amplificates. *Bioinformatics* (2004) 20:3005–12. doi: 10.1093/bioinformatics/bth346

48. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods* (2010) 1:97–111. doi: 10.1002/jrsm.12
49. Lopez JP, Mamdani F, Labonte B, Beaulieu MM, Yang JP, Berlim MT, et al. Epigenetic regulation of BDNF expression according to antidepressant response. *Mol Psychiatry* (2013) 18:398–9. doi: 10.1038/mp.2012.38
50. Keller S, Sarchiapone M, Zarrilli F, Videtic A, Ferraro A, Carli V, et al. Increased BDNF promoter methylation in the Wernicke area of suicide subjects. *Arch Gen Psychiatry* (2010) 67:258–67. doi: 10.1001/archgenpsychiatry.2010.9
51. Kang HJ, Kim JM, Lee JY, Kim SY, Bae KY, Kim SW, Shin IS, et al. BDNF promoter methylation and suicidal behavior in depressive patients. *J Affect Disord*. (2013) 151:679–85. doi: 10.1016/j.jad.2013.08.001
52. Papakostas GI, Ostergaard SD, Iovieno N. The nature of placebo response in clinical studies of major depressive disorder. *J Clin Psychiatry* (2015) 76:456–66. doi: 10.4088/JCP.14r09297
53. Brunoni AR, Padberg F, Vieira ELM, Teixeira AL, Carvalho AF, Lotufo PA, et al. Plasma biomarkers in a placebo-controlled trial comparing tDCS and escitalopram efficacy in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2018) 86:211–7. doi: 10.1016/j.pnpbp.2018.06.003
54. Tardito D, Perez J, Tiraboschi E, Musazzi L, Racagni G, Popoli M. Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol Rev*. (2006) 58:115–34. doi: 10.1124/pr.58.1.7
55. Hutchinson AN, Deng JV, Cohen S, West AE. Phosphorylation of MeCP2 at Ser421 contributes to chronic antidepressant action. *J Neurosci*. (2012) 32:14355–63. doi: 10.1523/jneurosci.2156-12.2012
56. Colle R, Trabado S, Rotenberg S, Brailly-Tabard S, Benyamina A, Aubin HJ, et al. Tobacco use is associated with increased plasma BDNF levels in depressed patients. *Psychiatry Res*. (2016) 246:370–2. doi: 10.1016/j.psychres.2016.10.012
57. Elzinga BM, Molendijk ML, Oude Voshaar RC, Bus BA, Prickaerts J, Spinhoven P, et al. The impact of childhood abuse and recent stress on serum brain-derived neurotrophic factor and the moderating role of BDNF Val66Met. *Psychopharmacology* (2011) 214:319–28. doi: 10.1007/s00213-010-1961-1
58. Bus BA, Molendijk ML, Penninx BJ, Buitelaar JK, Kenis G, Prickaerts J, et al. Determinants of serum brain-derived neurotrophic factor. *Psychoneuroendocrinology* (2011) 36:228–39. doi: 10.1016/j.psyneuen.2010.07.013
59. Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, et al. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *J Endocrinol*. (2008) 197:429–35. doi: 10.1677/joe-07-0376
60. Piccinni A, Marazziti D, Del Debbio A, Bianchi C, Roncaglia I, Mannari C, et al. Diurnal variation of plasma brain-derived neurotrophic factor (BDNF) in humans: an analysis of sex differences. *Chronobiol Int*. (2008) 25:819–26. doi: 10.1080/07420520802387773

Conflict of Interest Statement: KL, AT, HF, and SB are designated as inventors of the European patent number 12171541.1–2404 Method for predicting response or non-response to a mono-aminergic antidepressant. AT has received consultancy fees from Janssen and Novartis.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 20 November 2018
doi: 10.3389/fpsyt.2018.00609

Serum Markers of Inflammation Mediate the Positive Association Between Neuroticism and Depression

Frank M. Schmidt[1], Christian Sander[1], Juliane Minkwitz[2], Roland Mergl[1], Bethan Dalton[3], Lesca M. Holdt[4], Daniel Teupser[4], Ulrich Hegerl[1] and Hubertus Himmerich[2,3]*

[1] Department of Psychiatry and Psychotherapy, University Hospital Leipzig, Leipzig, Germany, [2] Leipzig University Medical Center, IFB Adiposity Diseases, Leipzig, Germany, [3] Department of Psychological Medicine, King's College London, London, United Kingdom, [4] Institute of Laboratory Medicine, University Hospital Munich, LMU Munich, Munich, Germany

OPEN ACCESS

Edited by:
Gianluca Serafini, Dipartimento di Neuroscienze e Organi di Senso, Ospedale San Martino (IRCCS), Italy

Reviewed by:
Catherine Toben, University of Adelaide, Australia
Atsuo Nakagawa, School of Medicine, Keio University, Japan

****Correspondence:***
Frank M. Schmidt
f.schmidt@medizin.uni-leipzig.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *10 August 2018*
Accepted: *30 October 2018*
Published: *20 November 2018*

Citation:
Schmidt FM, Sander C, Minkwitz J, Mergl R, Dalton B, Holdt LM, Teupser D, Hegerl U and Himmerich H (2018) Serum Markers of Inflammation Mediate the Positive Association Between Neuroticism and Depression. Front. Psychiatry 9:609. doi: 10.3389/fpsyt.2018.00609

Background: The personality trait neuroticism has been implicated in a poor response to stress, may relate to increased concentrations of cytokines and the development of depression. Inflammatory mechanisms may also be associated with the onset, severity and symptoms of depression. Both are related to poor antidepressant treatment outcome. Therefore, mediators of inflammation may bridge the relationship between neuroticism and depression.

Methods: To disentangle these interrelationships, the associations between neuroticism (according to NEO-PIR-N), depressive symptoms (BDI-II scores) and serum levels of hsCRP, TNF-α, IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, GM-CSF were investigated in a group of 212 participants, consisting of 37 depressed and 175 non-depressed subjects. A mediation model was used to investigate whether the impact of neuroticism on depressive symptoms may be mediated by cytokines.

Results: Regression analyses revealed that IFN-γ, IL-5, and IL-12-levels, but none of the anti-inflammatory cytokines, were associated with the overall neuroticism score and several of the cytokines were related to the different facets of neuroticism. TNF-α, IFN-γ, IL-5, IL-12, and IL-13 were further related to the severity of depressive symptoms, as well as the somatic-affective and the cognitive dimensions of depression. Pro-inflammatory IFN-γ, IL-5 and IL-12 were identified as mediators of the positive prediction of depression severity by the degree of neuroticism.

Conclusions: The current findings demonstrate that conditions related to long-term stress, such as depression and high neuroticism, are related to an up-regulation of inflammatory agents. Neuroticism may increase stress perception and, thus, increase the production of pro-inflammatory messenger molecules which are involved in the development of depression. This evidence may contribute to future anti-inflammatory

interventions, particularly in subjects with high neuroticism who are at risk for developing depression. Furthermore, depressed patients with high neuroticism and cytokine levels may require early escalations in the intensity of treatment, along with additional therapeutic elements to increase the rate of treatment success.

Keywords: neuroticism, depression, chronic stress, cytokines, TNF-α, mediation analyses

INTRODUCTION

Neuroticism, as one of the Big Five higher-order personality traits, represents the tendency to experience negative emotions, such as anxiety and anger, and to have an increased perception of stress, as well as the inability to relieve the self from and to cope with stress (1). Acute and chronic stress have been shown to significantly influence cytokine production in human and animal studies (2, 3). In accord, higher neuroticism as well as stress-related disorders, such as post-traumatic stress disorder (PTSD), have been found to be associated with higher levels of pro-inflammatory agents (4, 5). However, investigations on neuroticism have been limited to a few inflammatory parameters: for example, C-reactive protein (CRP) or interleukin (IL)-6, for which the relationship with neuroticism could not be confirmed (6). Importantly, neuroticism is considered a risk factor for the development and onset of major depression and certain subtypes of depression (7–9). However, the biological mechanisms for this relationship are not yet well understood.

Alongside neuroticism, a body of evidence supports the involvement of low-grade inflammation in the pathogenesis of depressive disorders (10). Accordingly, cross-sectional studies have demonstrated an association between pro-inflammatory markers and the presence, course and treatment outcome of major depression (11–14). Inconsistent results have been observed in the relationship between pro-inflammatory cytokines and the severity of depressive symptoms, as measured in cohorts of patients with major depression or physical illnesses concomitant with depressive syndromes, and in population-based studies (15–22). Additionally, anti-inflammatory cytokines have gained little attention in these investigations (15, 23). The relationship between the symptoms and severity of depression and cytokine regulation should be interpreted with caution given that the majority of investigations were limited to a few parameters, in particular CRP and IL-6, and relevant confounders, such as the distribution of sexes, have not always been taken into account sufficiently.

Given that only a small number of mediators of inflammation have yet been investigated with regards to neuroticism, results on inflammation and depressive symptomatology are inconclusive and the biological pathways for the relationship between neuroticism and depression are unidentified. Therefore, the aim of this study was firstly to investigate the hypothesis that pro- and anti-inflammatory markers are associated with the severity of depressive symptoms and the personality trait neuroticism. Secondly, we aimed to explore if cytokines mediate the relationship between depressive symptoms and neuroticism.

METHODS

Participants

The presented data were collected as part of the "OBDEP" research project (Obesity and Depression: pathogenetic role of sleep and wakefulness regulation, motor activity level and neurochemical aspects). Three-hundred and four participants were initially recruited from the outpatient clinic of the Integrated Research and Treatment Center (IFB) for Adiposity Diseases Leipzig, from the Department of Psychiatry and Psychotherapy at the University Hospital Leipzig and via advertisements (intranet, internet, local newspapers). Evaluation of inclusion and exclusion criteria for the study was performed in two stages, as previously reported (24). First, potential participants took part in a telephone screening interview, which involved collecting socio-demographic data, assessing the presence of physical illnesses and completing a checklist of the Structured Clinical Interview for DSM-IV [SCID-I; (25)]. Following this, potentially eligible participants were invited to the study center, where exclusion criteria were assessed in more detail. In cases of positive SCID-screening or known diagnosis of depression, the SCID-I was performed in full. Exclusion criteria were DSM-IV Axis 1 disorders for the non-depressed participants and DSM-IV Axis 1 disorders other than a major depression for the depressed subjects. For all subjects, further exclusion criteria were acute or chronic infections (according to clinical investigation and/or CRP $\geq$ 10 mg/l), current medication treatment with a non-steroidal anti-inflammatory drug (NSAID), the presence of current and/or past neurological disorders, and a history of head injury with loss of consciousness exceeding 1 h. Assessments for current and past history of health problems and current medication were performed using standardized questionnaires. Only data sets of participants who completed both the Beck Depression Inventory II [BDI-II; (26)] and the Revised NEO Personality Inventory [NEO-PIR-N; (27)] were included in the statistical analyses presented herewith, resulting in a total sample of 212 participants, including 175 non-depressed and 37 depressed subjects. All participants were aged 18–70 years. Written informed consent was obtained from all participants. The study was approved by Leipzig University Ethics Committee (#015-10-18012009).

Assessments

Depressive symptoms were assessed using the Beck Depression Inventory II [BDI-II; (26)]. Along with a total sum score, subscale scores for a somatic-affective factor and a cognitive factor were calculated (28). The somatic-affective factor was calculated by summation of scores on items 4, 11-13, and 15-21 and

the cognitive factor by summation of scores on items 1-3, 5-9, and 14. To assess neuroticism, the neuroticism domain (consisting of six dimensions: anxiety, hostility, depression, self-consciousness, stress vulnerability, impulsiveness) of the Revised NEO Personality Inventory [NEO-PIR-N; (27)] was used.

Cytokine Measurement

After blood drawing, serum probes were immediately centrifuged at 3,000 rpm for 10 min. The supernatant was aliquoted and stored in non-absorbing polypropylene tubes of 300 µl. Probes were shock-frozen in liquid nitrogen and stored in freezers at −80°C until required. Cytokines were measured using the Bio-Plex Pro human cytokine Th1/Th2 immunoassay from Bio Rad, Germany, a 96-well kit that uses coupled magnetic beads and detection antibodies. This multiplex assay detects IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, GM-CSF, IFN-γ, and TNF-α. The intraassay coefficient of variation (CV) for cytokines was between 1.6 and 3.8%. High sensitivity (hs)-CRP was measured using a turbidimetric assay on an AU 5800 analyzer, Beckman Coulter, Germany. For hs-CRP intraassay CV was 4.1%.

Statistics

CRP and the nine pro- and anti-inflammatory cytokines were log-transformed in order to obtain approximately normally

TABLE 1 | Sociodemographic details for the total sample, and for participants with and without a diagnosis of major depression, separately.

	Participants total (*N* = 212)	Participants non-depressed (*N* = 175)	Participants depressed (*N* = 37)
Sex [male/female]	81/131	62/113	19/18
Age [years] (mean ± SD)	37.14 ± 12.01	36.61 ± 13.04	39.62 ± 12.73
Smoker (yes/no)	48/164	37/138	11/26
BMI [kg/m²] (mean ± SD)	34.71 ± 13.00	35.28 ± 12.25	32.02 ± 10.61
Marital status			
married or cohabiting	53 (25.0%)	39 (22.3%)	14 (37.8%)
Divorced	23 (10.8%)	17 (9.7%)	6 (16.2%)
Widowed	3 (1.4%)	2 (1.1%)	1 (2.7%)
Single	131 (61.8%)	115 (65.7%)	16 (43.2%)
NA	1.2%	2 (1.2%)	0
Occupational status			
Employed	106 (50.0%)	85 (48.6%)	21 (56.8%)
Unemployed	47 (22.2%)	38 (21.7%)	9 (24.3%)
Retired	12 (5.7%)	8 (4.6%)	4 (10.8%)
Student	40 (18.9%)	37 (21.1%)	3 (8.1%)
Homemaker	4 (1.9%)	4 (2.3%)	0
NA	3 (1.4%)	3 (1.7%)	0
Comorbid Disorders (yes)	87 (41.0%)	73 (41,7%)	14 (37,8%)
None	125 (59.0%)	102 (58.3%)	23 (62.2%)
Arterial hypertension	50 (23.6%)	42 (24.0%)	8 (21.6%)
Hypothyreosis	36 (16.9%)	31 (17.7%)	5 (13.5%)
Diabetes	25 (11.8%)	24 (13.7%)	1 (2.7%)
Obstructive sleep apnea syndrome	8 (3.8%)	7 (4.0%)	1 (2.7%)
Asthma	7 (3.3%)	6 (4.1%)	1 (2.7%)
Cerebrovascular disorders/Myocardial infarction	12 (5.7%)	8 (4.6%)	4 (10.8%)
Medication (yes)	60 (28.3%)	52 (29.7%)	8 (21.6%)
None	152 (71.7%)	123 (70.3%)	29 (78.4%)
ACE-blocker	28 (13.2%)	26 (14.9%)	2 (5.4%)
Beta-blocker	25 (11.8%)	22 (12.6%)	3 (8.1%)
Hypoglycaemics	25 (11.8%)	24 (13.7%)	1 (2.7%)
AT1-blocker	18 (8.5%)	12 (6.9%)	5 (13.5%)
Calcium chanel blocker	9 (4.2%)	7 (5.7%)	2 (5.4%)
Statins	3 (1.4%)	3 (1.7%)	0

TABLE 2 | Sum total and dimension scores for depression and neuroticism, and concentrations of markers of inflammation in the total sample, and for participants with and without a diagnosis of major depression, separately.

	Participants total	Participants non-depressed	Participants depressed
	(*N* = 212)	(*N* = 175)	(*N* = 37)
BDI-II sum score (mean ± SD)	11.01 ± 11.34	7.65 ± 7.81	26.95 ± 11.94
Somatic-affective dimension (mean ± SD)	6.21 ± 6.37	4.33 ± 4.38	15.63 ± 6.47
Cognitive dimension (mean ± SD)	4.14 ± 4.89	2.98 ± 3.64	10.33 ± 5.98
NEO-PIR-N sum score (mean ± SD)	89.15 ± 28.01	83.23 ± 25.59	117.14 ± 21.39
Anxiety dimension (mean ± SD)	15.23 ± 6.04	14.03 ± 5.51	20.89 ± 5.17
Hostility dimension (mean ± SD)	15.15 ± 5.49	14.30 ± 5.19	19.16 ± 5.10
Depression dimension (mean ± SD)	14.49 ± 7.55	12.76 ± 6.70	22.65 ± 5.60
Self-consciousness dimension (mean ± SD)	16.56 ± 5.57	15.66 ± 5.35	20.81 ± 4.59
Impulsiveness dimension (mean ± SD)	14.72 ± 4.11	14.73 ± 4.14	14.68 ± 4.02
Stress vulnerability dimension (mean ± SD)	13.00 ± 6.05	11.75 ± 5.28	18.95 ± 5.95
CRP levels [mg/dl] (mean ± SD)	0.68 ± 0.78	0.69 ± 0.76	0.65 ± 0.85
TNF-α levels [pg/ml] (mean ± SD)	30.37 ± 18.02	28.69 ± 16.56	38.29 ± 22.34
IFN-γ levels [pg/ml] (mean ± SD)	149.67 ± 113.18	119.58 ± 67.38	292.97 ± 167.10
IL-2 levels [pg/ml] (mean ± SD)	6.46 ± 8.13	6.46 ± 8.42	6.46 ± 6.71
IL-4 levels [pg/ml] (mean ± SD)	3.78 ± 2.23	3.80 ± 2.30	3.66 ± 1.90
IL-5 levels [pg/ml] (mean ± SD)	3.14 ± 2.45	2.75 ± 1.98	4.99 ± 3.45
IL-10 levels [pg/ml] (mean ± SD)	6.54 ± 26.75	6.90 ± 29.31	4.83 ± 6.25
IL-12 levels [pg/ml] (mean ± SD)	14.35 ± 18.79	11.41 ± 17.01	28.26 ± 20.73
IL-13 levels [pg/ml] (mean ± SD)	6.20 ± 6.46	5.76 ± 6.46	8.26 ± 6.13
GM-CSF levels [pg/ml] (mean ± SD)	35.01 ± 20.08	33.73 ± 18.53	42.98 ± 25.01
Time of blood drawings (mean ± SD)	11 : 51 ± 2 : 28	11 : 56 ± 2 : 32	11 : 26 ± 2 : 10

distributed variables. We investigated the association between depressive symptoms and neuroticism with the log-transformed serum concentrations of CRP and the cytokines by linear regression analyses.

Multiple regression analyses were performed to determine the relationship between the dependent variable (i.e., depressive symptoms according to BDI-scores) and each of the log-transformed CRP and cytokine values. Regression analyses were controlled for confounding variables known to impact cytokine levels: age, sex, smoking status, BMI and time of blood drawing. Sum values of the physical illnesses and medication listed in **Table 1** were further included as control variables ranging from 0 ("none") to the maximum of 4. Levels of significance were adjusted for multiple testing using the False Discovery Rate [FDR; according to (29)] for BDI-II, NEO-PIR-N and the respective cytokines separately. For all other analyses, the level of significance was set at $p < 0.05$. Mediation analyses were performed for those cytokines significantly associated with sum scores of both BDI-II and NEO-PIR-N. For the mediation analyses, residuals were calculated for logTNF-α, logIFN-γ, logIL-5, logIL-12, logIL-13, BDI-II sum scores and NEO-PIR-N sum scores through listwise-regression analyses in order to exclude significant confounders. Covariates included into the analyses were: "age," "sex," "BMI," "smoking status," "time of blood sampling," "sum of physical illnesses," and "sum of medication." After excluding multicollinearity between the parameters, mediation analyses were performed with the freely available SPSS macro "sobel" (http://www.processmacro.org/download.html), which includes bootstrapping and Sobel test (30). All further analyses were performed with SPSS Version 24 (IBM Corporation; Armonk, NY, USA).

RESULTS

Two hundred twelve participants, consisting of 37 depressed and 175 non-depressed subjects, were included in the final analyses. Demographic data, including medication status and the presence of physical illnesses, are presented in **Table 1**. Serum concentrations of CRP and cytokines, depression symptoms and neuroticism scores are shown in **Table 2**.

CRP, Cytokines and Depression Symptoms

Linear regression analyses showed a significant increase in the severity of depressive symptoms associated with higher logTNF-α, logIFN-γ, logIL-5, logIL-12, and logIL13 (**Table 3**). The BDI-II sum score increased by a coefficient of up to 15.5 for each standard deviation of logIFN-γ, followed by 9.2 for each standard deviation of logTNF-α, by a coefficient of 9.2 for logIL-5, 6.7 for logIL-12 and 6.4 for logIL-13. Scores on the somatic-affective and cognitive dimensions of the BDI-II were significantly associated with logTNF-α, logIFN-γ, logIL-5, logIL-12, logIL13 and logGM-CSF. No significant association between the sum scores, the dimensions of depression and the cytokine levels were observed for logIL-2, logIL-4 or logIL-10 and also logCRP.

CRP, Cytokines and Neuroticism

Linear regression analyses between the pro-and anti-inflammatory markers, the six different dimensions of neuroticism and the sum score of neuroticism showed that a significant increase in the magnitude of neuroticism (NEO PI-R-N sum score) was dependent on higher logIFN-γ, logIL-5 and logIL-12 after correcting for multiple testing (**Table 4**). The increase in the anxiety dimension was predicted by logIFN-γ and logIL-12 levels. The depression dimension was found to significantly depend on logIFN-γ, logIL-5, and logIL-12. The self-consciousness dimension depended significantly on all parameters except logCRP and logIL4. The stress vulnerability dimension depended on logTNF-α, logIFN-γ, logIL-5, logIL-10, logIL-12, and logIL-13. The hostility dimension and the impulsiveness dimension were not associated with any of the cytokines.

Relationship Between Neuroticism, Depressive Symptoms and Inflammatory Markers

In a first step, the residuals of the BDI-II scores were predicted by the residuals of the NEO-scores. In a second step, the residuals of

TABLE 3 | Regression analyses between inflammatory agents and dimensions of depression.

	BDI-II sum score	Somatic-affective dimension	Cognitive dimension
$_{log}$CRP	6.109 (−6.737 to 18.955) = 0.350	3.574 (−3.663 to 10.811) = 0.331	1.788 (−3.795 to 7.370) = 0.528
$_{log}$TNF-α	**9.212 (3.017 to 15.408) =0.004***	**6.011 (2.528 to 9.494), <0.001***	**3.849 (1.116 to 6.582) = 0.006***
$_{log}$IFN-γ	**15.517 (10.463 to 20.571), <0.001***	**8.723 (5.854 to 11.593), <0.001***	**5.939 (3.697 to 8.180), <0.001***
$_{log}$IL-2	1.974 (−1.107 to 5.056) = 0.208	1.160 (−0.576–2.897) = 0.189	0.956 (−0.383 to 2.294) = 0.161
$_{log}$IL-4	3.219 (−4.954 to 11.392) = 0.438	2.216 (−2.425 to 6.858) = 0.348	1.801 (−1.753 to 5.354) = 0.319
$_{log}$IL-5	**9.248 (4.664 to 13.832), <0.001***	**5.904 (3.341 to 8.468), <0.001***	**3.244 (1.228 to 5.260) = 0.002***
$_{log}$IL-10	3.437 (−0.445 to 7.318) = 0.082	1.998 (−0.193 to 4.190) = 0.074	1.387 (−0.300 to 3.074) = 0.107
$_{log}$IL-12	**6.743 (3.647 to 9.839), <0.001***	**3.872 (2.118 to 5.626), <0.001***	**3.108 (1.749 to 4.466), <0.001***
$_{log}$IL-13	**6.370 (1.900 to 10.840) =0.005***	**3.904 (1.388 to 6.420) = 0.003***	**2.860 (0.897 to 4.823) = 0.005***
$_{log}$GM-CSF	**5.531 (0.119 to 10.944) = 0.045**	**3.198 (0.150 to 6.246) = 0.040**	**2.839 (0.491 to 5.187) = 0.018***

Coefficient (95%-CI), p-value. Significant correlations are highlighted in bold font. Significance after correction for multiple comparisons is labeled with an asterisk.

TABLE 4 | Regression analyses between inflammatory markers and the dimensions of neuroticism.

	NEO-PIR-N sum score	Anxiety dimension	Hostility dimension	Depression dimension	Self-consciousness dimension	Impulsiveness dimension	Stress vulnerability dimension
$_{log}$CRP	8.937 (−22.468 to 40.341) =0.575	5.052 (−1.738 to 11.843) = 0.144	1.826 (−4.392 to 8.044) = 0.563	−0.579 (−9.097 to 7.939) = 0.894	0.461 (−5.766 to 6.688) = 0.884	−0.580 (−5.205 to 4.044) = 0.805	2.757 (−4.130 to 9.643) = 0.431
$_{log}$TNF-α	**16.571 (1.300 to 31.842) = 0.034**	2.985 (−0.344 to 6.313) = 0.079	0.616 (−2.440 to 3.673) = 0.691	3.668 (−0.487 to 7.822) = 0.083	**4.797 (1.810 to 7.784) = 0.002***	0.678 (−1.593 to 2.948) = 0.557	**3.828 (0.481 to 7.175) = 0.025***
$_{log}$IFN-γ	**31.712 (19.046 to 44.378), <0.001***	**6.363 (3.588 to 9.138), <0.001***	**3.474 (0.864 to 6.084) = 0.009**	**8.134 (4.679 to 11.588), <0.001***	**5.510 (2.965 to 9.054), <0.001***	1.604 (−0.356 to 3.564) = 0.108	**6.628 (3.833 to 9.423), <0.001***
$_{log}$IL-2	3.972 (−3.561 to 11.505) =0.300	0.618 (−1.020 to 2.256) = 0.458	−0.574 (−2.072 to 0.24) = 0.451	0.910 (−1.149 to 2.969) = 0.384	**1.630 (0.140 to 3.119) = 0.032***	0.800 (−0.314 to 1.914) = 0.158	0.724 (−0.945 to 2.392) = 0.394
$_{log}$IL-4	6.204 (−13.759 to 26.167) = 0.541	1.632 (−2.702 to 5.966) = 0.459	−1.858 (−5.806 to 2.090) = 0.355	0.575 (−4.840 to 5.990) = 0.834	3.164 (−0.771 to 7.099) = 0.114	1.094 (−1.842 to 4.031) = 0.463	1.598 (−2.782 to 5.977) = 0.473
$_{log}$IL-5	**17.439 (6.074 to 28.803) = 0.003***	**2.823 (0.330 to 5.316) = 0.027**	1.774 (−0.513 to 4.062) = 0.128	**4.223 (1.129 to 7.317) = 0.008***	**3.833 (1.593 to 6.074), <0.001***	0.764 (−0.943 to 2.470) = 0.379	**4.021 (1.533 to 6.510) = 0.002***
$_{log}$IL-10	7.796 (−1.689 to 17.281) = 0.107	1.608 (−0.453 to 3.669) = 0.126	−0.171 (−2.061 to 1.719) = 0.859	1.710 (−0.867 to 4.286) = 0.192	**2.159 (0.291 to 4.027), 0.024***	0.296 (−1.108 to 1.700) = 0.678	**2.194 (0.121 to 4.267) = 0.038***
$_{log}$IL-12	**14.806 (7.183 to 22.429), <0.001***	**2.530 (0.852 to 4.208) = 0.003***	1.205 (−0.349 to 2.759) = 0.128	**4.074 (2.010 to 6.138), <0.001***	**3.059 (1.554 to 4.565), <0.001***	0.601 (−0.557 to 1.760) = 0.307	**3.336 (1.666 to 5.006), <0.001***
$_{log}$IL-13	**13.038 (2.062 to 24.014) = 0.020**	2.276 (−0.119 to 4.671) = 0.062	0.918 (−1.281 to 3.116) = 0.412	2.934 (−0.053 to 5.922) = 0.054	**3.329 (1.173 to 5.484) = 0.003***	0.637 (−0.998 to 2.217) = 0.443	**2.945 (0.538 to 5.351) = 0.017***
$_{log}$GM-CSF	9.703 (−3.574 to 22.980) = 0.151	1.708 (−1.181 to 4.597) = 0.245	0.436 (−2.205 to 3.078) = 0.745	1.625 (−1.984 to 5.235) = 0.376	**3.148 (0.540 to 5.757) = 0.018***	0.703 (−1.258 to 2.665) = 0.480	2.081 (−0.833 to 4.996) = 0.161

Coefficient (95%-CI), p-value. Significant correlations are highlighted in bold font. Significance after correction for multiple comparisons is labeled with an asterisk.

the cytokine levels (logTNF-α, logIFN-γ, logIL-5, logIL-12, logIL-13) were predicted by the residuals of the NEO-scores. In a third step, the residuals of the BDI-II-scores were predicted by the residuals of the cytokine levels and the NEO-scores. The results of the regression analyses are presented in **Table 5**

The bootstrapping analysis with $m = 5{,}000$ estimates revealed a significant indirect effect of the residuals of the NEO-scores on the residuals of the BDI-scores through the residuals of logIFN-γ (95% CI 0.0097, 0.0462), logIL-5 (95% CI 0.0027, 0.0230) and logIL-12 (95% CI 0.0013, 0.0291) -residuals. As an example, the standardized coefficients of regression for logIFN-γ are depicted in **Figure 1**. The Sobel-Z-test revealed a significant effect for the residuals through logIFN-γ ($Z = 2.88$, $p = 0.004$) that was trending for logIL-5 ($Z = 1.89$, $p = 0.059$) and IL-12 ($Z = 1.91$, $p = 0.056$).

DISCUSSION

The current findings on the relationship between both pro- and anti-inflammatory markers and characteristics of depression and neuroticism confirmed our hypothesis of a positive association between inflammatory agents and the degree of depression and neuroticism. The results further revealed that markers of inflammation may be significant mediators for the positive relationship between neuroticism and depressive symptoms.

After correcting for multiple testing and controlling for potential confounding variables, the analyses revealed that depressive symptoms were association with the cytokines TNF-α, IFN-γ, IL-5, IL-12, and IL-13. These results expand on the range of cytokines previously investigated. They also add to existing findings suggesting that a pro-inflammatory state is related to depressive symptoms in the general population as well as in

TABLE 5 | Mediation analyses Regression analyses between dimensions of depression, neuroticism and the inflammatory agents.

Criterion	Predictor	β	*p*
BDI-II	NEO	1.60	< 0.001
TNF-α	NEO	16.42	0.029
IFN-γ	NEO	32.11	< 0.001
IL-5	NEO	18.38	0.002
IL-12	NEO	15.07	< 0.001
IL-13	NEO	4.61	0.016
BDI-II	TNF-α	0.009	0.048
	NEO	1.566	< 0.001
BDI-II	IFN-γ	0.017	0.005
	NEO	1.448	< 0.001
BDI-II	IL-5	0.011	0.016
	NEO	1.54	< 0.001
BDI-II	IL-12	0.011	0.026
	NEO	1.527	< 0.001
BDI-II	IL-13	0.0004	0.123
	NEO	1.572	< 0.001

β, *standardized coefficient of regression.*

cohorts of depressed subjects [e.g., (15–17, 31)]. Extending on previous research that focussed on pro-inflammatory markers, our analyses could not demonstrate an affection of the degree of depressive mood and cognition by the anti-inflammatory mediators IL-4 and IL-10. Though anti-inflammatory cytokines, have been found to be elevated in major depression and in response to pro-depressive agents (24, 32, 33), the degree of depression could previously not be statistically explained by IL-10 or IL-4 (24, 31). However, the sample sizes here and in previous studies may, in part, account for the negative results for which small effect sizes were to be expected (34). Results of a decreased IL-4-responsiveness of microglia as well as the inhibition of IL-10-signaling leading to depression-like behavior in animal models of depression demonstrate the need for further investigations on the role of anti-inflammatory agents in depression (35, 36).

The present results do not support the involvement of CRP as a relevant mediator once relevant covariates were accounted for (13, 15). In line with this, most of the mechanisms involved in the inflammation-depression -relationship have been described for TNF-α and IFN-γ, but not CRP, including the stimulation of the indolamine-2,3-dioxygenase (IDO) (10), the relationship with the psychopathology of depression (37–39) and the potential as an antidepressant drug target (14, 40). As for CRP, the differences in results between correlation and regression analyses (41) and the variability in results across other studies, showing no relationship between cytokines and depressive symptoms (20, 24), illustrates the need to account for potential confounders. Studies not including essential confounding parameters, such as BMI, smoking habits and inflammation-modulating drugs, should therefore be considered with caution.

Overall neuroticism and also several dimensions of neuroticism, in particular the self-consciousness and the stress vulnerability dimensions, were related to multiple cytokines, such as IFN-γ and TNF-α; however no such relationship was observed for the anti-inflammatory IL-4 and IL-10. These associations highlight the relationship between personality traits leading to long-term impairments in stress response and the regulation of mediators of inflammation. There is little data to which we can compare our findings; however, for CRP, the absence of associations are in line with a recent meta-analysis showing CRP and IL-6 not to be related to neuroticism (6). To our knowledge, this is the first study in the English language to examine the relationship between neuroticism and the anti-inflammatory cytokines IL-4 and IL-10, which warrants replication. For TNF-α, the findings from the limited number of previous studies conducted are consistent with ours; for example, in patients with hepatitis C, TNF-α correlated with scores of neuroticism after therapy (4). Hypothetically, the finding of up-regulation of markers of inflammation in neuroticism may explain the increased incidence of a wide range of physical illnesses in people vulnerable to neuroticism (42). Also, neuroticism may be associated with increased cytokine levels due to its relationship with a higher prevalence of obesity and smoking habits, factors known to up-regulate pro-inflammatory cytokines (9, 43, 44).

With regards to our findings of a relationship between neuroticism and depressive symptoms, neuroticism has repeatedly been shown to be associated with or to be a risk factor for the development of depressive disorders (7–9). To the best of our knowledge, this is the first time that inflammatory markers have been identified as a relevant mediator of this association. One possible patho-biological pathway for this link could be hypothalamic-pituitary-adrenal (HPA) axis activity: chronic stress induces the upregulation of HPA axis activity as well as the synthesis of cytokines (45–47). Some cytokines, for example IFN-γ, in turn stimulate the HPA axis (48). Of note, HPA axis activity has been found to be altered both in major depression and neuroticism (45). Other identified links between neuroticism and depression include a decreased expression of brain-derived neurotrophic factor (BDNF), as well as the activity of the anterior cingulate cortex [ACC; (45, 46)], for which an involvement of cytokines has been described (11, 49). Regarding the integration of this relationship into antidepressant treatment strategies, it should be of note that both inflammation (13, 18) and neuroticism (50, 51) impact on the treatment outcome of depression. Therefore, depressed patients with high neuroticism may require more specialized clinical efforts (50). In addition, the determination of inflammatory mediators as well as factors associated with increased cytokine levels and neuroticism, such as obesity and smoking should be taken into account (9, 19, 43, 44). Psychotherapeutic interventions may exert anti-depressant effects via modulation of neuroticism (52), however the question as to whether this is related to observed reductions of inflammation (53) is yet unknown.

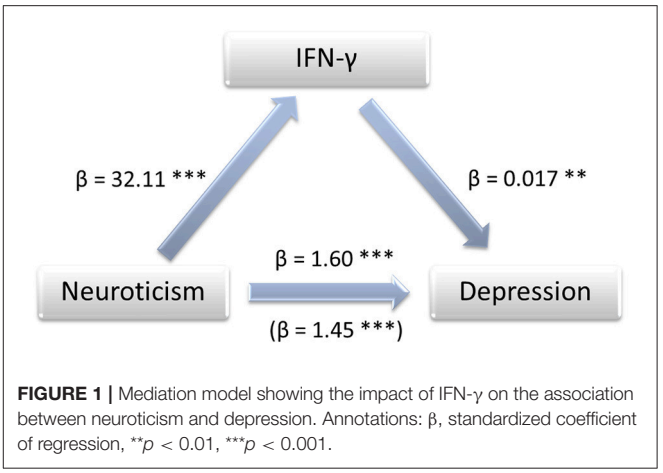

FIGURE 1 | Mediation model showing the impact of IFN-γ on the association between neuroticism and depression. Annotations: β, standardized coefficient of regression, $^{**}p < 0.01$, $^{***}p < 0.001$.

This study needs to be considered in light of several limitations: The analyses were performed in participants with a high proportion of obesity which is unlikely to represent the general population. The sample size of the group of depressed patients was too small to perform separate analysis in this group and to rule out type II errors. This may also be the case for the total number of participants and potential effects with small effect sizes, like the relationship between depressiveness and serum levels of IL-4 and IL-10.

Our results show a relationship between depressive symptoms, neuroticism and cytokine levels, but cannot provide information on the molecular mechanisms underlying this association. A

longitudinal design could more clearly demonstrate whether the personality trait neuroticism contributes to the risk of developing depression. Further, our analyses highlight the importance of including several potential covariates into the statistical analyses, such as BMI, medication status, and concurrent physical illnesses, due to their potential impact on inflammation. However, a more distinct impact, e.g., of the dosage of the separate drugs or the activity and acuity of the diagnoses, remains unclear.

In conclusion, the current study included 37 depressed and 175 non-depressed subjects, finding significant associations between depressive symptoms, the degree and dimensions of neuroticism and serum levels of pro-inflammatory, but not anti-inflammatory, cytokines. Further, IFN-γ, IL-5 and IL-12 were found to be significant mediators of the effect of neuroticism on depressive symptoms. These findings support the relationship between depressive psychopathology and pro-inflammatory conditions. Neuroticism as a long-term psychological stressor may exert pro-depressive features by facilitating a persistent low-grade inflammation. Since neuroticism and inflammation are related to the course of depression, psychotherapeutic emphasis on neuroticism and pharmacological targeting of inflammation may contribute to more personalized antidepressant therapeutic interventions, helping to prevent therapeutic non-response and the development of a chronic course of illness.

AUTHOR CONTRIBUTIONS

FS and HH designed the study. FS, CS, and HH wrote the manuscript. LH and DT conducted the chemical analyses. FS and RM performed the statistical analyses. JM, RM, BD, and UH revised the manuscript. All authors approved the final manuscript.

FUNDING

This study was supported by the Integrated Research and Treatment Centre for Adiposity Diseases (IFB), University of Leipzig, the Federal Ministry of Education and Research (BMBF), FKZ: 01EO1001, and the Claussen-Simon-Foundation. We acknowledge support from the German Research Foundation (DFG) and Universität Leipzig within the program of Open Access Publishing. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

REFERENCES

1. Abbas IS. The role of neuroticism in the maintenance of chronic baseline stress perception and negative affect. *Span J Psychol.* (2016) 19:E9. doi: 10.1017/sjp.2016.7
2. Wirtz PH, von Känel R. Psychological stress, inflammation, and coronary heart disease. *Curr Cardiol Rep*. (2017) 19:111. doi: 10.1007/s11886-017-0919-x
3. Himmerich H, Fischer J, Bauer K, Kirkby KC, Sack U, Krügel U. Stress-induced cytokine changes in rats. *Eur Cytokine Netw*. (2013) 24:97–103. doi: 10.1684/ecn.2013.0338
4. Pawlowski T, Radkowski M, Małyszczak K, Inglot M, Zalewska M, Jablonska J, et al. Depression and neuroticism in patients with chronic hepatitis C: correlation with peripheral blood mononuclear cells activation. *J Clin Virol.* (2014) 60:105–11. doi: 10.1016/j.jcv.2014.03.004
5. Hussein S, Dalton B, Willmund GD, Ibrahim MAA, Himmerich H. A systematic review of tumor necrosis factor-α in post-traumatic stress disorder: evidence from human and animal studies. *Psychiatr Danub*. (2017) 29:407–20. doi: 10.24869/psyd.2017.407
6. Luchetti M, Barkley JM, Stephan Y, Terracciano A, Sutin AR. Five-factor model personality traits and inflammatory markers: new data and a meta-analysis. *Psychoneuroendocrinology* (2014) 50:181–93. doi: 10.1016/j.psyneuen.2014.08.014
7. Rudaz DA, Vandeleur CL, Gebreab SZ, Gholam-Rezaee M, Strippoli MF, Lasserre AM, et al. Partially distinct combinations of psychological, metabolic and inflammatory risk factors are prospectively associated with the onset of the subtypes of major depressive disorder in midlife. *J Affect Disord*. (2017) 222:195–203. doi: 10.1016/j.jad.2017.07.016
8. Jeronimus BF, Kotov R, Riese H, Ormel J. Neuroticism's prospective association with mental disorders halves after adjustment for baseline symptoms and psychiatric history, but the adjusted association hardly decays with time: a meta-analysis on 59 longitudinal/prospective studies with 443 313 participants. *Psychol Med*. (2016) 46:2883–906. doi: 10.1017/S0033291716001653
9. Gale CR, Hagenaars SP, Davies G, Hill WD, Liewald DC, Cullen B, et al. Pleiotropy between neuroticism and physical and mental health: findings from 108 038 men and women in UK Biobank. *Transl Psychiatry* (2016) 6:e791. doi: 10.1038/tp.2016.56
10. Lichtblau N, Schmidt FM, Schumann R, Kirkby KC, Himmerich H. Cytokines as biomarkers in depressive disorder: current standing and prospects. *Int Rev Psychiatry* (2013) 25:592–603. doi: 10.3109/09540261.2013.813442
11. Schmidt FM, Kirkby KC, Lichtblau N. Inflammation and immune regulation as potential drug targets in antidepressant treatment. *Curr Neuropharmacol.* (2016) 14:674–87. doi: 10.2174/1570159X14666160115130414
12. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1β, tumour necrosis factor α and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun*. (2015) 49:206–15. doi: 10.1016/j.bbi.2015.06.001
13. Uher R, Tansey KE, Dew T, Maier W, Mors O, Hauser J, et al. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. *Am J Psychiatry* (2014) 171:1278–86. doi: 10.1176/appi.ajp.2014.14010094
14. Schmidt FM, Kirkby KC, Himmerich H. The TNF-alpha inhibitor etanercept as monotherapy in treatment-resistant depression - report of two cases. *Psychiatr Danub*. (2014) 26:288–90.
15. Köhler-Forsberg O, Buttenschøn HN, Tansey KE, Maier W, Hauser J, Dernovsek MZ, et al. Association between C-reactive protein (CRP) with depression symptom severity and specific depressive symptoms in major depression. *Brain Behav Immun*. (2017) 62:344–50. doi: 10.1016/j.bbi.2017.02.020
16. Jokela M, Virtanen M, Batty GD, Kivimäki M. Inflammation and Specific Symptoms of Depression. *JAMA Psychiatry* (2016) 73:87–8. doi:10.1001/jamapsychiatry.2015.1977
17. White J, Kivimäki M, Jokela M, Batty GD. Association of inflammation with specific symptoms of depression in a general population of older people: the english Longitudinal study of ageing. *Brain Behav Immun*. (2017) 61:27–30. doi: 10.1016/j.bbi.2016.08.012
18. Schmidt FM, Schröder T, Kirkby KC, Sander C, Suslow T, Holdt LM, et al. Pro- and anti-inflammatory cytokines, but not CRP, are inversely correlated with severity and symptoms of major depression. *Psychiatry Res*. (2016) 239:85–91. doi: 10.1016/j.psychres.2016.02.052
19. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et al. Inflammatory cytokines in general and central obesity and

modulating effects of physical activity. *PLoS ONE* (2015) 10:e0121971. doi: 10.1371/journal.pone.0121971
20. Dahl J, Ormstad H, Aass HC, Malt UF, Bendz LT, Sandvik L, et al. The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* (2014) 45:77–86. doi: 10.1016/j.psyneuen.2014.03.019
21. Thomas AJ, Davis S, Morris C, Jackson E, Harrison R, O'Brien JT. Increase in Interleukin-1beta in late-life depression. *Am J Psychiatry* (2005) 162:175–7. doi: 10.1176/appi.ajp.162.1.175
22. Steptoe A, Kunz-Ebrecht SR, Owen N. Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. *Psychol Med*. (2003) 33:667–74. doi: 10.1017/S0033291702007250
23. Ramsey JM, Cooper JD, Bot M, Guest PC, Lamers F, Weickert CS, et al. Sex differences in serum markers of major depressive disorder in the netherlands study of depression and anxiety (NESDA). *PLoS ONE* (2016) 11:e0156624. doi: 10.1371/journal.pone.0156624
24. Schmidt FM, Lichtblau N, Minkwitz J, Chittka T, Thormann J, Kirkby KC, et al. Cytokine levels in depressed and non-depressed subjects, and masking effects of obesity. *J Psychiatr Res*. (2014) 55:29–34. doi: 10.1016/j.jpsychires.2014.04.021
25. Wittchen HU, Zaudig M, Fydrich T. *SKID Strukturiertes Klinisches Interview für DSM-IV (Achse I und II) [Structured Clinical Interview for DSM-IV (axis I and II)]*. Göttingen: Hogrefe (1997).
26. Beck AT, Steer RA, Brown GK. *BDI–II, Beck Depression Inventory: Manual*. 2nd edn. Boston: Harcourt Brace (1996).
27. Costa PT, McCrae RR. *NEO PI-R Professional Manual*. Odessa, FL: Psychological Assessment Resources, Inc (1992).
28. Huang C, Chen JH. Meta-Analysis of the factor structures of the beck depression inventory-II. *Assessment* (2015) 22:459–72. doi: 10.1177/1073191114548873
29. Benjamini Y, Hochberg Y. *Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B*. (1995) 57:289–300.
30. Preacher KJ, Hayes AF. SPSS and SAS procedures for estimating indirect effects in simple mediation models. *Behav Res Methods Instrum Comput*. (2004) 36:717–31. doi: 10.3758/BF03206553
31. Zou W, Feng R, Yang Y. Changes in the serum levels of inflammatory cytokines in antidepressant drug-naïve patients with major depression. *PLoS ONE* (2018) 13:e0197267. doi: 10.1371/journal.pone.0197267
32. Simon NM, McNamara K, Chow CW, Maser RS, Papakostas GI, Pollack MH, et al. A detailed examination of cytokine abnormalities in major depressive disorder. *Eur Neuropsychopharmacol*. (2008) 18:230–3. doi: 10.1016/j.euroneuro.2007.06.004
33. Hernández ME, Mendieta D, Martínez-Fong D, Loría F, Moreno J, Estrada I, et al. Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. *Eur Neuropsychopharmacol*. (2008) 18:917–24. doi: 10.1016/j.euroneuro.2008.08.001
34. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*. (2017) 135:373–87. doi: 10.1111/acps.12698
35. Wachholz S, Knorr A, Mengert L, Plümper J, Sommer R, Juckel G, et al. Interleukin-4 is a participant in the regulation of depressive-like behavior. *Behav Brain Res*. (2017) 326:165–72. doi: 10.1016/j.bbr.2017.03.02
36. Laumet G, Edralin JD, Chiang AC, Dantzer R, Heijnen CJ, Kavelaars A. Resolution of inflammation-induced depression requires T lymphocytes and endogenous brain interleukin-10 signaling. *Neuropsychopharmacology* (2018). doi: 10.1038/s41386-018-0154-1
37. Duivis HE, Vogelzangs N, Kupper N, de Jonge P, Penninx BW. Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: findings from the Netherlands study of depression and anxiety (NESDA). *Psychoneuroendocrinology* (2013) 38:1573–85. doi: 10.1016/j.psyneuen.2013.01.002
38. Black C, Miller BJ. Meta-Analysis of cytokines and chemokines in suicidality: distinguishing suicidal versus nonsuicidal patients. *Biol Psychiatry* (2015) 78:28–37. doi: 10.1016/j.biopsych.2014.10.014
39. Eller T, Aluoja A, Maron E, Vasar V. Soluble interleukin-2 receptor and tumor necrosis factor levels in depressed patients in Estonia. *Medicina* (2009) 45:971–7. doi: 10.3390/medicina45120124
40. Tyring S, Gottlieb A, Papp K, Gordon K, Leonardi C, Wang A, et al. Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *Lancet* (2006) 367:29–35 doi: 10.1016/S0140-6736(05)67763-X
41. Krogh J, Benros ME, Jørgensen MB, Vesterager L, Elfving B, Nordentoft M. The association between depressive symptoms, cognitive function, and inflammation in major depression. *Brain Behav Immun*. (2014) 35:70–6. doi: 10.1016/j.bbi.2013.08.014
42. Goodwin RD, Cox BJ, Clara I. Neuroticism and physical disorders among adults in the community: results from the National Comorbidity Survey. *J Behav Med*. (2006) 29:229–38. doi: 10.1007/s10865-006-9048-5
43. Sutin AR, Ferrucci L, Zonderman AB, Terracciano A. Personality and obesity across the adult life span. *J Pers Soc Psychol*. (2011) 101:579–92. doi: 10.1037/a0024286
44. Terracciano A, Costa PTJr. Smoking and the five-factor model of personality. *Addiction* (2004) 99:472–81. doi: 10.1111/j.1360-0443.2004.00687.x
45. Foster JA, MacQueen G. Neurobiological factors linking personality traits and major depression. *Can J Psychiatry* (2008) 53:6–13. doi: 10.1177/070674370805300103
46. Barlow DH, Ellard KK, Sauer-Zavala S, Bullis JR, Carl JR. The origins of neuroticism. *Perspect Psychol Sci*. (2014) 9:481–96. doi: 10.1177/1745691614544528
47. Krügel U, Fischer J, Radicke S, Sack U, Himmerich H. Antidepressant effects of TNF-α blockade in an animal model of depression. *J Psychiatr Res*. (2013) 47:611–6.
48. Besedovsky HO, del Rey A, Klusman I, Furukawa H, Monge Arditi G, Kabiersch A. Cytokines as modulators of the hypothalamus-pituitary-adrenal axis. J. Steroid. Biochem. Mol. *Biol*. (1991) 40:613–8. doi: 10.1016/0960-0760(91)90284-C
49. van Velzen LS, Schmaal L, Milaneschi Y, van Tol MJ, van der Wee NJ, Veltman DJ, et al. Immunometabolic dysregulation is associated with reduced cortical thickness of the anterior cingulate cortex. *Brain Behav Immun*. (2017) 60:361–8. doi: 10.1016/j.bbi.2016.10.019
50. Wardenaar KJ, Conradi HJ, Bos EH, de Jonge P. Personality modulates the efficacy of treatment in patients with major depressive disorder. *J Clin Psychiatry* (2014) 75:e916–23. doi: 10.4088/JCP.13m08855
51. Bukh JD, Andersen PK, Kessing LV. Personality and the long-term outcome of first-episode depression: a prospective 5-year follow-up study. *J Clin Psychiatry* (2016) 77:e704–10. doi: 10.4088/JCP.15m09823
52. Spinhoven P, Huijbers MJ, Ormel J, Speckens AEM. Improvement of mindfulness skills during mindfulness-based cognitive therapy predicts long-term reductions of neuroticism in persons with recurrent depression in remission. *J Affect Disord*. (2017) 213:112–7. doi: 10.1016/j.jad.2017.02.011
53. Lopresti AL. Cognitive behaviour therapy and inflammation: a systematic review of its relationship and the potential implications for the treatment of depression. *Aust N Z J Psychiatry* (2017) 51:565–82. doi: 10.1177/0004867417701996

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 29 November 2018
doi: 10.3389/fpsyt.2018.00634

Cerebellar Contributions to Major Depression

*Malte S. Depping, Mike M. Schmitgen, Katharina M. Kubera and Robert C. Wolf**

Department of General Psychiatry, Center for Psychosocial Medicine, University of Heidelberg, Heidelberg, Germany

Extending beyond the motor domain, the cerebellum is involved in various aspects of cognition and affect. Multidisciplinary evidence has demonstrated topographic organization of higher-order cognitive functions within the cerebellum. We here review recent neuroimaging research that indicates cerebellar contributions to major depressive disorder (MDD). At the structural level, increased volume of lobule IX has been demonstrated in MDD patients, independent of acute or remitted disease state. Successful treatment with electroconvulsive therapy has been associated with increased lobule VIIA volume in depressed patients. At the functional level, connectivity analyses have shown reduced cerebro-cerebellar coupling of lobules VI and VIIA/B with prefrontal, posterior parietal, and limbic regions in patients with MDD. As a limitation, most of this evidence is based on smaller patient samples with incomplete phenotypic and neuropsychological characterization and with heterogenous medication. Some studies did not apply cerebellum-optimized data analysis protocols. Taken together, MDD pathophysiology affects distinct subregions of the cerebellum that communicate with cortical networks subserving cognitive and self-referential processing. This mini-review synthesizes research evidence from cerebellar structural and functional neuroimaging in depression, and provides future perspectives for neuroimaging of cerebellar contributions to MDD.

Keywords: major depression, cerebellum, cerebro-cerebellar networks, VBM, intrinsic connectivity

OPEN ACCESS

Edited by:
Martin Walter,
University of Tubingen, Germany

Reviewed by:
Aislinn Joanmarie Williams,
University of Iowa, United States
Sebastian Walther,
Universität Bern, Switzerland

****Correspondence:***
Robert C. Wolf
christian.wolf@med.uni-heidelberg.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *08 August 2018*
Accepted: *08 November 2018*
Published: *29 November 2018*

Citation:
Depping MS, Schmitgen MM, Kubera KM and Wolf RC (2018) Cerebellar Contributions to Major Depression. Front. Psychiatry 9:634. doi: 10.3389/fpsyt.2018.00634

INTRODUCTION

Lesions of the cerebellar posterior hemispheres and the cerebellar vermis frequently result in cognitive and/or affective symptoms, sometimes referred to as the "cerebellar cognitive affective syndrome" (1). It is characterized by deficits in executive function and linguistic processing, as well as by emotion dysregulation (emotional lability, impaired social cognition, apathy, or depressed mood) (2). Electrical stimulation experiments in animal models have additionally related cerebellar neural activity to anxious and impulsive behavior (3–5). Over the past decade, cerebellar contributions to non-motor functions have attracted increasing scientific interest. In particular, structural and functional neuroimaging has allowed for a more detailed understanding of the "cognitive/affective cerebellum" (6, 7). Against this background, the question has been raised whether abnormal cerebellar structure or function may contribute to major depressive disorder (MDD) (8).

In this mini-review, we briefly summarize the available structural and functional neuroimaging data in humans regarding the role of the cerebellum in depression. We identify current research limitations and discuss future perspectives for neuroimaging of cerebellar contributions to MDD. To describe cerebellar anatomy, we use a revised version of the Larsell nomenclature (9), as suggested by Schmahmann et al. (10).

FUNCTIONAL NEUROANATOMY OF THE "COGNITIVE-AFFECTIVE CEREBELLUM"

The cerebellum's histology is uniform throughout cortex, i.e., unlike the cerebral cortex, it has no discernable areal boundaries (11). The spatial organization of somatosensory, cognitive and affective representations within the cerebellum relies on polysynaptic connections that different subregions of the cerebellum form with functionally distinct regions of the cerebrum (12). MRI-Analyses of intrinsic functional connectivity (fcMRI) provide a means to discern theses cerebro-cerebellar circuits, thereby revealing the functional topography of the cerebellum. This evidence is corroborated by older electrophysiological and tract tracing experiments in animal models (13, 14). In healthy human subjects, several fcMRI studies of cerebro-cerebellar coupling are available (15–18). So far, the most comprehensive cerebellar mapping has been performed by Buckner et al. (17). Including 1,000 healthy subjects and employing different fcMRI approaches, the authors demonstrated that approximately half of the cerebellar cortex is associated with higher-level cognitive and affective functions (17). Non-motor functions are represented in the posterior lobe of the cerebellum, i.e., within cerebellar lobules VI–IX. **Figure 1** shows an illustration of the unfolded cerebellar cortex. The intrinsic connectivity networks (ICN) (19) in which the non-motor cerebellum participates, include the "cognitive control network" (CCN) (20), the "salience network" (SN) (20), and the "default mode network" (DMN) (21). Additionally, a "cerebello-amygdaloid network" (CAN) has been suggested (18). Consistent evidence for cerebellar functional topography has emerged, especially within the cognitive domain. All available MRI studies of cerebellar connectivity in healthy subjects suggest that the anterior part of lobule VIIA, crus I and II, is related to the CCN (15–17, 22, 23). Two studies suggest that major connections to the DMN originate from the posterior part of crus I and II of lobule VIIA (15, 17); one study suggests that lobule IX is an essential cerebellar representation of the DMN (22). Cerebellar contributions to the SN seem to come from lobule VI (18, 23) and from lobules VIIB and VIII (23). Finally, lobules VI and VIII (18, 23), as well as the vermal portions of lobules VIIB, VIII, and IX (23) seem to have functional connections to the amygdala, potentially representing an independent ICN (18). In summary, there are functional subregions within, and sometimes extending beyond, each of the lobules VI–IX. Within one cerebellar lobule, segregated subregions are associated with distinct functional networks, differentially supporting cognitive or affective processing. An open research question is whether the hemispheric and the vermal portions of one lobule have the same (17) or separate (23) connectivity patterns.

Task-related cerebellar activations have been investigated in several MRI and PET studies. Verbal working memory, executive functions and emotional functions have been probed. These studies build on a cumulative number of ∼100–150 subjects per task category. Stoodley and Schmahmann (6) conducted an "activation likelihood estimate" (ALE) meta-analysis of these findings. Consistent with the fcMRI studies, lobule VIIA, crus I was associated with cognitive functions (6). Further cognitive representations were detected in lobules VI and VIIB. The substrates of emotional processing were localized in lobule VI and in the vermal portion of lobule VII, in agreement with the functional connectivity results by Sang et al. (23) and Habas (18). Additional affective representations were localized in lobule VIIA, crus I (6).

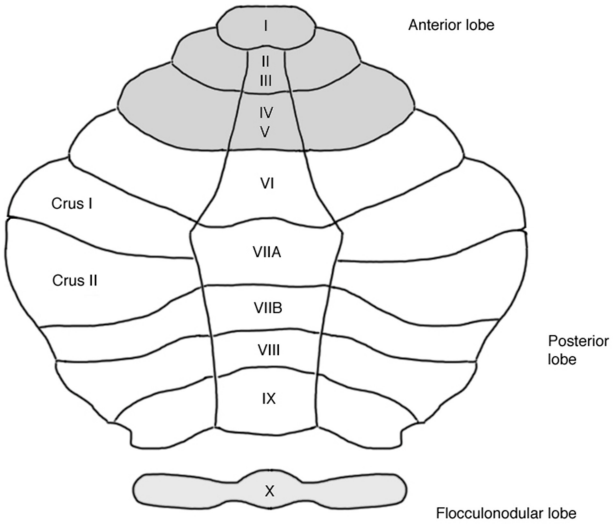

FIGURE 1 | Unfolded view of the cerebellar cortex showing lobes and lobules **(I–X)**. Anatomical labeling according to a revised version of the Larsell nomenclature (9), as described by Schmahmann et al. (10). Each cerebellar lobule comprises an unpaired medial (i.e., vermal) portion and a bilateral hemispheric portion. The posterior lobe represents the "cognitive/affective" cerebellum. Approximately half of the cerebellar cortex is associated with non-motor functions (17), see text for details.

In summary, the topographic organization of cognitive and affective representations in the cerebellum is complex. The cerebellar lobules within the "cognitive/affective cerebellum" consist of subregions with distinct connectivity patterns and functions. Across different imaging methodologies, there is most consistent evidence for the involvement of the anterior part of lobule VIIA, crus I and II, in cognitive processing (6, 15–18, 23). Clearly, there are also cerebellar contributions to the DMN, likely involving the posterior part of lobule VIIA, crus I and II (15, 17, 23), and potentially also lobule IX (15, 23). Furthermore, consistent evidence suggests that that lobule VI is associated with emotional processing (6, 18, 23). Finally, there is preliminary evidence for lobule VI involvement in the salience network (22, 23). Convergent findings are summarized in **Table 1**.

ABNORMAL CEREBELLAR STRUCTURE AND FUNCTION IN PATIENTS WITH DEPRESSION

Structural and functional changes of non-motor cerebellar regions in patients with depression have been subject to several MRI studies: Cerebellar connectivity has been investigated by

TABLE 1 | Topographic organization of the non-motor cerebellum, as suggested by cerebellar neuroimaging.

Psychological domain	Cerebellar subregion	Neuroimaging study
Cognitive representations (CCN)	Lobule VIIA, crus I and II (anterior part)	(6, 15–17, 22, 23)
Self-referential representations (DMN)	Lobule VIIA, crus I and II (posterior part)	(15, 17, 23)
	Lobule VIIA (vermal part)	(17)
	Lobule IX	(15, 23)
Affective representations (CAN)	Lobule VI	(6, 18, 23)
Representations of the salience network (SN)	Lobule VI	(22, 23)

Within one cerebellar lobule, segregated subregions are associated with distinct functional networks, differentially supporting cognitive, self-referential, affective, or salience processing, see text for details. CCN, cognitive control network; DMN, default mode-network; CAN, cerebello-amygdaloid network.

six studies (24–29), cerebellar resting-state blood flow has been studied by one study (30), and cerebellum-optimized structural data analysis have been applied by two investigations (31, 32). Task-based imaging studies of the cerebellum in depressed patients are lacking at present.

Cognitive Representations

Consistent findings indicate that lobule VIIA, crus I and II, shows reduced connectivity to the cerebral components of the CCN in patients with depression, particularly to the dorsolateral prefrontal cortex (dlPFC) (24, 25, 27), but also to the ventromedial prefrontal cortex (vmPFC) (24, 28). In further support for the pathophysiological relevance of abnormal cerebellar-CCN coupling in depression, reduced lobule VIIA-vmPFC connectivity has been found to significantly correlate with impaired verbal working memory performance in depressed individuals (24). Finally, treatment with electroconvulsive therapy (ECT) seems to modulate the structure of an anterior subregion within lobule VIIA, crus I, in patients with severe acute depression (32). Notably, this structural change was associated with the antidepressant efficacy of ECT (32).

Self-Referential Representations (DMN)

Aberrant activity of the DMN's cerebellar components has been repeatedly demonstrated in patients with depression (24, 25, 27, 28), however, the specific spatial distribution and the direction of change is unclear at present. In depressed patients, Guo et al. (27) reported reduced connectivity of lobule VII, crus I, to the inferior parietal cortex and to the inferior temporal cortex, Liu et al. (25) reported reduced connectivity of lobule VIIA, crus II, to the posterior cingulate cortex (PCC), while Alalade et al. (24) reported increased connectivity of the vermal portion of lobule VIIA to the PCC. The latter finding was associated with the severity of depressive symptoms (24). Aberrant functional connectivity of the cerebellar vermis with key components of the DMN, i.e., with the PCC (25), as well as with the anterior cingulate cortex and the vmPFC (28) have also been described. However, it is important to note that these studies did not consider the functional segmentation of the vermis. Taking into consideration that vermal topography is highly complex (7, 17, 23), only limited conclusions can be drawn from these investigations. In patients with depression, more sophisticated approaches are required to specify aberrant cerebellar DMN activity. Finally, preliminary evidence has been presented for structural abnormalities of a cerebellar DMN component in depression. Independent of acute or remitted disease stage, lobule IX volume appears to be increased in patients with recurrent depression (31).

Representations of Other Domains

In depressed patients, no studies have yet been published on the structure or connectivity of cerebellar affective representations or of cerebellar representations of the salience network.

THE CEREBELLUM IN DEPRESSION-STATE OF KNOWLEDGE AND FUTURE PERSPECTIVES

There is a special role for lobule VII within the "affective/cognitive cerebellum." Unlike all other cerebellar lobules, lobule VII is not connected with the somatomotor system. Lobule VII communicates exclusively with cognitive and affective cerebral association cortices and with (para-) limbic structures (17). Cerebellar lobule VII contains subregions that are associated with cognitive, self-referential, and affective processing. In particular, strong evidence has accumulated for the involvement of lobule VII in cognitive processes. Convincing evidence has been provided for abnormal structure and function of lobule VII in patients with MDD. Multiple studies report decreased connectivity of lobule VII with cortical components of the CCN (24, 25, 27, 28). Preliminary findings also highlight the neuropsychological relevance of abnormal lobule VII-CCN coupling in patients with depression, i.e., an association between lobule VII-CCN connectivity and delayed memory recall (24). There are also cerebellar representations of the DMN and of the SN, as well as a putative cerebro-cerebellar ICN involving the amygdala (18), but their functional neuroanatomy is incompletely characterized at present.

There are two major limitations in current imaging approaches toward understanding the role of the cerebellum in depression. First, due to methodological constraints, the complex functional topography of the cerebellar vermis has been insufficiently addressed. At present, there is a considerable dearth of knowledge on dysfunction of the cerebellar vermis in depression. This is in contrast to the significance of the vermis for affective processes as illustrated by lesion studies (1). To accurately map the vermis' functional topography in depression, cerebellum-optimized data analysis protocols should be used by future MRI investigations. Cerebellum-optimized protocols have proven to result in superior neuroanatomical precision compared to conventional methods (33). Second, it has been a key observation of recent MRI studies that cerebellar lobules are polymodal structures with segregated, functionally distinct subregions. In fcMRI studies, it is of

critical importance to consider these topographical details when placing the seed regions for connectivity analyses. The available fcMRI studies of cerebro-cerebellar connectivity in depression may not have been sufficiently accurate in this regard, potentially explaining the inconsistent cerebellar DMN findings in patients with MDD. Future studies should prefer data-driven extraction of cerebro-cerebellar ICNs. Validation of these findings by means of seed-based connectivity analyses may be performed, if accurate seed placement can be guaranteed based on previous data-driven identification of cerebellar ICN components (17).

Some general limitations of available brain imaging data in depression also apply to cerebellar imaging in MDD patients. First, depression is a clinically and biologically heterogeneous disorder, yet only few brain imaging studies have attempted to subtype patients based on clinical, neuropsychological or neurophysiological features (34). Second, in many studies, heterogeneity of psychotropic medication is a potential confound. Third, there is a lack of longitudinal brain imaging data in depression research. As a consequence, a significant number of fundamental research questions may not be answered. In particular, there is a need to discern the causal influence of depression risk factors on brain morphology and function. These limitations should be addressed when designing future studies to investigate cerebellar contributions to major depression.

This review emphasizes the role of non-motor cerebellar regions in patients with depression. Psychomotor disturbances, particularly psychomotor slowing, can be an important feature of major depression (35). The cerebral correlates of aberrant psychomotor functioning have been subject of several neuroimaging investigations (36–38). It should not be forgotten that future studies of the cerebellum in MDD will clearly benefit from investigating cerebro-cerebellar motor systems.

AUTHOR CONTRIBUTIONS

MD and RW wrote the manuscript. MS and KK contributed to the interpretation of data.

ACKNOWLEDGMENTS

We acknowledge financial support by Deutsche Forschungsgemeinschaft (funding programme Open Access Publishing), by the Baden-Württemberg Ministry of Science, Research and the Arts, and by Ruprecht-Karls-Universität Heidelberg. RW was supported by Deutsche Forschungsgemeinschaft, grant number WO1883/4-1.

REFERENCES

1. Schmahmann JD, Weilburg JB, Sherman JC. The neuropsychiatry of the cerebellum - insights from the clinic. *Cerebellum* (2007) 6:254–67. doi: 10.1080/14734220701490995
2. Hoche F, Guell X, Vangel MG, Sherman JC, Schmahmann JD. The cerebellar cognitive affective/Schmahmann syndrome scale. *Brain* (2018) 141:248–70. doi: 10.1093/brain/awx317
3. Moers-Hornikx VM, Sesia T, Basar K, Lim LW, Hoogland G, Steinbusch HW, et al. Cerebellar nuclei are involved in impulsive behaviour. *Behav Brain Res.* (2009) 203:256–63. doi: 10.1016/j.bbr.2009.05.011
4. Moers-Hornikx VM, Vles JS, Lim LW, Ayyildiz M, Kaplan S, Gavilanes AW, et al. Periaqueductal grey stimulation induced panic-like behaviour is accompanied by deactivation of the deep cerebellar nuclei. *Cerebellum* (2011) 10:61–9. doi: 10.1007/s12311-010-0228-z
5. Huguet G, Kadar E, Temel Y, Lim LW. Electrical stimulation normalizes c-Fos expression in the deep cerebellar nuclei of depressive-like rats: implication of antidepressant activity. *Cerebellum* (2017) 16:398–410. doi: 10.1007/s12311-016-0812-y
6. Stoodley CJ, Schmahmann JD. Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *Neuroimage* (2009) 44:489–501. doi: 10.1016/j.neuroimage.2008.08.039
7. Stoodley CJ, Schmahmann JD. Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex* (2010) 46:831–44. doi: 10.1016/j.cortex.2009.11.008
8. Konarski JZ, Mcintyre RS, Grupp LA, Kennedy SH. Is the cerebellum relevant in the circuitry of neuropsychiatric disorders? *J Psychiatry Neurosci.* (2005) 30:178–86.
9. Larsell O. *The Comparative Anatomy and Histology of the Cerebellum From Monotremes Through Apes*. Minneapolis, MN: The University of Minnesota Press (1970).
10. Schmahmann JD, Doyon J, Mcdonald D, Holmes C, Lavoie K, Hurwitz AS, et al. Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. *Neuroimage* (1999) 10:233–60. doi: 10.1006/nimg.1999.0459
11. Ito M. *The Cerebellum and Neural Control*. New York, NY: Raven (1984).
12. Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. *Annu Rev Neurosci.* (2009) 32:413–34. doi: 10.1146/annurev.neuro.31.060407.125606
13. Heath RG, Harper JW. Ascending projections of the cerebellar fastigial nucleus to the hippocampus, amygdala, and other temporal lobe sites: evoked potential and histological studies in monkeys and cats. *Exp Neurol.* (1974) 45:268–87. doi: 10.1016/0014-4886(74)90118-6
14. Kelly RM, Strick PL. Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J Neurosci.* (2003) 23:8432–44. doi: 10.1523/JNEUROSCI.23-23-08432.2003
15. Krienen FM, Buckner RL. Segregated fronto-cerebellar circuits revealed by intrinsic functional connectivity. *Cereb Cortex* (2009) 19:2485–97. doi: 10.1093/cercor/bhp135
16. O'reilly JX, Beckmann CF, Tomassini V, Ramnani N, Johansen-Berg H. Distinct and overlapping functional zones in the cerebellum defined by resting state functional connectivity. *Cereb Cortex* (2010) 20:953–65. doi: 10.1093/cercor/bhp157
17. Buckner RL, Krienen FM, Castellanos A, Diaz JC, Yeo BT. The organization of the human cerebellum estimated by intrinsic functional connectivity. *J Neurophysiol.* (2011) 106:2322–45. doi: 10.1152/jn.00339.2011
18. Habas C. Research note: a resting-state, cerebello-amygdaloid intrinsically connected network. *Cereb Ataxias* (2018) 5:4. doi: 10.1186/s40673-018-0083-0
19. Yeo BT, Krienen FM, Sepulcre J, Sabuncu MR, Lashkari D, Hollinshead M, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *J Neurophysiol.* (2011) 106:1125–65. doi: 10.1152/jn.00338.2011
20. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci.* (2007) 27:2349–56. doi: 10.1523/JNEUROSCI.5587-06.2007
21. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc Natl Acad Sci USA.* (2003) 100:253–8. doi: 10.1073/pnas.0135058100
22. Habas C, Kamdar N, Nguyen D, Prater K, Beckmann CF, Menon V, et al. Distinct cerebellar contributions to intrinsic connectivity networks. *J Neurosci.* (2009) 29:8586–94. doi: 10.1523/JNEUROSCI.1868-09.2009

23. Sang L, Qin W, Liu Y, Han W, Zhang Y, Jiang T, et al. Resting-state functional connectivity of the vermal and hemispheric subregions of the cerebellum with both the cerebral cortical networks and subcortical structures. *Neuroimage* (2012) 61:1213–25. doi: 10.1016/j.neuroimage.2012.04.011
24. Alalade E, Denny K, Potter G, Steffens D, Wang L. Altered cerebellar-cerebral functional connectivity in geriatric depression. *PLoS ONE* (2011) 6:e20035. doi: 10.1371/journal.pone.0020035
25. Liu L, Zeng LL, Li Y, Ma Q, Li B, Shen H, et al. Altered cerebellar functional connectivity with intrinsic connectivity networks in adults with major depressive disorder. *PLoS ONE* (2012) 7:e39516. doi: 10.1371/journal.pone.0039516
26. Guo W, Liu F, Liu J, Yu L, Zhang Z, Zhang J, et al. Is there a cerebellar compensatory effort in first-episode, treatment-naive major depressive disorder at rest? *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 46:13–8. doi: 10.1016/j.pnpbp.2013.06.009
27. Guo W, Liu F, Xue Z, Gao K, Liu Z, Xiao C, et al. Abnormal resting-state cerebellar-cerebral functional connectivity in treatment-resistant depression and treatment sensitive depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 44:51–7. doi: 10.1016/j.pnpbp.2013.01.010
28. Ma Q, Zeng LL, Shen H, Liu L, Hu D. Altered cerebellar-cerebral resting-state functional connectivity reliably identifies major depressive disorder. *Brain Res.* (2013) 1495:86–94. doi: 10.1016/j.brainres.2012.12.002
29. Guo W, Liu F, Liu J, Yu M, Zhang Z, Liu G, et al. Increased cerebellar-default-mode-network connectivity in drug-naive major depressive disorder at rest. *Medicine* (2015) 94:e560. doi: 10.1097/MD.0000000000000560
30. Depping MS, Wolf ND, Vasic N, Sosic-Vasic Z, Schmitgen MM, Sambataro F, et al. Aberrant resting-state cerebellar blood flow in major depression. *J Affect Disord.* (2018) 226:227–31. doi: 10.1016/j.jad.2017.09.028
31. Depping MS, Wolf ND, Vasic N, Sambataro F, Hirjak D, Thomann PA, et al. Abnormal cerebellar volume in acute and remitted major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2016) 71:97–102. doi: 10.1016/j.pnpbp.2016.06.005
32. Depping MS, Nolte HM, Hirjak D, Palm E, Hofer S, Stieltjes B, et al. Cerebellar volume change in response to electroconvulsive therapy in patients with major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2017) 73:31–5. doi: 10.1016/j.pnpbp.2016.09.007
33. Diedrichsen J. A spatially unbiased atlas template of the human cerebellum. *Neuroimage* (2006) 33:127–38. doi: 10.1016/j.neuroimage.2006.05.056
34. Drysdale AT, Grosenick L, Downar J, Dunlop K, Mansouri F, Meng Y, et al. Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat Med.* (2017) 23:28–38. doi: 10.1038/nm.4246
35. Buyukdura JS, Mcclintock SM, Croarkin PE. Psychomotor retardation in depression: biological underpinnings, measurement, and treatment. *Prog Neuropsychopharmacol Biol Psychiatry* (2011) 35:395–409. doi: 10.1016/j.pnpbp.2010.10.019
36. Bracht T, Federspiel A, Schnell S, Horn H, Hofle O, Wiest R, et al. Cortico-cortical white matter motor pathway microstructure is related to psychomotor retardation in major depressive disorder. *PLoS ONE* (2012) 7:e52238. doi: 10.1371/journal.pone.0052238
37. Liberg B, Rahm C. The functional anatomy of psychomotor disturbances in major depressive disorder. *Front Psychiatry* (2015) 6:34. doi: 10.3389/fpsyt.2015.00034
38. Hyett MP, Perry A, Breakspear M, Wen W, Parker GB. White matter alterations in the internal capsule and psychomotor impairment in melancholic depression. *PLoS ONE* (2018) 13:e0195672. doi: 10.1371/journal.pone.0195672

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

PERSPECTIVE
published: 19 December 2018
doi: 10.3389/fpsyt.2018.00687

The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder

Elena Bartkiene[1], Vesta Steibliene[2], Virginija Adomaitiene[2], Vita Lele[1], Darius Cernauskas[3,4], Daiva Zadeike[3], Dovile Klupsaite[3] and Grazina Juodeikiene[3]*

[1] Department of Food Safety and Quality, Lithuanian University of Health Sciences, Kaunas, Lithuania, [2] Psychiatric Clinic, Lithuanian University of Health Sciences, Kaunas, Lithuania, [3] Department of Food Science and Technology, Kaunas University of Technology, Kaunas, Lithuania, [4] Food Institute, Kaunas University of Technology, Kaunas, Lithuania

OPEN ACCESS

Edited by:
Martin Walter, University of Tubingen, Germany

Reviewed by:
Matthew Paul Hyett, Queensland Institute of Medical Research, Australia
Steffi Riedel-Heller, Universitätsklinikum Leipzig, Germany
Marie Dorow, Leipzig University, in collaboration with reviewer SRH, Germany

***Correspondence:**
Elena Bartkiene
elena.bartkiene@lsmuni.lt

Specialty section:
This article was submitted to Computational Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *17 March 2018*
Accepted: *26 November 2018*
Published: *19 December 2018*

Citation:
Bartkiene E, Steibliene V, Adomaitiene V, Lele V, Cernauskas D, Zadeike D, Klupsaite D and Juodeikiene G (2018) The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder. Front. Psychiatry 9:687. doi: 10.3389/fpsyt.2018.00687

Depressive disorder (DD) shortens a healthy and productive human life, has significant public health costs is and associated with high suicide rates. In depression sadness and emotional misery manifest in facial expressions, as psychomotor slowing, lack of energy, high tension, and attenuated sensory perception. Loss of appetite, changes to the taste of food, and the loss of pleasure in eating are important criteria in the diagnosis of DD. We hypothesized that a patient's facial expressions and emotional responses to different tastes can be used as the diagnostic moderators for the development of a new contactless, computer-based method for diagnosis of DD. The confirmation of this hypothesis can shed a new perspective on early contactless, computer-based psychiatric diagnostic strategies and early identification of DD symptoms, as DD is an important issue in public mental health. The benefits of this method are evidence from several perspectives (I) patients can use a self-rating instrument to assess DD symptoms; this may act as an incentive to seek professional help; (II) family and community can use an instrument for early recognition of DD symptoms and suicidal tendencies, making it possible to encourage the individual to seek professional health care; (III) general practitioners have a reliable instrument for preliminary diagnosis of DD in primary care, thus saving the time and resources; (IV) public health benefits include early diagnosis and treatment of DD and better outcomes, reductions in disability-adjusted life years and the global burden of the disease. It is nevertheless important to recognize the limitations and risks of contactless diagnosis of DD. As it is a self-assessment method it is not possible to rule out false positives and false negatives. However, this method might be used for early diagnosis of DD symptoms. Also, it should be mentioned that further evaluation and an experts opinion about this method is needed. The clinical diagnosis of DD should continue to be made by healthcare professionals. Finally, this method may perspectively predict DD at an early stage and may ensure a higher quality of the patients' primary care in the public health system.

Keywords: depressive disorder, food, food taste, emotions, FaceReader, computer based diagnostic

Depressive disorder (DD) is a common, often progressive and recurrent disorder. If left untreated or treated inadequately it may become chronic and treatment-resistant, with a significant negative impact on everyday functioning. It shortens a healthy and productive human life, has significant public health costs and is associated with high suicide rates. According to the World Health According (WHO), DD affects more than 300 million people worldwide and close to 800,000 people die by suicide every year; the WHO estimates that by 2020 the prevalence of DD will reach 5.7% and it will have become the second most common cause of disability-adjusted life years worldwide (1). DD develops gradually and in many cases the sufferer does not recognize the initial symptoms and seeks help only when the disorder has become severe. Although effective treatments for DD are available, globally fewer than half of those affected (in many countries, fewer than 10%) receive such treatment (2). If DD remains untreated for a long period this has a negative impact on treatment effectiveness, the duration of the disorder, outcomes and survival of somatic comorbidities (3–5). DD is associated with an imbalance in neurotransmitters, pituitary-hypothalamic-adrenal axis dysfunction, and changes in levels of neurotrophic factors that are considered direct precursors of neurodegenerative disorders and dementia (6–9). In primary care DD often remains undiagnosed and untreated. Due to the stigma associated with the disease patients are often reluctant to seek psychiatric help and long waiting times and gaps in the healthcare system can also reduce access to treatment. Moreover, patients often do not report depressive symptoms to their family doctor; due to lack of time family doctors do not use screening instruments and often fail to recognize symptoms of DD or offer only inadequate treatment (10). This increases suicide rates dramatically (11).

Better understanding of the pathophysiology of DD has led to evaluation of different genetic, neuroimaging, and biochemical biomarkers and instruments for objective diagnosis of DD in clinical settings (12). Given the need for a reliable, brief, and easy-to-administer screening instrument to help diagnose DD, the development of a contactless, computer-based method of early diagnosis should be a priority (12).

There is a growing body of evidence on the influence of nutrition on mental health, particularly affective symptoms (13). It has been established that diet and individual food

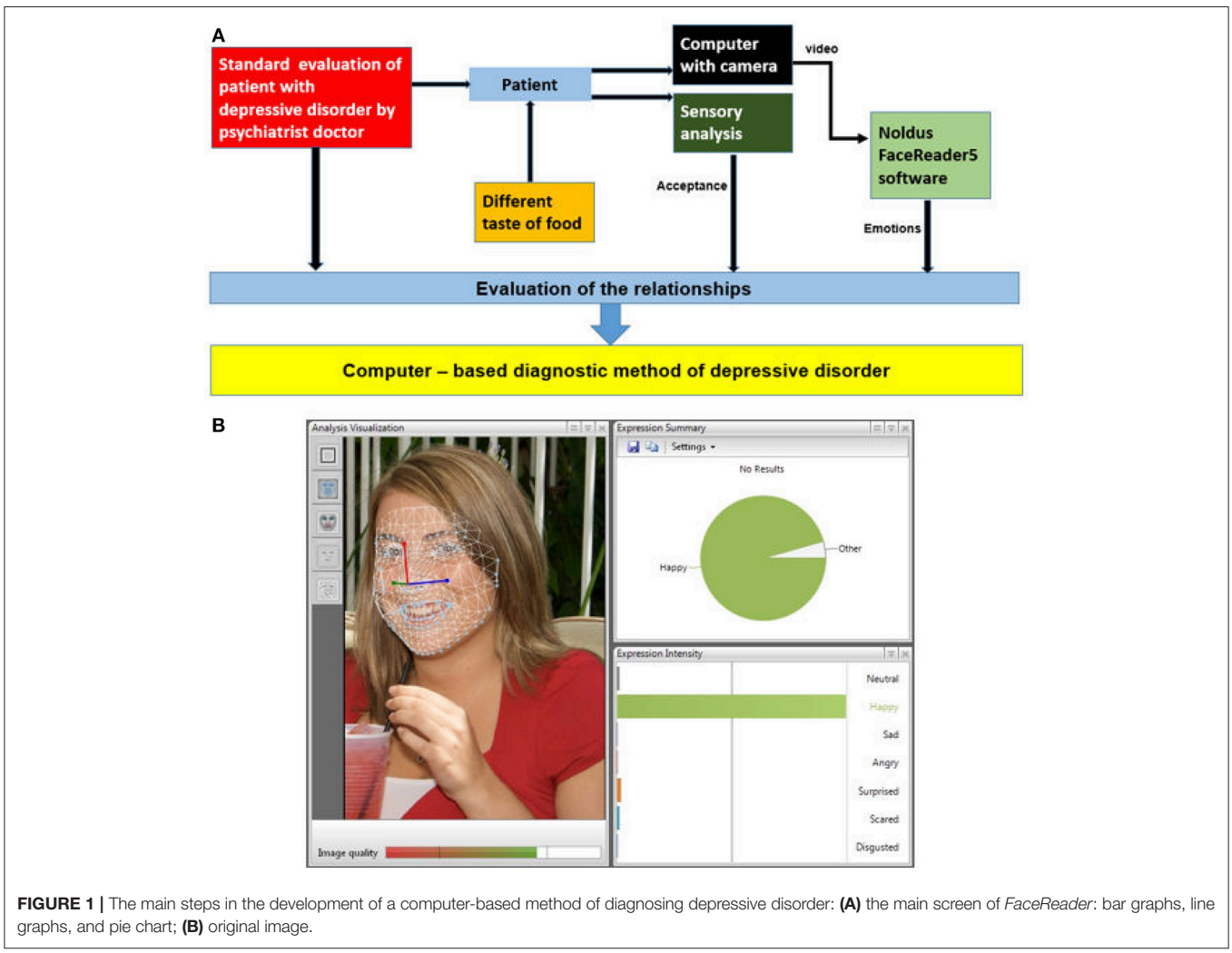

FIGURE 1 | The main steps in the development of a computer-based method of diagnosing depressive disorder: **(A)** the main screen of *FaceReader*: bar graphs, line graphs, and pie chart; **(B)** original image.

products influence the occurrence, onset, severity, and duration of DD (14). Newly developed effective public health nutritional strategies could be used to prevent clinical depression (15). Depression manifests as sadness, and emotional misery that is visible in facial expressions, as psychomotor slowing, low energy, high tension, and attenuated sensory perception. Loss of appetite, loss of sense of taste, and pleasure in eating are important DD diagnostic criteria, although atypical depression may be characterized by a strong preference for energy-dense foods, fatty foods, and sweet foods. Uncontrolled eating, satiation and altered food preferences as well as appetite may indicate negative mood (16). The mentioned changes could be due to the influence of carbohydrates and proteins on the tryptophan ratio in the brain, as well as to the effects of such changes on mood and arousal given that there could be individual differences in susceptibility to nutritional effects on mood, emotion and aspects of brain function (17).

It is known that most depressed patients show impaired production of emotional facial expressions, particularly positive expressions (18). It has been reported that affective facial reactions to the food tastes are important factors in food intake and even predict body mass index (19). Facial expressions and emotional response to difference tastes can be measured using *FaceReader 4* software (Noldus Information Technology, The Netherlands). The data obtained from the facial expression of emotions to the different food tastes have been shown to correlate with data of conventional sensory analysis (20). Our previous research showed that the evaluations of facial expressions obtained using FaceReader 4 software were strongly correlated with self-reported hedonic liking, indicating that computer-based technology can be a sufficiently accurate tool for the food tastes induced emotions evaluation (21).

On this basis we hypothesized that seven categories of emotional response (neutral; angry; disgusted; happy; sad; scared; surprised) to different tastes (neutral; acid; sweet; salt; bitter) would be strongly correlated with clinical symptoms of DD as evaluated using clinical diagnostic instruments and depression severity rating scales (Montgomery and Asberg Depression Rating Scale MADRS; (22)]. We also hypothesized that a patient's facial expression of emotions to different tastes of food can be used as a diagnostic moderator for the development of a new contactless, computer-based diagnostic method and support the creation of algorithm for DD diagnosis (**Figure 1**).

The benefits of this method are evidence from several perspectives (I) patients can use a self-rating instrument to assess DD symptoms; this may act as an incentive to seek professional help; (II) family and community can use an instrument for early recognition of DD symptoms and suicidal tendencies, making it possible to encourage the individual to seek professional health care; (III) general practitioners have a reliable instrument for preliminary diagnosis of DD in primary care, thus saving the time and resources; (IV) public health benefits include early diagnosis and treatment of DD and better outcomes, reductions in disability-adjusted life years and the global burden of the disease.

It is nevertheless important to discuss the limitations and the risks of contactless diagnosis of DD. Since this is a self-assessment method it is not possible to rule out false positives and false negatives.

However, this method might be used for early diagnosis of DD symptoms. Also, it should be mentioned that further evaluation and experts opinion regarding relevance and accuracy of this method is needed. The clinical validation of DD diagnosis should be performed by healthcare professionals. Finally, this method may perspectively predict DD at an early stage and may ensure a higher quality of the patients' primary care in the public health system.

AUTHOR CONTRIBUTIONS

EB and VS designed the study and wrote up the initial draft. VA and GJ are principle supervisors overseeing the study. VL, DZ, DK, and DC were involved with the design of the study and the drafting of the paper.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Research Council of Lithuania for financial support (Project EMOPSYCHOSCREEN No. P-MIP-17-49).

REFERENCES

1. World Health Organisation (WHO). *Depression and Other Common Mental Disorders*. Geneva: Global Health Estimates (2017).
2. Reddy MS. Depression: the disorder and the burden. *Indian J Psychol Med*. (2010) 32:1–2. doi: 10.4103/0253-7176.70510
3. Bukh JD, Bock C, Vinberg M, Kessing LV. The effect of prolonged duration of untreated depression on antidepressant treatment outcome. *J Affect Disord*. (2013) 145:42–8. doi: 10.1016/j.jad.2012.07.008
4. Hung CI, Yu NW, Liu CY, Wu KY, Yang CH. The impact of the duration of an untreated episode on improvement of depression and somatic symptoms. *Neuropsychiatr Dis Treat*. (2015) 11:2245–52. doi: 10.2147/NDT.S89498
5. Ghio L, Gotelli S, Cervetti A, Respino M, Natta W, Marcenaro M, et al. Duration of untreated depression influences clinical outcomes and disability. *J Affect Disord*. (2015) 175:224–8. doi: 10.1016/j.jad.2015.01.014
6. Byers AL, Yaffe K. Depression and risk of developing dementia. *Nat Rev Neurol*. (2011) 7:323–31. doi: 10.1038/nrneurol.2011.60
7. Naert G, Ixart G, Maurice T, Tapia-Arancibia L, Givalois L. Brain-derived neurotrophic factor and hypothalamic-pituitary-adrenal axis adaptation processes in a depressive-like state induced by chronic restraint stress. *Mol Cell Neurosci*. (2011) 46:55–66. doi: 10.1016/j.mcn.2010.08.006
8. Du X, Pang TT. Is dysregulation of the hpa-axis a core pathophysiology mediating co-morbid depression in neurodegenerative diseases? *Front Psychiatry* (2015) 6:32. doi: 10.3389/fpsyt.2015.00032
9. Lebedeva AK, Westman E, Borza T, Beyer MK, Engedal K, Aarsland D, et al. MRI-Based classification models in prediction of mild cognitive impairment and dementia in late-life depression. *Front Aging Neurosci*. (2017) 2:9–13. doi: 10.3389/fnagi.2017.00013
10. Craven MA, Bland R. Depression in primary care: current and future challenges. *Can J Psychiatry* (2013) 58:442–8. doi: 10.1177/070674371305800802
11. Rihmer Z, Gonda X. Prevention of depression-related suicides in primary care. *Psychiatr Hung*. (2012) 27:72–81.

12. Young JJ, Silber T, Bruno D, Galatzer-Levy IR, Pomara N, Marmar CR. Is there progress? An overview of selecting biomarker candidates for major depressive disorder. *Front Psychiatry* (2016) 7:72. doi: 10.3389/fpsyt.2016.00072
13. Zepf FD, Hood S, Guillemin GJ. Food and your mood: nutritional psychiatry. *Lancet Psychiatry* (2015) 2:19. doi: 10.1016/S2215-0366(15)00241-2
14. Roca M, Kohls E, Gili M, Watkins E, Owens M, Hegerl U, et al. Prevention of depression through nutritional strategies in high-risk persons: rationale and design of the MooDFOOD prevention trial. *BMC Psychiatry* (2016) 16:192. doi: 10.1186/s12888-016-0900-z
15. O'Neil A, Berk M, Itsiopoulos C, Castle D, Opie R, Pizzinga J, et al. A randomised, controlled trial of a dietary intervention for adults with major depression (the "SMILES" trial): study protocol. *BMC Psychiatry* (2013) 13:114. doi: 10.1186/1471-244X-13-114
16. Singh M. Mood, food, and obesity. *Front Psychol.* (2014) 5:925. doi: 10.3389/fpsyg.2014.00925
17. Rao TS, Asha MR, Ramesh BN, Rao KS. Understanding nutrition, depression and mental illnesses. *Indian, J Psychol Med*. (2008) 50:77–82. doi: 10.4103/0019-5545.42391
18. Jaeger J, Borod JC, Peselow E. Facial expression of positive and negative emotions in patients with unipolar depression. *J Affect Disord*. (1986) 11:43–50. doi: 10.1016/0165-0327(86)90058-3
19. Garcia-Burgos D, Zamora MC. Facial affective reactions to bitter-tasting foods and body mass index in adults. *Appetite* (2013) 71:178–86. doi: 10.1016/j.appet.2013.08.013
20. Danner L, Haindl S, Joechl M, Duerrschmid K. Facial expressions and autonomous nervous system responses elicited by tasting different juices. *Food Res Int*. (2014) 64:81–90. doi: 10.1016/j.foodres.2014.06.003
21. Juodeikiene G, Basinskiene L, Vidmantiene D, Klupsaite D, Bartkiene E. The use of face reading technology to predict consumer acceptance of baked goods. In: *7th International Congress "Flour-Bread'13" and 9th Croatian Congress of Cereal Technologists "Brašno-Kruh'13"*. Opatija (2013). p. 72.
22. Williams JB, Kobak KA. Development and reliability of a structured interview guide for the Montgomery Asberg Depression Rating Scale (SIGMA). *Br J Psychiatry* (2008) 192:52–8. doi: 10.1192/bjp.bp.106.032532

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

CORRECTION
published: 29 January 2019
doi: 10.3389/fpsyt.2019.00012

Corrigendum: The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder

Elena Bartkiene[1], Vesta Steibliene[2], Virginija Adomaitiene[2], Vita Lele[1], Darius Cernauskas[3,4], Daiva Zadeike[3], Dovile Klupsaite[3] and Grazina Juodeikiene[3]*

[1] Department of Food Safety and Quality, Lithuanian University of Health Sciences, Kaunas, Lithuania, [2] Psychiatric Clinic, Lithuanian University of Health Sciences, Kaunas, Lithuania, [3] Department of Food Science and Technology, Kaunas University of Technology, Kaunas, Lithuania, [4] Food Institute, Kaunas University of Technology, Kaunas, Lithuania

Keywords: depressive disorder, food, food taste, emotions, FaceReader, computer based diagnostic

OPEN ACCESS

Approved by:
Frontiers in Psychiatry Editorial Office, Frontiers Media SA, Switzerland

****Correspondence:***
Elena Bartkiene
elena.bartkiene@lsmuni.lt

Specialty section:
This article was submitted to Computational Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *03 January 2019*
Accepted: *09 January 2019*
Published: *29 January 2019*

Citation:
Bartkiene E, Steibliene V, Adomaitiene V, Lele V, Cernauskas D, Zadeike D, Klupsaite D and Juodeikiene G (2019) Corrigendum: The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder. Front. Psychiatry 10:12. doi: 10.3389/fpsyt.2019.00012

A Corrigendum on

The Perspectives Associated With the Computer-Based Diagnostic Method of Depressive Disorder

by Bartkiene, E., Steibliene, V., Adomaitiene, V., Lele, V., Cernauskas, D., Zadeike, D., et al. (2018). Front. Psychiatry 9:687. doi: 10.3389/fpsyt.2018.00687

In the published article, there was an error regarding the affiliations for Darius Cernauskas. As well as having affiliation 3, they should also have "Food Institute, Kaunas University of Technology, Kaunas, Lithuania."

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

frontiers in Psychiatry

REVIEW
published: 09 January 2019
doi: 10.3389/fpsyt.2018.00739

Mitochondria, Microglia, and the Immune System—How Are They Linked in Affective Disorders?

Carsten Culmsee[1,2]*, *Susanne Michels*[1,2], *Stefanie Scheu*[3], *Volker Arolt*[4], *Udo Dannlowski*[4] and *Judith Alferink*[4,5]*

[1] Institute of Pharmacology and Clinical Pharmacy, University of Marburg, Marburg, Germany, [2] Center for Mind, Brain and Behavior – CMBB, Marburg, Germany, [3] Institute of Medical Microbiology and Hospital Hygiene, University of Düsseldorf, Düsseldorf, Germany, [4] Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany, [5] Cells in Motion, Cluster of Excellence, University of Münster, Münster, Germany

Major depressive disorder (MDD) is a severe mood disorder and frequently associated with alterations of the immune system characterized by enhanced levels of circulating pro-inflammatory cytokines and microglia activation in the brain. Increasing evidence suggests that dysfunction of mitochondria may play a key role in the pathogenesis of MDD. Mitochondria are regulators of numerous cellular functions including energy metabolism, maintenance of redox and calcium homeostasis, and cell death and therefore modulate many facets of the innate immune response. In depression-like behavior of rodents, mitochondrial perturbation and release of mitochondrial components have been shown to boost cytokine production and neuroinflammation. On the other hand, pro-inflammatory cytokines may influence mitochondrial functions such as oxidative phosphorylation, production of adenosine triphosphate, and reactive oxygen species, thereby aggravating inflammation. There is strong interest in a better understanding of immunometabolic pathways in MDD that may serve as diagnostic markers and therapeutic targets. Here, we review the interaction between mitochondrial metabolism and innate immunity in the pathophysiology of MDD. We specifically focus on immunometabolic processes that govern microglial and peripheral myeloid cell functions, both cellular components involved in neuroinflammation in depression-like behavior. We finally discuss microglial polarization and associated metabolic states in depression-associated behavior and in MDD.

Keywords: major depressive disorder, immune system, metabolic pathways, mitochondria, immune cells, microglia, neuroinflammation, immunometabolism

OPEN ACCESS

Edited by:
Frank M. Schmidt, Universitätsklinikum Leipzig, Germany

Reviewed by:
Hubertus Himmerich, King's College London, United Kingdom
Ole Köhler-Forsberg, Aarhus University, Denmark

***Correspondence:**
Carsten Culmsee
culmsee@staff.uni-marburg.de
Judith Alferink
judith.alferink@uk.muenster.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *25 September 2018*
Accepted: *14 December 2018*
Published: *09 January 2019*

Citation:
Culmsee C, Michels S, Scheu S, Arolt V, Dannlowski U and Alferink J (2019) Mitochondria, Microglia, and the Immune System—How Are They Linked in Affective Disorders? Front. Psychiatry 9:739. doi: 10.3389/fpsyt.2018.00739

INTRODUCTION

Major depression is a serious mood disorder and characterized by marked functional impairment and increased health care utilization (1, 2). In particular, major depressive disorder (MDD) is estimated to affect more than 300 million people worldwide and treatment resistant depression occurs in about 20–30% of patients (3). Therefore, a better understanding of the underlying mechanisms is warranted to improve the therapeutic options in MDD.

Although the pathophysiology of MDD is not yet fully understood, genetic and environmental factors have been identified as major risk factors for the development of depression (4–6). In addition, a plethora of findings point toward an association between inflammation and depression. Immune alterations such as increased levels of circulating pro-inflammatory cytokines and polymorphisms in immune–associated genes have been frequently found in depressed individuals. Additional observations that immunotherapy with type I interferons may induce depressive symptoms and that depression-like "sickness behavior" in rodents is caused by treatment with inflammatory mediators underscored the bidirectional relationship between the immune response and depression (7–11). Accordingly, the "inflammation hypothesis of depression" has been proposed over 20 years ago (12, 13). The multi-faceted inflammatory process in depression has been reviewed in detail before (13–18). In this review, we focus on the intricate interplay between metabolic processes and innate immunity in the pathophysiology of MDD. We further discuss the link between mitochondrial dysfunction and neuroinflammation in depression-associated behavior in the rodent model. Finally, we highlight the concept that specific metabolic processes are associated with distinct microglial activation states that may contribute to the pathogenesis of depression.

INNATE IMMUNE RESPONSE IN DEPRESSION

Mitochondrial Function in the Innate Immune Response

The innate immune system represents the first line of defense against invading microbial pathogens and comprises a variety of cell types, molecules, and signaling cascades (19). Myeloid cells are the cellular components of innate immunity and represent a heterogeneous group of bone marrow (BM)—derived cells including monocytes/macrophages, dendritic cells (DCs), and granulocytes (20). Within minutes of encountering pathogens, highly conserved pathogen-associated molecular patterns (PAMPs) bind to pattern recognition receptors (PRR) expressed within the cytosol or on membranes of innate immune cells, such as RIG-I-like receptors (RLR), NOD-like receptors (NLR), or Toll-like receptors (TLR) (21) (**Figure 1**). Activated PRR can trigger the release of cytokines, chemokines, and additional inflammatory factors via various intracellular pathways to ultimately control infection (22). Even in the absence of overt pathogenic infection, cell damage or stress responses may alert the innate immune response and induce a "sterile inflammatory immune response." In this context, endogenous or "self-molecules" (e.g., high mobility group box 1, S100 proteins, RNA, and DNA) are recognized as danger signals when released into the extracellular space. These damage-associated molecular pattern molecules (DAMPs) trigger innate immune responses also via binding of PRRs (23).

Multiple lines of evidence strongly suggest that mitochondrial integrity and function, and innate immunity are closely interlinked processes. Mitochondria are intracellular organelles required for numerous cellular functions including energy metabolism, regulation of reactive oxygen species (ROS) signaling, Ca^{2+} homeostasis, and apoptosis. In addition, several mitochondrial components such as adenosine triphosphate (ATP), N-formyl peptides or mitochondrial DNA (mtDNA) function as DAMPs and are sensed by distinct PRRs thereby promoting an inflammatory response (24). Accordingly, it has been demonstrated in humans that injury induces release of N-formyl peptides and mtDNA into the circulation and activates neutrophils via binding of formyl peptide receptor-1 and TLR9, respectively (25). Studies in mice demonstrated that mtDNA aggravated the inflammatory response, while inflammation was reduced in animals deficient for TLR9 or the adaptor protein, myeloid differentiation primary response gene 88 (Myd88) (26). Furthermore, also ATP has been found to induce mitochondrial dysfunction, enhanced generation of ROS, and apoptosis, resulting in cytosolic release of oxidized mtDNA, that binds to and activates the NLR family pyrin domain containing 3 (NLRP3) inflammasome (27).

The inflammasome is a multi-protein signaling complex that triggers caspase-1-dependent secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (25, 28, 29). A particular role has been described for intracellular mtDNA and mitochondria-derived ROS in pathways that activate the inflammasome (30–32). For example, mtDNA accumulation in the cytosol of macrophages was identified as a prerequisite for caspase 1-dependent IL-1β release in response to combined lipopolysaccharide (LPS) and ATP exposure. The essential role of mitochondria in this process was further demonstrated by depletion of autophagic proteins that enhanced the accumulation of dysfunctional mitochondria in macrophages thereby increasing mitochondrial ROS production and susceptibility to stimulation by LPS and ATP (30, 33). Further, extracellular ATP can induce NLRP3 inflammasome activation through engagement of P2x7 receptors

Abbreviations: AIF, Apoptosis inducing factor; Arg-1, Arginase-1; ATP, Adenosine triphosphate; BD, Bipolar disorder; BHI, Bioenergetic health index; BID, BH3-interacting domain death agonist; BM, Bone marrow; CCL2, Chemokine ligand 2; CCR2, Chemokine receptor 2; CMPK2, Cytidine/uridine monophosphate kinase-2; DAMP, Damage-associated molecular pattern; DC, Dendritic cell; $\Delta\Psi m$, Mitochondrial membrane potential; DISC1, Disrupted in schizophrenia 1; Drp1, Dynamin-related protein 1; FAO, Fatty acid oxidation; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GxE, Gene-environment interaction; HIF1-α, Hypoxia-inducible factor 1-alpha; iNOS, Inducible nitric oxide synthase; L-Arg, L-Arginine; LPS, Lipopolysaccharide; LTCC, L-type calcium channel; MDD, Major depressive disorder; Mfn, Mitofusin; mtDNA, Mitochondrial deoxyribonucleic acid; Myd88, Myeloid differentiation primary response gene 88; NF, Nuclear factor; NLR, NOD-like receptor; NLRP3, NLR family pyrin domain containing 3; NMDA, N-Methyl-D-aspartate; NO, Nitric oxide; OCD, Obsessive-compulsive disorder; OPA1, Optical atrophy 1; OXPHOS, Oxidative phosphorylation; P2X7, ATP-binding purinoceptor; PAMP, Pathogen-associated molecular pattern; PBMCs, Peripheral blood mononuclear cells; PET, Positron emission tomography; PPP, Pentose phosphate pathway; PRR, Pattern recognition receptor; PTSD, Post-traumatic stress disorder; RLR, RIG-I-like receptor; ROS, Reactive oxygen species; SSRI, Selective serotonin reuptake inhibitors; TLR, Toll-like receptor; TRIF, Toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon beta; TSPO, Microglial translocator protein.

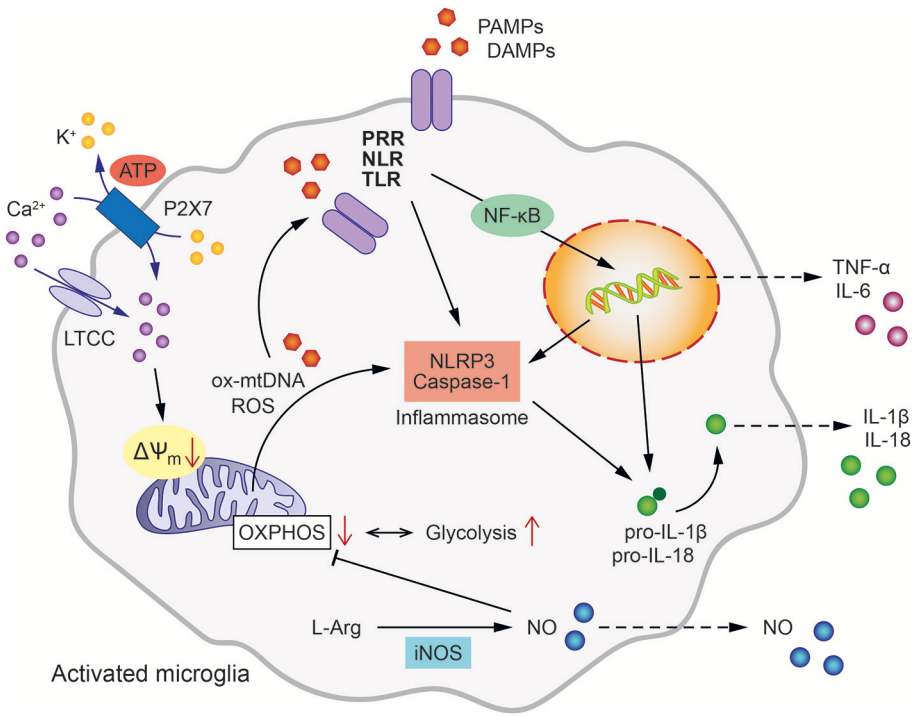

FIGURE 1 | Mitochondrial involvement in microglial activation and inflammatory signaling. Microglial activation pathways and inflammatory cytokine release are initiated by the binding of pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) to intracellular or membrane-bound pattern recognition receptors (PRR), such as NOD-like receptors (NLR) or Toll-like receptors (TLR). This triggers NLRP3 and Caspase-1 assembly to form the inflammasome leading to the processing of pro-IL-1β as well as pro-IL-18 to active IL-1β and IL-18, respectively. In addition, oxidized mitochondrial DNA (ox-mtDNA) and mitochondrial reactive oxygen species (ROS) are canonical activators of inflammasome formation. Tumor necrosis factor (TNF) and Interleukin-6 (IL-6) are generated via nuclear factor NF-κB-dependent transcriptional activation. The oxidation of L-arginine (L-Arg) by inducible nitric oxide synthase (iNOS) produces NO (Nitric oxide), which in turn inhibits oxidative phosphorylation (OXPHOS) shifting the energy metabolism of the cell toward glycolysis. Ca^{2+} influx via L-type calcium channels (LTCC) and ATP-binding purinoceptors (P2X7) results in the loss of mitochondrial membrane potential ($\Delta\Psi m$), enhanced mitochondrial ROS formation and further contributes to inflammasome formation and pro-inflammatory activation of microglia.

and downstream mitochondrial dysfunction. The purinergic P2x7 receptor is an ATP-gated ion channel that is expressed by virtually all immune cell subsets and its activation has been associated with inflammation (34). A recent study demonstrated in macrophages that K^+ efflux and Ca^{2+} influx through P2x7 were required for sustained reduction of the mitochondrial membrane potential and generation of mitochondrial ROS formation upstream of NLRP3 inflammasome assembly and pyroptotic cell death (35). Earlier studies pointed toward ROS as the key regulators of the NLRP inflammasome in response to PAMPS and DAMPS, such as oxidized mtDNA or other DAMPs resulting from metabolic dysregulation (36, 37). More recent studies, however, underscored the importance of new mtDNA synthesis for NLRP3 inflammasome activation. According to these findings, LPS-induced TLR signaling via MyD88 and Toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon beta (TRIF) triggers transcription of the mitochondrial cytidine/uridine monophosphate kinase-2 (CMPK2). CMPK2 belongs to a family of nucleotide kinases that are required for mtDNA synthesis and production of oxidized mtDNA fragments that ultimately act as activating ligands for the NLRP3 inflammasome complex in stimulated macrophages (38).

Innate Immune Responses in MDD

Dysregulation of innate immune responses has been linked to stress-associated psychiatric disorders such as MDD (16, 39–43). A plethora of studies and meta-analyses have demonstrated that patients with MDD frequently show elevated levels of TNF, IL-6, as well as the T helper cell differentiation cytokine IL-12 (44–48). Stress may induce the activation of the innate immune system and stressful experiences such as adverse childhood events induce long-term alterations of the immune response and increase the susceptibility to depression (49–55). In analogy to depression, exposure to early life stressors in humans has been shown to elevate blood levels of pro-inflammatory cytokines (56). Mechanistically, pro-inflammatory cytokines can activate the hypothalamic–pituitary–adrenal axis leading to hypercortisolism and increased glucocorticoid receptor resistance, both mechanisms involved in the etiology of MDD (57). In addition, pro-inflammatory cytokines modulate the tryptophan–kynurenine pathway and enhance synthesis of the neurotoxic N-methyl-D-aspartate (NMDA) glutamate receptor agonist quinolinic acid and 3-hydroxykynurenine with detrimental effects on brain function (41). Recent studies support the idea that inflammation contributes to depression in a subgroup of patients characterized by enhanced disease severity

and potentially neurovegetative symptoms. In addition, somatic comorbidities associated with an ongoing inflammatory process and elevated circulating inflammatory factors have shown better treatment responses to anti-inflammatory agents in MDD [for review see Raison and Miller (42)].

It is important to note, however, that altered blood cytokine levels as discussed here are not specific for affective disorders, but have also been found elevated in post-traumatic stress disorders (PTSD) (58, 59), obsessive-compulsive disorders (OCD) (60) or eating disorders (61). Additionally, low grade inflammation and cytokine elevation play a role in a number of physical diseases for example metabolic diseases such as diabetes and obesity as hallmarks of the metabolic syndrome. It has also to be considered that overall altered cytokine levels may result from distinct immune activation patterns on the level of immune cell subsets. Therefore, in addition to the analysis of overall cytokine levels in the peripheral blood, characterization of cytokine and receptor expression profiles of specific immune cell subsets (i.e., immune signatures) may better represent an individual's psychiatric disease risk and progression. Longitudinal studies are required in MDD patients and healthy participants, including those with familial genetic risk and exposure to early life stress (e.g., childhood maltreatment) to identify such immune signatures. In the multicenter cohort study FOR2107, we established a large-scale multi-parameter flow cytometry screen for characterization of immune activation profiles on a single cell level with prognostic potential in MDD patients with genetic (G), environmental (E), or GxE risk factors. In this cohort study, established immune signatures in patients are now compared to those identified in peripheral immune cells and microglia of the CNS in genetic and behavioral rat models of depression in defined GxE risk settings (62). This translational approach will provide a better understanding of the functional impact of (neuro-) inflammatory responses in MDD and the mechanisms by which GxE alters immune activation profiles and the risk to develop MDD.

Microglia in MDD and Depression-Associated Behavior

In analogy to humans, also in rodent models, depression-associated behavior after stress exposure is frequently associated with "low-grade immune activation" characterized by enhanced levels of circulating pro-inflammatory cytokines and immigration of myeloid immune cells into the brain (63–66). Specifically, trafficking of "inflammatory" Ly6C^{hi} monocytes that co-express CC chemokine receptor 2 (CCR2), the receptor for the CC chemokine ligand 2 (CCL2), to the brain has been shown to promote neuroinflammation in the stress response (67–69). The treatment of mice with the TLR4 ligand LPS is well-known to induce an innate immune response and trigger sickness behavior, i.e., anhedonia and weight loss. Mice deficient for the inflammatory caspase-1 exhibit enhanced resistance to LPS-induced depressive-like behavior underscoring the involvement of the inflammasome in depression (70). Also pretreatment with an NLRP3 inflammasome inhibitor abrogated the depressive-like behaviors induced by LPS in mice (71). Interesting findings further identified TRIF, one of the key mediators of oxidized mtDNA production in NLRP3 inflammasome activation, as an important inflammatory signaling mediator of LPS-induced sickness behavior through regulation of CCL2 in the hypothalamus (72). This CCR2-CCL2 signaling mechanism may thus link metabolic and behavioral adaptation to inflammation in the brain (73).

Peripheral immune alterations are closely linked to microglia activation that plays a prominent role in the pathogenesis of MDD and depression-associated behavior (65, 74–77). Microglia express PRRs and thus recognize PAMPs and DAMPs. Upon ligand binding to PRRs, microglia acquire an amoeboid-like phenotype, migrate to inflammatory sites, and release pro-inflammatory cytokines (e.g., IL-1β, IL-6, IL-18, TNF), chemokines, and neurotoxic factors such as nitric oxide (NO) generated by the inducible NO synthase (iNOS) and ROS (78–81). Classically activated M1 microglia are induced by stimulation with LPS, granulocyte-macrophage colony-stimulating factor (GM-CSF) or interferon-γ (IFN-γ) and express enhanced levels of major histocompatibility complex (MHC) class II, and CD86. They are involved in the defense against pathogens but may also occur in stress responses. Upon alternative activation with IL-1 and anti-inflammatory IL-10, M2 microglia express arginase-1 (Arg-1), the chitinase-like protein Ym1, Fizz1 (found in inflammatory zone), anti-inflammatory cytokines, extracellular matrix proteins, and glucocorticoids (82). In analogy to peripheral macrophages, the M2 microglia phenotype has further been sub-classified into M2a, M2b, and M2c activation states, each subtype specifically equipped to contribute to immune regulation, phagocytosis, and/or tissue repair [for review, see Singhal and Baune (79)].

To study microglial activation in depression *in vivo*, positron emission tomography (PET) imaging studies using various PET ligands for the microglial marker translocator protein 18 kDa (TSPO) have been conducted. TSPO predominantly localizes to the outer mitochondrial membrane and is expressed in brain microglia (83). Depression-associated elevations in TSPO in the prefrontal cortex, insula, and anterior cingulate cortex have been correlated with the severity and duration of depression (84). Post-mortem and PET imaging studies further identified microglia activation in individuals who committed suicide (74, 85–87). However, also negative results demonstrating lack of microglia activation or even suppressed microglia activation states have been reported in depressed individuals [for review, see Yirmiya et al. (88)].

Several studies investigated substances that may affect M1 to M2 polarization in microglia. For example, inhibition of the JAK/STAT pathway is known to suppress M1-associated downstream genes in inflammatory CNS disorders (89). Treatment with the PPARγ agonist, pioglitazone, has been shown to cause a phenotypic switch of M1 microglia to the anti-inflammatory M2 state in various CNS disease models and to mediate antidepressant properties in several studies (90). Furthermore, Glatiramer acetate, approved for the treatment of relapsing-remitting MS, mediates neuroprotective effects by inducing an anti-inflammatory microglial M2 phenotype and thus harbors potential for treatment of MDD (91). A

study in cultured mouse microglial cells demonstrated that the antidepressant selective serotonin reuptake inhibitors (SSRI) fluoxetine and S-citalopram can inhibit M1 activation and enhanced M2 activation of these immune cells *in vitro* (92). With regard to microglia activation in depression-associated behavior, several studies have been conducted in rodent models. One study demonstrated that mice exposed to social defeat stress, an established stress/depression model, exhibit microglia activation and increased expression of microglial-derived pro-inflammatory cytokines specifically in brain regions associated with fear and anxiety (31). Furthermore, inhibition of microglial activation or NLRP3 deletion has been proven to impair stress-induced alterations associated with depression in rodents (93). Treatment with substances mediating antidepressant effects has further been shown to suppress classical microglial activation and increased the microglial M2 markers in the brain of C57BL/6 mice exposed to chronic mild stress (94) Moreover, anti-inflammatory effects of tricyclic antidepressants, SSRI, and lithium have been described *in vivo*, in animal models of IFNα-induced sickness behavior and inflammation-induced cytokine production in the brain (45, 95).

As has been discussed in this chapter, exposure to early life stressors represents a risk factor for MDD and depression-like behavior and is associated with alterations of the innate immune response. Elevated blood levels of pro-inflammatory cytokines in depressed individuals may affect microglia activation, a pathophysiological hallmark of major depression.

INVOLVEMENT OF MITOCHONDRIA IN THE NEUROBIOLOGY OF AFFECTIVE DISORDERS

Mitochondrial Impairments Associated With Depression

Intracellular and intercellular mechanisms of stress adaptation in the brain such as in the course of MDD lead to a significant increase in energy demand (96). In neural cells mitochondria are pivotal for energy production through oxidative phosphorylation that converts the chemical energy stored in glucose to ATP. Furthermore, mitochondria are essential for Ca^{2+} homeostasis, generation of ROS, neuronal outgrowth and differentiation, synaptic plasticity, and cell death signaling. Thus, they are highly important for cellular resilience and stress adaptation in the brain. More recent reports suggested a role for mitochondrial dysfunction and related major hallmarks of cellular stress, such as impaired redox balance and deregulation of intracellular Ca^{2+} homeostasis in the development of MDD and bipolar disorders (BD) (97–100).

While affective disorders such as MDD or BD are not considered as classic mitochondrial diseases, emerging evidence suggests a substantial link between mitochondrial dysfunction and these disorders in genetic and behavioral animal models, as well as in patients (99, 101, 102). For example, patients suffering from mitochondrial diseases caused by genetic alterations affecting mitochondrial metabolism frequently develop symptoms of MDD, BD, psychosis, and personality changes (103–105). Further, mood disorders are often prevalent years before the onset of cognitive and motor symptoms in patients later diagnosed with neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's disease (106, 107), which all feature mitochondrial dysfunction in neurons as a major hallmark of the underlying pathology (107–109). Concurring reports are derived from genetic studies as well as post-mortem brain analysis, brain imaging or biomarker studies in patients diagnosed with affective disorders, and in the respective animal models (99, 110). Mitochondrial impairments are characterized by morphological, biochemical, and functional hallmarks which all contribute to disturbed energy metabolism, but also to reduced Ca^{2+} buffering, loss of membrane potential, and increased mitochondrial ROS production. Finally, fatal mitochondrial dysfunction can result in disruption of the mitochondrial membrane and release of pro-apoptotic proteins such as cytochrome c or apoptosis-inducing factor (AIF) which mediate caspase-dependent or caspase-independent cell death, respectively.

Disturbed oxidative phosphorylation (OXPHOS) and reduced mitochondrial ATP production may significantly contribute to impaired neuronal plasticity and neurogenesis which are considered hallmarks in the neurobiology of depression (102). Several studies detected lower ATP levels in the brain tissue of MDD patients compared to healthy controls (111, 112). Similar correlations of depressive behavior and mitochondrial dysfunction in energy supply were confirmed in animal models of depression. In a mouse model of chronic restraint stress depressive behavior in the tail suspension and forced swim tests was associated with decreased oxygen consumption rate in isolated brain mitochondria (113). Further, impaired mitochondrial respiration and additional features of mitochondrial damage such as altered mitochondrial membrane potential and changes in the mitochondrial ultrastructure were also detected in other rodent models of depression induced by chronic mild stress such as learned helplessness in mice (114) or anhedonia in rats (115). Interestingly, treatment with the antidepressant fluoxetine reversed the depressive behavior and restored ATP production in brain tissue in a rat model of unpredictive chronic stress (116).

Mitochondria are highly dynamic organelles that undergo permanent fission and fusion processes allowing for the transport, reorganization, and regeneration of these organelles within the cells. In a model of streptozotocin-induced diabetes in mice, the associated depressive behavior was accompanied by increased expression of mitochondrial fission genes fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1), and a decreased expression of mitochondrial fusion genes mitofusin 1 (Mfn1), mitofusin 2 (Mfn2), and optical atrophy 1 (Opa1) in the brain tissue (117). Further, the DISC1 protein is an important regulator of mitochondrial dynamics and mediates the transport, fusion, and regeneration of these organelles in neuronal axons and dendrites (118). Pathological DISC1 isoforms disrupt mitochondrial dynamics leading to abnormal neuronal development and DISC1 mutations have been implicated in major mental disorders including MDD and BD (119). Intact mitochondrial fission and fusion dynamics are also important

for the proper cristae formation, respiratory functions of these organelles, and quality control through mitophagy. Impairments in the structural dynamics lead to reduced energy supply, accumulation of dysfunctional mitochondria and increased ROS production. These phenomena are closely associated with both, enhanced inflammatory responses (120, 121) and the risk of psychiatric disorders, including MDD (122).

In particular, oxidative stress has been frequently linked to the pathophysiology of depression. In MDD patients and in animal models, decreased levels of antioxidants and antioxidant enzymes were detected, suggesting an impaired antioxidant defense associated with depressive behavior. For example, in a rat model of restraint stress glutathione levels were significantly decreased in the brain tissue for weeks after stress exposure (123). In neurons, glutathione depletion leads to increased lipid peroxidation and the activation of pro-apoptotic signaling pathways that involve the activation and mitochondrial translocation of BH3-interacting domain death agonist (BID) and the fission-inducing GTPase Drp1. Upon mitochondrial transactivation, these proteins mediate mitochondrial fission, mitochondrial ROS production, ATP depletion, and disruption of the mitochondrial membrane (124–126). Notably, lipid peroxidation was enhanced in mouse brain tissue after restraint stress (123), and increased oxidative damage and altered expression levels of the electron transport chain complex I were also detected in brain tissue of MDD patients (127). Inhibition of complex I leads to a rapid increase in mitochondrial ROS formation which further impairs mitochondrial respiration, integrity, and function. As outlined before, complex I inhibition and the associated increase in mitochondrial ROS formation and oxidized mtDNA have been established as a trigger mechanism for inflammatory responses in macrophages through activation of the NLRP3 (36–38).

The Risk Gene CACNA1C and Mitochondrial Dysfunction

How mitochondrial functions are affected by genetic risk factors and environmental stress in the context of affective disorders is an emerging field of research. The trigger mechanisms of mitochondrial pathology, such as oxidative stress, impaired intracellular Ca^{2+} homeostasis, and molecular signaling pathways causing loss of mitochondrial function and integrity have been associated with the pathology of affective disorders. In particular, recent findings closely connected the psychiatric risk gene CACNA1C to mitochondrial dysfunction in conditions of oxidative stress. CACNA1C codes for the α1c subunit of the L-type Ca^{2+} channel (LTCC) Cav1.2, and has been identified by several genome-wide association studies as one of the strongest and most replicable risk factors for MDD and BD (128). In cultured mouse neuronal cells, reduction of CACNA1C expression or pharmacological inhibition of LTCC prevented excessive ROS formation, mitochondrial damage and ATP depletion, and rescued the neurons from cell death in a model of oxidative stress (129, 130). Our data corroborate earlier reports demonstrating that CACNA1C depletion or pharmacological LTCC inhibition was associated with antidepressant-like behavior and resilience to chronic stress, while activation of CACNA1C was detrimental for synaptic plasticity and cognitive functions (131, 132). In gene/genetic x environmental risk interactions, mitochondrial dysfunction may represent a converging point of the complex interdependent processes of energy metabolism, cellular stress, and calcium homeostasis in the neurobiology of affective disorders.

Overall, increased cellular ROS levels and the ensuing oxidative stress may be cause as well as consequence of mitochondrial dysfunction and metabolic impairments involved in neuroinflammatory responses in the neurobiology of depression. The according signaling pathways may serve as future therapeutic targets. Similar to therapeutic effects on innate immune responses and mitochondrial impairments, antidepressants attenuate parameters of oxidative stress in MDD patients and animal models [for review, see Allen et al. (102) and Adzic et al. (133)]. Further, a recent study exposed functional perturbations of apoptotic mitochondrial stress signaling induced by BID as a potential therapeutic target in rodent models of depression (134). Targeting such mechanisms of mitochondrial damage may provide novel therapeutic approaches in both, age-related disorders of the nervous system and psychiatric disorders (135–137). Systematic studies investigating the impact of genetic risk factors and environmental stress on mitochondrial functions and morphological alterations are highly warranted for a better understanding of the proposed link between the course of psychiatric disorders and mitochondrial demise.

IMMUNOMETABOLISM AND NEUROINFLAMMATORY RESPONSES IN MDD

Metabolic Programs in M1 and M2 Like Macrophages and Microglia

The research field termed immunometabolism has significantly advanced our understanding on the link between immunological and metabolic processes in immune cell differentiation and effector function. Naïve as well as activated immune cells require the capacity to produce ATP as energy supply for cellular function and it has been demonstrated that myeloid cells primarily use glycolysis as a source of ATP that represents a major mechanism of pro-inflammatory adaptation (138, 139). It is well-established that inflammatory factors such as pro-inflammatory cytokines influence mitochondrial function and can shift ATP production from OXPHOS to glycolysis. In this regard, TNF produced e.g., by activated microglia inhibited OXPHOS and concomitantly induced enhanced mitochondrial ROS production (120).

Immunometabolism may fine-tune myeloid cell functions and thereby influence activation states and polarization of myeloid cells. In accordance, M1 and M2 macrophages have been linked to distinct metabolic programs (139). It has been shown that classically activated M1 macrophages exhibit enhanced aerobic glycolysis and increased pentose phosphate pathway (PPP), while mitochondrial fatty acid oxidation (FAO), the Krebs-cycle, and OXPHOS were reduced (140, 141). This metabolic shift

in M1 cells allows for conserving and generating metabolites necessary for pro-inflammatory activation, cell proliferation and concomitant supply of the required amount of ATP. For example, succinate from the inactive Krebs-cycle activates hypoxia-inducible factor 1-alpha (HIF1-α) which stimulates IL-1β production, and, together with the increased glycolysis, supports cell activity and survival in hypoxic-inflammatory environments (141). Enhanced NO production through oxidation of L-arginine by iNOs activity is another hallmark of activated M1 macrophages and microglia (142, 143). NO reduces Krebs-cycle activity through inhibition of the pyruvate dehydrogenase, i.e., by reducing the production of acetyl-CoA from pyruvate (144, 145). Further, increased NO levels can also reversibly inhibit OXPHOS through inhibition of the mitochondrial cytochrome oxidase.

Similar to peripheral immune cells, LPS stimulation of mouse microglial cell lines and primary microglia revealed a metabolic switch from mitochondrial respiration to glycolysis (146) (and own observations in primary rat microglia). The LPS-mediated activation of microglia was accompanied by increased lactate production and activation of glycolysis-driving enzymes such as hexokinase, glucose-6-phosphate dehydrogenase, phosphofructokinase-1, and lactate dehydrogenase. The metabolic shift upon TLR activation in macrophages and microglia appears to occur in two steps that allows for utilizing OXPHOS, glycolysis, and the PPP simultaneously in the first phase, while glycolytic metabolism and the PPP support survival and pro-inflammatory activity after full M1 transformation (146). In contrast, anti-inflammatory M2 macrophages, supporting e.g., wound-healing, utilize fatty acid oxidation as the primary energy source which results in generation of acetyl-CoA that is shuttled to the catabolic Krebs-cycle in the mitochondrial matrix (144, 147). This metabolic state represents the phenotype of resident macrophages and features reduced glucose utilization and the synthesis of ornithine and polyamines to promote cell proliferation and tissue repair, collagen synthesis, fibrosis, and tissue remodeling (142). In cultured mouse microglia, induction of the M2 phenotype by exposure to IL-4 was also accompanied by reduced glucose consumption and lactate production, and mitochondrial respiration was preserved to control levels in non-stimulated cells (146). These findings are in contrast to peripheral human macrophages, where IL-4 stimulation enhanced glucose uptake, fatty acid metabolism, and mitochondrial biogenesis (148), thus pointing to differences in the M2 states between these two immune cell populations. More insight into the molecular mechanisms of microglia polarization is required to identify potential targets for pharmacological intervention at the level of mitochondrial metabolism or at the level of cytokine regulation and signaling (149).

Metabolic Programs in PMBCs

Up to now there is limited knowledge on the effect of mitochondria-derived metabolic pathways on (neuro-)inflammation in MDD. Furthermore, the impact of pro-inflammatory cytokines on mitochondrial functioning in depression is yet unresolved. During neuroinflammation in depression-associated behavior, inflammatory mediators such as TNF produced by activated microglia and brain-infiltrating immune cells trigger intracellular signaling cascades that can alter mitochondrial metabolism, ROS formation, and programmed cell death as outlined before. In contrast to microglia, which are hardly accessible from MDD patients, peripheral blood mononuclear cells (PBMCs) may provide an accessible source of the mitochondrial pool with relevance to alterations of mitochondrial functions in the brain. It has been shown recently in non-human primates that the mitochondrial bioenergetics profile of blood monocytes and platelets is positively related to frontal cortex mitochondrial function and metabolism (150). Brain mitochondrial dysfunction, in turn, is significantly involved in the pathophysiology of psychiatric disorders as supported by a growing body of literature (102, 151). In fact, a few studies already assessed mitochondrial function in circulating blood cells of psychiatric patients (152–154). For example, basal and maximal mitochondrial respiration was significantly lower in platelets (153) as well as in PBMCs (152) of depressed patients vs. healthy controls. Fresh intact platelets of depressive patients in partial remission showed decreased basal and maximal respiration, whereas the ratio of both values remained unchanged compared to healthy individuals (153). Basal and maximal mitochondrial respiration, and ATP production were significantly lower in cryopreserved PBMCs of female patients with a current diagnosis of major depression (152). As outlined before, compromised mitochondrial metabolism often leads to excess superoxide production thereby modulating redox-sensitive inflammatory pathways and inducing oxidative stress, which most likely play a role in MDD pathophysiology (155, 156). The Bioenergetic Health Index (BHI) comprises several parameters of a person's respiration profile and overall mitochondrial function (157, 158). By considering the spare respiratory capacity, the BHI may even have predictive value for the development of affective disorders because it may already identify alterations in mitochondrial performance before cellular energy failure occurs.

In this chapter we reviewed studies providing compelling evidence for metabolic re-programming in peripheral innate immune cells and in microglia upon activation. This is characterized by a switch from mitochondrial respiration to glycolysis and the PPP in the pro-inflammatory "M1" phenotype and, in contrast, to enhanced utilization of fatty acid and acetyl-CoA shuttling to the Krebs-cycle in anti-inflammatory M2 macrophages/microglia. The pro-inflammatory M1 phenotype has been associated with enhanced disease status in MDD, whereas a switch toward M2-activated microglia was associated with the therapeutic effect of antidepressants. Whether bioenergetic profiles of peripheral immune cells could serve as predictive biomarkers in affective disorders or even as therapeutic target with relevance for both, peripheral immune cells and microglia in the brain, requires further investigation.

SUMMARY

As discussed in this review, certain metabolic pathways may determine microglia differentiation to shape the effector

function of these cells. Consequently, manipulating these pathways may constitute a novel target to combat detrimental inflammatory responses in affective disorders. For example, the potential to promote an M1 to M2 shift in microglia during neuroinflammation in MDD may have beneficial therapeutic implications. In patients, PBMCs may be a valuable surrogate model of brain function and established mitochondrial perturbations in PBMCs may serve as biomarkers for neuropsychiatric disorders. In most studies, impaired mitochondrial respiration in the PBMCs was linked to an enhanced risk for or already established psychiatric disorders in the donor patients. Limitations in the overall comparability of the reported findings are attributed to differences in study cohort characteristics, antidepressant medication, cell type, cell storage, and detection methods of mitochondrial function. Whether mitochondrial dysfunction precedes the onset of psychiatric disorders has not been investigated in detail so far. Therefore, it remains to be elucidated, if changes in mitochondrial bioenergetics are already present in healthy individuals with psychiatric disease-relevant genetic or environmental risk factors and thus can serve as prognostic marker before clinical symptoms manifest. However, the impact of metabolic regulation in immune cell activation on the pathophysiology of depression and the question how increasing knowledge on immunometabolism could be translated into potential therapies for affective disorders remains to be answered.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work is part of the DFG FOR2107 and supported by a DFG grant to CC (CU 43/9-1 and CU43/9-2), UD (DA 1151/5-1 and DA 1151/5-2), and JA (AL 1145/5-2). SS receives research support from the DFG (RTG 2158), the Manchot Graduate School Molecules of Infection II and III, the Research Commission of the Medical Faculty of the University of Düsseldorf, Germany (30/2016). VA receives research support from EU FP7-People-IAPP-2011 (Project 286334), EU Grant Moodstratification (grant: 754740), the Interdisciplinary Center for Clinical Studies (IZKF) of the medical faculty of Münster (Ju3/024/15). UD receives further research support from the German Research Foundation (SFB-TRR58, Projects C09 and Z02) and the Interdisciplinary Center for Clinical Research (IZKF) of the medical faculty of Münster (grant Dan3/012/17). JA receives further support from the DFG EXC 1003 Cells in Motion–Cluster of Excellence (grant: FF-2014-01), the Alzheimer Forschung Initiative e.V. (AFI e.V.) (grant: No. 14835), the Interdisciplinary Center for Clinical Studies (IZKF) of the medical faculty of Münster (grant: Alf3/018/16), and the IMF of the medical faculty of Münster (grant: I-ST111423).

REFERENCES

1. Greden JF. The burden of recurrent depression: causes, consequences, and future prospects. *J Clin Psychiatry* (2001) 62(Suppl. 22):5–9.
2. Greenberg PE, Kessler RC, Birnbaum HG, Leong SA, Lowe SW, Berglund PA, et al. The economic burden of depression in the United States: how did it change between 1990 and 2000? *J Clin Psychiatry* (2003) 64:1465–75. doi: 10.4088/JCP.v64n1211
3. Nemeroff CB. Prevalence and management of treatment-resistant depression. *J Clin Psychiatry* (2007) 68(Suppl. 8):17–25.
4. Kupfer DJ, Frank E, Phillips ML. Major depressive disorder: new clinical, neurobiological, and treatment perspectives. *Lancet* (2012) 379:1045–55. doi: 10.1016/S0140-6736(11)60602-8
5. Direk N, Williams S, Smith JA, Ripke S, Air T, Amare AT, et al. An analysis of two genome-wide association meta-analyses identifies a new locus for broad depression phenotype. *Biol Psychiatry* (2017) 82:322–9. doi: 10.1016/j.biopsych.2016.11.013
6. Yu C, Baune BT, Wong ML, Licinio J. Investigation of short tandem repeats in major depression using whole-genome sequencing data. *J Affect Disord.* (2018) 232:305–9. doi: 10.1016/j.jad.2018.02.046
7. Mills NT, Scott JG, Wray NR, Cohen-Woods S, Baune BT. Research review: the role of cytokines in depression in adolescents: a systematic review. *J Child Psychol Psychiatry Allied Discip.* (2013) 54:816–35. doi: 10.1111/jcpp.12080
8. Prager G, Hadamitzky M, Engler A, Doenlen R, Wirth T, Pacheco-Lopez G, et al. Amygdaloid signature of peripheral immune activation by bacterial lipopolysaccharide or staphylococcal enterotoxin B. *J Neuroimmune Pharmacol.* (2013) 8:42–50. doi: 10.1007/s11481-012-9373-0
9. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* (2006) 27:24–31. doi: 10.1016/j.it.2005.11.006
10. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. *Biol Psychiatry* (2004) 56:819–24. doi: 10.1016/j.biopsych.2004.02.009
11. Dantzer R. Cytokine, sickness behavior, and depression. *Neurol Clin.* (2006) 24:441–60. doi: 10.1016/j.ncl.2006.03.003
12. Maes M. Evidence for an immune response in major depression: a review and hypothesis. *Prog Neuro Psychopharmacol Biol Psychiatry* (1995) 19:11–38. doi: 10.1016/0278-5846(94)00101-M
13. Maes M. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. *Neuro Endocrinol Lett.* (2008) 29:287–91.
14. Leonard BE. Inflammation and depression: a causal or coincidental link to the pathophysiology? *Acta Neuropsychiatr.* (2018) 30:1–16. doi: 10.1017/neu.2016.69
15. Bauer ME, Teixeira AL. Inflammation in psychiatric disorders: what comes first? *Ann NY Acad Sci.* (2018). doi: 10.1111/nyas.13712. [Epub ahead of print].
16. Eyre HA, Stuart MJ, Baune BT. A phase-specific neuroimmune model of clinical depression. *Prog Neuro Psychopharmacol Biol Psychiatry* (2014) 54:265–74. doi: 10.1016/j.pnpbp.2014.06.011
17. Miller AH, Raison CL. Are anti-inflammatory therapies viable treatments for psychiatric disorders?: Where the rubber meets the road. *JAMA Psychiatry* (2015) 72:527–8. doi: 10.1001/jamapsychiatry.2015.22
18. Bufalino C, Hepgul N, Aguglia E, Pariante CM. The role of immune genes in the association between depression and inflammation: a review of recent clinical studies. *Brain Behav Immun.* (2013) 31:31–47. doi: 10.1016/j.bbi.2012.04.009
19. Medzhitov R, Shevach EM, Trinchieri G, Mellor AL, Munn DH, Gordon S, et al. Highlights of 10 years of immunology in Nature Reviews Immunology. *Nat Rev Immunol.* (2011) 11:693–702. doi: 10.1038/nri3063
20. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* (2010) 327:656–61. doi: 10.1126/science.1178331

21. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* (2010) 140:805–20. doi: 10.1016/j.cell.2010.01.022
22. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat. Immunol.* (2015) 16:343–53. doi: 10.1038/ni.3123
23. Rock KL, Latz E, Ontiveros F, Kono H. The sterile inflammatory response. *Ann Rev Immunol.* (2010) 28:321–42. doi: 10.1146/annurev-immunol-030409-101311
24. Krysko DV, Agostinis P, Krysko O, Garg AD, Bachert C, Lambrecht BN, et al. Emerging role of damage-associated molecular patterns derived from mitochondria in inflammation. *Trends Immunol.* (2011) 32:157–64. doi: 10.1016/j.it.2011.01.005
25. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* (2010) 464:104–7. doi: 10.1038/nature08780
26. Boyapati RK, Tamborska A, Dorward DA, Ho GT. Advances in the understanding of mitochondrial DNA as a pathogenic factor in inflammatory diseases. *F1000Res.* (2017) 6:169. doi: 10.12688/f1000research.10397.1
27. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* (2012) 36:401–14. doi: 10.1016/j.immuni.2012.01.009
28. Sutterwala FS, Ogura Y, Szczepanik M, Lara-Tejero M, Lichtenberger GS, Grant EP, et al. Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* (2006) 24:317–27. doi: 10.1016/j.immuni.2006.02.004
29. Heneka MT, McManus RM, Latz E. Inflammasome signalling in brain function and neurodegenerative disease. *Nat Rev Neurosci.* (2018) 19:610–21. doi: 10.1038/s41583-018-0055-7
30. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol.* (2011) 12:222–30. doi: 10.1038/ni.1980
31. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* (2011) 469:221–5. doi: 10.1038/nature09663
32. Fleshner M, Frank M, Maier SF. Danger signals and inflammasomes: stress-evoked sterile inflammation in mood disorders. *Neuropsychopharmacology* (2017) 42:36–45. doi: 10.1038/npp.2016.125
33. Sadatomi D, Nakashioya K, Mamiya S, Honda S, Kameyama Y, Yamamura Y, et al. Mitochondrial function is required for extracellular ATP-induced NLRP3 inflammasome activation. *J Biochem.* (2017) 161:503–12. doi: 10.1093/jb/mvw098
34. Deussing JM, Arzt E. P2X7 receptor: a potential therapeutic target for depression? *Trends Mol Med.* (2018) 24:736–47. doi: 10.1016/j.molmed.2018.07.005
35. Yaron JR, Gangaraju S, Rao MY, Kong X, Zhang L, Su F, et al. K(+) regulates Ca(2+) to drive inflammasome signaling: dynamic visualization of ion flux in live cells. *Cell Death Dis.* (2015) 6:e1954. doi: 10.1038/cddis.2015.277
36. Tschopp J, Schroder K. NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? *Nat Rev Immunol.* (2010) 10:210–5. doi: 10.1038/nri2725
37. Schroder K, Tschopp J. The inflammasomes. *Cell* (2010) 140:821–32. doi: 10.1016/j.cell.2010.01.040
38. Zhong Z, Liang S, Sanchez-Lopez E, He F, Shalapour S, Lin XJ, et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature* (2018) 560:198–203. doi: 10.1038/s41586-018-0372-z
39. Gibney SM, Drexhage HA. Evidence for a dysregulated immune system in the etiology of psychiatric disorders. *J Neuroimmune Pharmacol.* (2013) 8:900–20. doi: 10.1007/s11481-013-9462-8
40. Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G, et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metabol Brain Dis.* (2009) 24:27–53. doi: 10.1007/s11011-008-9118-1
41. Dantzer R, O'Connor JC, Lawson MA, Kelley KW. Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology* (2011) 36:426–36. doi: 10.1016/j.psyneuen.2010.09.012
42. Raison CL, Miller AH. Is depression an inflammatory disorder? *Curr Psychiatry Rep.* (2011) 13:467–75. doi: 10.1007/s11920-011-0232-0
43. Grosse L, Carvalho LA, Wijkhuijs AJ, Bellingrath S, Ruland T, Ambree O, et al. Clinical characteristics of inflammation-associated depression: monocyte gene expression is age-related in major depressive disorder. *Brain Behav Immun.* (2015) 44:48–56. doi: 10.1016/j.bbi.2014.08.004
44. Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, et al. A meta-analysis of cytokines in major depression. *Biol. Psychiatry* (2010) 67:446–57. doi: 10.1016/j.biopsych.2009.09.033
45. Kohler CA, Freitas TH, Stubbs B, Maes M, Solmi M, Veronese N, et al. Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: systematic review and meta-analysis. *Mol Neurobiol.* (2018) 55:4195–206. doi: 10.1007/s12035-017-0632-1
46. Baune BT, Konrad C, Grotegerd D, Suslow T, Birosova E, Ohrmann P, et al. Interleukin-6 gene (IL-6): a possible role in brain morphology in the healthy adult brain. *J Neuroinflamm.* (2012) 9:125. doi: 10.1186/1742-2094-9-125
47. Baune BT, Konrad C, Grotegerd D, Suslow T, Ohrmann P, Bauer J, et al. Tumor necrosis factor gene variation predicts hippocampus volume in healthy individuals. *Biol. Psychiatry* (2012) 72:655–62. doi: 10.1016/j.biopsych.2012.04.002
48. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry* (2016) 21:1696–709. doi: 10.1038/mp.2016.3
49. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun.* (2007) 21:901–12. doi: 10.1016/j.bbi.2007.03.011
50. Redlich R, Stacey D, Opel N, Grotegerd D, Dohm K, Kugel H, et al. Evidence of an IFN-gamma by early life stress interaction in the regulation of amygdala reactivity to emotional stimuli. *Psychoneuroendocrinology* (2015) 62:66–73. doi: 10.1016/j.psyneuen.2015.08.008
51. Opel N, Redlich R, Zwanzger P, Grotegerd D, Arolt V, Heindel W, et al. Hippocampal atrophy in major depression: a function of childhood maltreatment rather than diagnosis? *Neuropsychopharmacology* (2014) 39:2723–31. doi: 10.1038/npp.2014.145
52. Hazel NA, Hammen C, Brennan PA, Najman J. Early childhood adversity and adolescent depression: the mediating role of continued stress. *Psychol Med.* (2008) 38:581–9. doi: 10.1017/S0033291708002857
53. Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature* (2008) 455:894–902. doi: 10.1038/nature07455
54. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M et al. Major depressive disorder. *Nat Rev Dis Primers* (2016) 2:16065. doi: 10.1038/nrdp.2016.65
55. Schedlowski M, Engler H, Grigoleit JS. Endotoxin-induced experimental systemic inflammation in humans: a model to disentangle immune-to-brain communication. *Brain Behav Immun.* (2014) 35:1–8. doi: 10.1016/j.bbi.2013.09.015
56. Cattaneo A, Macchi F, Plazzotta G, Veronica B, Bocchio-Chiavetto L, Riva MA, et al. Inflammation and neuronal plasticity: a link between childhood trauma and depression pathogenesis. *Front Cell Neurosci.* (2015) 9:40. doi: 10.3389/fncel.2015.00040
57. Cohen S, Janicki-Deverts D, Doyle WJ, Miller GE, Frank E, Rabin BS, et al. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proc Natl Acad Sci USA.* (2012) 109:5995–9. doi: 10.1073/pnas.1118355109
58. Waheed A, Dalton B, Wesemann U, Ibrahim MAA, Himmerich H. A systematic review of interleukin-1beta in post-traumatic stress disorder: evidence from human and animal studies. *J Interferon Cytokine Res.* (2018) 38:1–11. doi: 10.1089/jir.2017.0088
59. Hussein S, Dalton B, Willmund GD, Ibrahim MAA, Himmerich H. A systematic review of tumor necrosis factor-alpha in post-traumatic stress disorder: evidence from human and animal studies. *Psychiatr Danub.* (2017) 29:407–20. doi: 10.24869/psyd.2017.407
60. Rao NP, Venkatasubramanian G, Ravi V, Kalmady S, Cherian A, Yc JR. Plasma cytokine abnormalities in drug-naive, comorbidity-free obsessive-compulsive disorder. *Psychiatry Res.* (2015) 229:949–52. doi: 10.1016/j.psychres.2015.07.009

61. Dalton B, Bartholdy S, Robinson L, Solmi M, Ibrahim MAA, Breen G, et al. A meta-analysis of cytokine concentrations in eating disorders. *J Psychiatr Res.* (2018) 103:252–64. doi: 10.1016/j.jpsychires.2018.06.002
62. Kircher T, Wohr M, Nenadic I, Schwarting R, Schratt G, Alferink J, et al. Neurobiology of the major psychoses: a translational perspective on brain structure and function-the FOR2107 consortium. *Eur Arch Psychiatry Clin Neurosci.* (2018). doi: 10.1007/s00406-018-0943-x. [Epub ahead of print].
63. Krishnan V, Berton O, Nestler E. The use of animal models in psychiatric research and treatment. *Am J Psychiatry* (2008) 165:1109. doi: 10.1176/appi.ajp.2008.08071076
64. Toyoda A. Social defeat models in animal science: what we have learned from rodent models. *Anim Sci J.* (2017) 88:944–52. doi: 10.1111/asj.12809
65. Ramirez K, Fornaguera-Trias J, Sheridan JF. Stress-induced microglia activation and monocyte trafficking to the brain underlie the development of anxiety and depression. *Curr Top Behav Neurosci.* (2017) 31:155–72. doi: 10.1007/7854_2016_25
66. Engler H, Bailey MT, Engler A, Sheridan JF. Effects of repeated social stress on leukocyte distribution in bone marrow, peripheral blood and spleen. *J Neuroimmunol.* (2004) 148:106–15. doi: 10.1016/j.jneuroim.2003.11.011
67. Wohleb ES, Powell ND, Godbout JP, Sheridan JF. Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. *J Neurosci.* (2013) 33:13820–33. doi: 10.1523/JNEUROSCI.1671-13.2013
68. Zheng X, Ma S, Kang A, Wu M, Wang L, Wang Q, et al. Chemical dampening of Ly6C(hi) monocytes in the periphery produces anti-depressant effects in mice. *Sci Rep.* (2016) 6:19406. doi: 10.1038/srep19406
69. Ambree O, Ruland C, Scheu S, Arolt V, Alferink J. Alterations of the innate immune system in susceptibility and resilience after social defeat stress. *Front Behav Neurosci.* (2018) 12:141. doi: 10.3389/fnbeh.2018.00141
70. Li MX, Zheng HL, Luo Y, He JG, Wang W, Han J, et al. Gene deficiency and pharmacological inhibition of caspase-1 confers resilience to chronic social defeat stress via regulating the stability of surface AMPARs. *Mol Psychiatry* (2018) 23:556–68. doi: 10.1038/mp.2017.76
71. Zhang Y, Liu L, Peng YL, Liu YZ, Wu TY, Shen XL, et al. Involvement of inflammasome activation in lipopolysaccharide-induced mice depressive-like behaviors. *CNS Neurosci Ther.* (2014) 20:119–24. doi: 10.1111/cns.12170
72. Burfeind KG, Zhu X, Levasseur PR, Michaelis KA, Norgard MA, Marks DL. TRIF is a key inflammatory mediator of acute sickness behavior and cancer cachexia. *Brain Behav Immun.* (2018) 73:364–74. doi: 10.1016/j.bbi.2018.05.021
73. Le Thuc O, Cansell C, Bourourou M, Denis RG, Stobbe K, Devaux N, et al. Central CCL2 signaling onto MCH neurons mediates metabolic and behavioral adaptation to inflammation. *EMBO Rep.* (2016) 17:1738–52. doi: 10.15252/embr.201541499
74. Reus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* (2015) 300:141–54. doi: 10.1016/j.neuroscience.2015.05.018
75. Tay TL, Bechade C, D'Andrea I, St-Pierre MK, Henry MS, Roumier A, et al. Microglia gone rogue: impacts on psychiatric disorders across the lifespan. *Front Mol Neurosci.* (2017) 10:421. doi: 10.3389/fnmol.2017.00421
76. Kalkman HO, Feuerbach D. Antidepressant therapies inhibit inflammation and microglial M1-polarization. *Pharmacol Ther.* (2016) 163:82–93. doi: 10.1016/j.pharmthera.2016.04.001
77. Reader BF, Jarrett BL, McKim DB, Wohleb ES, Godbout JP, Sheridan JF. Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. *Neuroscience* (2015) 289:429–42. doi: 10.1016/j.neuroscience.2015.01.001
78. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. *J Clin Invest.* (2012) 122:1164–71. doi: 10.1172/JCI58644
79. Singhal G, Baune BT. Microglia: an interface between the loss of neuroplasticity and depression. *Front Cell Neurosci.* (2017) 11:270. doi: 10.3389/fncel.2017.00270
80. Tay TL, Savage JC, Hui CW, Bisht K, Tremblay ME. Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. *J Physiol.* (2017) 595:1929–45. doi: 10.1113/JP272134
81. Napoli I, Neumann H. Microglial clearance function in health and disease. *Neuroscience* (2009) 158:1030–8. doi: 10.1016/j.neuroscience.2008.06.046
82. Amici SA, Dong J, Guerau-de-Arellano M. Molecular mechanisms modulating the phenotype of macrophages and microglia. *Front Immunol.* (2017) 8:1520. doi: 10.3389/fimmu.2017.01520
83. Yrondi A, Aouizerate B, El-Hage W, Moliere F, Thalamas C, Delcourt N, et al. Assessment of translocator protein density, as marker of neuroinflammation, in major depressive disorder: a pilot, multicenter, comparative, controlled, Brain PET Study (INFLADEP Study). *Front. Psychiatry* (2018) 9:326. doi: 10.3389/fpsyt.2018.00326
84. Setiawan E, Attwells S, Wilson AA, Mizrahi R, Rusjan PM, Miler L, et al. Association of translocator protein total distribution volume with duration of untreated major depressive disorder: a cross-sectional study. *Lancet Psychiatry* (2018) 5:339–47. doi: 10.1016/S2215-0366(18)30048-8
85. Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol.* (2013) 39:19–34. doi: 10.1111/j.1365-2990.2012.01306.x
86. Steiner J, Bielau H, Brisch R, Danos P, Ullrich O, Mawrin C, et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J Psychiatr Res.* (2008) 42:151–7. doi: 10.1016/j.jpsychires.2006.10.013
87. Schnieder TP, Trencevska I, Rosoklija G, Stankov A, Mann JJ, Smiley J, et al. Microglia of prefrontal white matter in suicide. *J Neuropathol Exp Neurol.* (2014) 73:880–90. doi: 10.1097/NEN.0000000000000107
88. Yirmiya R, Rimmerman N, Reshef R. Depression as a microglial disease. *Trends Neurosci.* (2015) 38:637–58. doi: 10.1016/j.tins.2015.08.001
89. Liu Y, Holdbrooks AT, De Sarno P, Rowse AL, Yanagisawa LL, McFarland BC, et al. Therapeutic efficacy of suppressing the Jak/STAT pathway in multiple models of experimental autoimmune encephalomyelitis. *J Immunol.* (2014) 192:59–72. doi: 10.4049/jimmunol.1301513
90. Colle R, de Larminat D, Rotenberg S, Hozer F, Hardy P, Verstuyft C, et al. PPAR-gamma agonists for the treatment of major depression: a review. *Pharmacopsychiatry* (2017) 50:49–55. doi: 10.1055/s-0042-120120
91. English C, Aloi JJ. New FDA-approved disease-modifying therapies for multiple sclerosis. *Clin Ther.* (2015) 37:691–715. doi: 10.1016/j.clinthera.2015.03.001
92. Su F, Yi H, Xu L, Zhang Z. Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia *in vitro*. *Neuroscience* (2015) 294:60–8. doi: 10.1016/j.neuroscience.2015.02.028
93. Alcocer-Gomez E, Ulecia-Moron C, Marin-Aguilar F, Rybkina T, Casas-Barquero N, Ruiz-Cabello J, et al. Stress-induced depressive behaviors require a functional NLRP3 inflammasome. *Mol Neurobiol.* (2016) 53:4874–82. doi: 10.1007/s12035-015-9408-7
94. Zhao Q, Wu X, Yan S, Xie X, Fan Y, Zhang J, et al. The antidepressant-like effects of pioglitazone in a chronic mild stress mouse model are associated with PPARgamma-mediated alteration of microglial activation phenotypes. *J Neuroinflam.* (2016) 13:259. doi: 10.1186/s12974-016-0728-y
95. Kenis G, Maes M. Effects of antidepressants on the production of cytokines. *Int J Neuropsychopharmacol.* (2002) 5:401–12. doi: 10.1017/S1461145702003164
96. Herculano-Houzel S. Scaling of brain metabolism with a fixed energy budget per neuron: implications for neuronal activity, plasticity and evolution. *PLoS ONE* (2011) 6:e17514. doi: 10.1371/journal.pone.0017514
97. Anglin RE, Garside SL, Tarnopolsky MA, Mazurek MF, Rosebush PI. The psychiatric manifestations of mitochondrial disorders: a case and review of the literature. *J Clin Psychiatry* (2012) 73:506–12. doi: 10.4088/JCP.11r07237
98. Kato T. Role of mitochondrial DNA in calcium signaling abnormality in bipolar disorder. *Cell Calcium* (2008) 44:92–102. doi: 10.1016/j.ceca.2007.11.005
99. Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, et al. Impaired mitochondrial function in psychiatric disorders. *Nat Rev Neurosci.* (2012) 13:293–307. doi: 10.1038/nrn3229
100. Moylan S, Eyre HA, Maes M, Baune BT, Jacka FN, Berk M. Exercising the worry away: how inflammation, oxidative and nitrogen stress mediates the beneficial effect of physical activity on anxiety disorder symptoms and behaviours. *Neurosci Biobehav Rev.* (2013) 37:573–84. doi: 10.1016/j.neubiorev.2013.02.003
101. Morava E, Kozicz T. Mitochondria and the economy of stress (mal)adaptation. *Neurosci Biobehav Rev.* (2013) 37:668–80. doi: 10.1016/j.neubiorev.2013.02.005

102. Allen J, Romay-Tallon R, Brymer KJ, Caruncho HJ, Kalynchuk LE. Mitochondria and mood: mitochondrial dysfunction as a key player in the manifestation of depression. *Front Neurosci.* (2018) 12:386. doi: 10.3389/fnins.2018.00386
103. Fattal O, Link J, Quinn K, Cohen BH, Franco K. Psychiatric comorbidity in 36 adults with mitochondrial cytopathies. *CNS Spectr.* (2007) 12:429–38. doi: 10.1017/S1092852900015303
104. Cataldo AM, McPhie DL, Lange NT, Punzell S, Elmiligy S, Ye NZ, et al. Abnormalities in mitochondrial structure in cells from patients with bipolar disorder. *Am J Pathol.* (2010) 177:575–85. doi: 10.2353/ajpath.2010.081068
105. Petschner P, Gonda X, Baksa D, Eszlari N, Trivaks M, Juhasz G, et al. Genes linking mitochondrial function, cognitive impairment and depression are associated with endophenotypes serving precision medicine. *Neuroscience* (2018) 370:207–17. doi: 10.1016/j.neuroscience.2017.09.049
106. Woolley JD, Khan BK, Murthy NK, Miller BL, Rankin KP. The diagnostic challenge of psychiatric symptoms in neurodegenerative disease: rates of and risk factors for prior psychiatric diagnosis in patients with early neurodegenerative disease. *J Clin Psychiatry* (2011) 72:126–33. doi: 10.4088/JCP.10m06382oli
107. Galindo MF, Ikuta I, Zhu X, Casadesus G, Jordan J. Mitochondrial biology in Alzheimer's disease pathogenesis. *J Neurochem.* (2010) 114:933–45. doi: 10.1111/j.1471-4159.2010.06814.x
108. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. *Nat Clin Pract Neurol.* (2008) 4:600–9. doi: 10.1038/ncpneuro0924
109. Turner C, Schapira AH. Mitochondrial matters of the brain: the role in Huntington's disease. *J Bioenerg Biomembr.* (2010) 42:193–8. doi: 10.1007/s10863-010-9290-y
110. Scaini G, Santos PM, Benedet J, Rochi N, Gomes LM, Borges LS, et al. Evaluation of Krebs cycle enzymes in the brain of rats after chronic administration of antidepressants. *Brain Res Bull.* (2010) 82:224–7. doi: 10.1016/j.brainresbull.2010.03.006
111. Moretti A, Gorini A, Villa RF. Affective disorders, antidepressant drugs and brain metabolism. *Mol Psychiatry* (2003) 8:773–85. doi: 10.1038/sj.mp.4001353
112. Martins-de-Souza D, Guest PC, Harris LW, Vanattou-Saifoudine N, Webster MJ, Rahmoune H, et al. Identification of proteomic signatures associated with depression and psychotic depression in post-mortem brains from major depression patients. *Transl Psychiatry* (2012) 2:e87. doi: 10.1038/tp.2012.13
113. Kambe Y, Miyata A. Potential involvement of the mitochondrial unfolded protein response in depressive-like symptoms in mice. *Neurosci Lett.* (2015) 588:166–71. doi: 10.1016/j.neulet.2015.01.006
114. Gong Y, Chai Y, Ding JH, Sun XL, Hu G. Chronic mild stress damages mitochondrial ultrastructure and function in mouse brain. *Neurosci Lett.* (2011) 488:76–80. doi: 10.1016/j.neulet.2010.11.006
115. Gamaro GD, Streck EL, Matte C, Prediger ME, Wyse AT, Dalmaz C. Reduction of hippocampal Na+, K+-ATPase activity in rats subjected to an experimental model of depression. *Neurochem Res.* (2003) 28:1339–44. doi: 10.1023/A:1024988113978
116. Wen L, Jin Y, Li L, Sun S, Cheng S, Zhang S, et al. Exercise prevents raphe nucleus mitochondrial overactivity in a rat depression model. *Physiol Behav.* (2014) 132:57–65. doi: 10.1016/j.physbeh.2014.04.050
117. Chen C, Wang Y, Zhang J, Ma L, Gu J, Ho G. Contribution of neural cell death to depressive phenotypes of streptozotocin-induced diabetic mice. *Dis Models Mech.* (2014) 7:723–30. doi: 10.1242/dmm.016162
118. Norkett R, Modi S, Birsa N, Atkin TA, Ivankovic D, Pathania M, et al. DISC1-dependent regulation of mitochondrial dynamics controls the morphogenesis of complex neuronal dendrites. *J Biol Chem.* (2016) 291:613–29. doi: 10.1074/jbc.M115.699447
119. Kato T. Molecular genetics of bipolar disorder and depression. *Psychiatry Clin Neurosci.* (2007) 61:3–19. doi: 10.1111/j.1440-1819.2007.01604.x
120. Urrutia PJ, Mena NP, Nunez MT. The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. *Front Pharmacol.* (2014) 5:38. doi: 10.3389/fphar.2014.00038
121. Gardner A, Boles RG. Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. *Prog Neuro Psychopharmacol Biol Psychiatry* (2011) 35:730–43. doi: 10.1016/j.pnpbp.2010.07.030
122. Cordeiro JL, Marques W, Hallak JE, Osorio FL. Charcot-Marie-Tooth disease, psychiatric indicators and quality of life: a systematic review. *ASN Neuro* (2014) 6:185–92. doi: 10.1042/AN20130048
123. Madrigal JL, Olivenza R, Moro MA, Lizasoain I, Lorenzo P, Rodrigo J, et al. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. *Neuropsychopharmacology* (2001) 24:420–9. doi: 10.1016/S0893-133X(00)00208-6
124. Neitemeier S, Jelinek A, Laino V, Hoffmann L, Eisenbach I, Eying R, et al. BID links ferroptosis to mitochondrial cell death pathways. *Redox Biol.* (2017) 12:558–70. doi: 10.1016/j.redox.2017.03.007
125. Jelinek A, Heyder L, Daude M, Plessner M, Krippner S, Grosse R, et al. Mitochondrial rescue prevents glutathione peroxidase-dependent ferroptosis. *Free Radic Biol Med.* (2018) 117:45–57. doi: 10.1016/j.freeradbiomed.2018.01.019
126. Grohm J, Kim SW, Mamrak U, Tobaben S, Cassidy-Stone A, Nunnari J, et al. Inhibition of Drp1 provides neuroprotection *in vitro* and *in vivo*. *Cell Death Diff.* (2012) 19:1446–58. doi: 10.1038/cdd.2012.18
127. Ben-Shachar D, Karry R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. *PLoS ONE* (2008) 3:e3676. doi: 10.1371/journal.pone.0003676
128. Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM, Perlis RH, et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet.* (2013) 45:984–94. doi: 10.1038/ng.2711
129. Michels S, Wohr M, Schwarting RK, Culmsee C. Psychiatric risk gene Cacna1c determines mitochondrial resilience against oxidative stress in neurons. *Cell Death Dis.* (2018) 9:645. doi: 10.1038/s41419-018-0676-9
130. Michels S, Ganjam GK, Martins H, Schratt GM, Wohr M, Schwarting RKW, et al. Downregulation of the psychiatric susceptibility gene Cacna1c promotes mitochondrial resilience to oxidative stress in neuronal cells. *Cell Death Discov.* (2018) 4:54. doi: 10.1038/s41420-018-0061-6
131. Kabir ZD, Lee AS, Burgdorf CE, Fischer DK, Rajadhyaksha AM, Mok E, et al. Cacna1c in the prefrontal cortex regulates depression-related behaviors via REDD1. *Neuropsychopharmacology* (2017) 42:2032–42. doi: 10.1038/npp.2016.271
132. Yoshimizu T, Pan JQ, Mungenast AE, Madison JM, Su S, Ketterman J, et al. Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. *Mol Psychiatry* (2015) 20:284. doi: 10.1038/mp.2014.181
133. Adzic M, Brkic Z, Bulajic S, Mitic M, Radojcic MB. Antidepressant action on mitochondrial dysfunction in psychiatric disorders. *Drug Dev Res.* (2016) 77:400–6. doi: 10.1002/ddr.21332
134. Malkesman O, Austin DR, Tragon T, Henter ID, Reed JC, Pellecchia M, et al. Targeting the BH3-interacting domain death agonist to develop mechanistically unique antidepressants. *Mol Psychiatry* (2012) 17:770–80. doi: 10.1038/mp.2011.77
135. Cardoso AL, Costa P, de Almeida LP, Simoes S, Plesnila N, Culmsee C, et al. Tf-lipoplex-mediated c-Jun silencing improves neuronal survival following excitotoxic damage *in vivo*. *J Control. Release* (2010) 142:392–403. doi: 10.1016/j.jconrel.2009.11.004
136. Culmsee C, Plesnila N. Targeting Bid to prevent programmed cell death in neurons. *Biochem Soc Trans.* (2006) 34 (Pt 6):1334–40. doi: 10.1042/BST0341334
137. Pereira C, Chavarria V, Vian J, Ashton MM, Berk M, Marx W, et al. Mitochondrial agents for bipolar disorder. *Int J Neuropsychopharmacol.* (2018) 21:550–69. doi: 10.1093/ijnp/pyy018
138. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* (2015) 25:771–84. doi: 10.1038/cr.2015.68
139. Zhu L, Zhao Q, Yang T, Ding W, Zhao Y. Cellular metabolism and macrophage functional polarization. *Int Rev Immunol.* (2015) 34:82–100. doi: 10.3109/08830185.2014.969421
140. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab.* (2012) 15:813–26. doi: 10.1016/j.cmet.2012.04.023

141. Galvan-Pena S, O'Neill LA. Metabolic reprograming in macrophage polarization. *Front Immunol.* (2014) 5:420. doi: 10.3389/fimmu.2014.00420
142. Mills CD. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol.* (2012) 32:463–88. doi: 10.1615/CritRevImmunol.v32.i6.10
143. Rath M, Muller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. *Front Immunol.* (2014) 5:532. doi: 10.3389/fimmu.2014.00532
144. De Santa F, Vitiello L, Torcinaro A, Ferraro E. The role of metabolic remodeling in macrophage polarization and its effect on skeletal muscle regeneration. *Antioxid Redox Signal.* (2018). doi: 10.1089/ars.2017.742. [Epub ahead of print].
145. Klimaszewska-Lata J, Gul-Hinc S, Bielarczyk H, Ronowska A, Zysk M, Gruzewska K, et al. Differential effects of lipopolysaccharide on energy metabolism in murine microglial N9 and cholinergic SN56 neuronal cells. *J Neurochem.* (2015) 133:284–97. doi: 10.1111/jnc.12979
146. Orihuela R, McPherson CA, Harry GJ. Microglial M1/M2 polarization and metabolic states. *Br J Pharmacol.* (2016) 173:649–65. doi: 10.1111/bph.13139
147. Van den Bossche J, Baardman J, de Winther MP. Metabolic characterization of polarized M1 and M2 bone marrow-derived macrophages using real-time extracellular flux analysis. *J Visual Exp.* (2015) 105:e53424. doi: 10.3791/53424
148. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab.* (2006) 4:13–24. doi: 10.1016/j.cmet.2006.05.011
149. Cherry JD, Olschowka JA, O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J Neuroinflam.* (2014) 11:98. doi: 10.1186/1742-2094-11-98
150. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Shively C, Andrews RN, et al. Blood-based bioenergetic profiling reflects differences in brain bioenergetics and metabolism. *Oxid Med Cell Longevity* (2017) 2017:7317251. doi: 10.1155/2017/7317251
151. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. *Mol Psychiatry* (2004) 9:684–97, 643. doi: 10.1038/sj.mp.4001511
152. Karabatsiakis A, Bock C, Salinas-Manrique J, Kolassa S, Calzia E, Dietrich DE, et al. Mitochondrial respiration in peripheral blood mononuclear cells correlates with depressive subsymptoms and severity of major depression. *Transl Psychiatry* (2014) 4:e397. doi: 10.1038/tp.2014.44
153. Hroudova J, Fisar Z, Kitzlerova E, Zverova M, Raboch J. Mitochondrial respiration in blood platelets of depressive patients. *Mitochondrion* (2013) 13:795–800. doi: 10.1016/j.mito.2013.05.005
154. Gubert C, Stertz L, Pfaffenseller B, Panizzutti BS, Rezin GT, Massuda R, et al. Mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells of patients with bipolar disorder, schizophrenia, and healthy subjects. *J Psychiatr Res.* (2013) 47:1396–402. doi: 10.1016/j.jpsychires.2013.06.018
155. Lopez-Armada MJ, Riveiro-Naveira RR, Vaamonde-Garcia C, Valcarcel-Ares MN. Mitochondrial dysfunction and the inflammatory response. *Mitochondrion* (2013) 13:106–18. doi: 10.1016/j.mito.2013.01.003
156. Ng DS, Chu T, Esposito B, Hui P, Connelly PW, Gross PL. Paraoxonase-1 deficiency in mice predisposes to vascular inflammation, oxidative stress, and thrombogenicity in the absence of hyperlipidemia. *Cardiovasc Pathol.* (2008) 17:226–32. doi: 10.1016/j.carpath.2007.10.001
157. Chacko BK, Kramer PA, Ravi S, Benavides GA, Mitchell T, Dranka BP, et al. The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci.* (2014) 127:367–73. doi: 10.1042/CS20140101
158. Chacko BK, Zhi D, Darley-Usmar VM, Mitchell T. The Bioenergetic Health Index is a sensitive measure of oxidative stress in human monocytes. *Redox Biol.* (2016) 8:43–50. doi: 10.1016/j.redox.2015.12.008

Conflict of Interest Statement: VA is member of advisory boards and/or gave presentations for the following companies: Astra-Zeneca, Allergan, Janssen-Organon, Lundbeck, Otsuka, Servier, and Trommsdorff. JA gave a presentation for the company Servier.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 14 January 2019
doi: 10.3389/fpsyt.2018.00768

Leveraging Machine Learning Approaches for Predicting Antidepressant Treatment Response Using Electroencephalography (EEG) and Clinical Data

Natalia Jaworska[1,2,3], *Sara de la Salle*[1], *Mohamed-Hamza Ibrahim*[4], *Pierre Blier*[1,2,3] *and Verner Knott*[1,2,3]*

[1] Institute of Mental Health Research, University of Ottawa, Ottawa, ON, Canada, [2] Cellular & Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada, [3] Brain and Mind Research Institute, University of Ottawa, Ottawa, ON, Canada, [4] Department of Mathematics, Faculty of Science, Zagazig University, Zagazig, Egypt

Background: Individuals with major depressive disorder (MDD) vary in their response to antidepressants. However, identifying objective biomarkers, prior to or early in the course of treatment that can predict antidepressant efficacy, remains a challenge.

Methods: Individuals with MDD participated in a 12-week antidepressant pharmacotherapy trial. Electroencephalographic (EEG) data was collected before and 1 week post-treatment initiation in 51 patients. Response status at week 12 was established with the Montgomery-Asberg Depression Scale (MADRS), with a ≥50% decrease characterizing responders (N = 27/24 responders/non-responders). We used a machine learning (ML)-approach for predicting response status. We focused on Random Forests, though other ML methods were compared. First, we used a tree-based estimator to select a relatively small number of significant features from: (a) demographic/clinical data (age, sex, individual item/total MADRS scores at baseline, week 1, change scores); (b) scalp-level EEG power; (c) source-localized current density (via exact low-resolution electromagnetic tomography [eLORETA] software). Second, we applied kernel principal component analysis to reduce and map important features. Third, a set of ML models were constructed to classify response outcome based on mapped features. For each dataset, predictive features were extracted, followed by a model of all predictive features, and finally by a model of the *most* predictive features.

Results: Fifty eLORETA features were predictive of response (across bands, both time-points); $alpha_1$/theta eLORETA features showed the highest predictive value. Eighty-eight scalp EEG features were predictive of response (across bands, both time-points), with theta/$alpha_2$ being most predictive. Clinical/demographic data consisted of 31 features, with the most important being week 1 "concentration difficulty" scores. When all features were included into one model, its predictive utility was high (88% accuracy). When the *most* important features were extracted in the final model, 12 predictive features emerged (78% accuracy), including baseline scalp-EEG frontopolar theta, parietal $alpha_2$ and frontopolar $alpha_1$.

OPEN ACCESS

Edited by:
Frank M. Schmidt,
Universitätsklinikum Leipzig, Germany

Reviewed by:
Wajid Mumtaz,
Universiti Teknologi Petronas, Malaysia
Neil Bailey,
Monash Alfred Psychiatry Research Centre, Australia

***Correspondence:**
Verner Knott
verner.knott@theroyal.ca

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *15 October 2018*
Accepted: *21 December 2018*
Published: *14 January 2019*

Citation:
Jaworska N, de la Salle S, Ibrahim M-H, Blier P and Knott V (2019) Leveraging Machine Learning Approaches for Predicting Antidepressant Treatment Response Using Electroencephalography (EEG) and Clinical Data. Front. Psychiatry 9:768. doi: 10.3389/fpsyt.2018.00768

Conclusions: These findings suggest that ML models of pre- and early treatment-emergent EEG profiles and clinical features can serve as tools for predicting antidepressant response. While this must be replicated using large independent samples, it lays the groundwork for research on personalized, "biomarker"-based treatment approaches.

Keywords: major depressive disorder (MDD), antidepressants, biomarker, quantitative EEG, machine learning (ML), classification and regression trees, predictive models, personalized treatment

INTRODUCTION

Worldwide, major depressive disorder (MDD) carries a large burden of disease (1), is associated with impaired daily functioning (2), and worsening of co-morbid medical illness (3, 4). It is also linked with shorter life expectancies (5), including increased death by suicide. However, one of the largest naturalistic clinical trials assessing treatment outcomes in depressed patients found that fewer than ∼50% responded (>50% symptom decreases), and only ∼30% remitted (absence/near absence of symptoms), to intervention with a serotonin reuptake inhibitor (SSRI) antidepressant (6, 7); SSRIs are the most common antidepressant pharmacotherapy for treating MDD. Unfortunately, partial or inadequate response carries serious consequences, as each attempt to improve outcome either by switching or combining pharmacotherapies may require weeks to evaluate effectiveness (8, 9). This represents a substantial amount of time during which patients live with lingering, debilitating and even fatal symptoms.

Current approaches for treating MDD rely on trial-and-error sequential treatment strategies, as there is currently no established method of predicting whether a medication will lead to response. Identifying markers of response, either by *a priori* prediction or by distinguishing eventual responders from non-responders shortly after commencing treatment, would significantly increase the efficiency and efficacy of MDD interventions. Evidenced-based decision-making regarding treatment selection may be aided by biomarkers. Biomarkers are measurable and objective indicators of biological processes, or biological responses to interventions (10). To date, there have been no identified biomarkers of sufficient clinical utility to inform antidepressant treatment selection (11, 12). Nevertheless, growing evidence supporting mood disorders as brain disorders with putative structural and functional abnormalities in certain neural circuits (13) has positioned neuroimaging techniques as candidates for prognostic biomarkers in MDD (14–21), and as potential indices of treatment response prediction.

For routine clinical use, predictive biomarkers must have a high specificity/sensitivity, be reproducible, yet also be relatively inexpensive, non-invasive and accessible (22). Although not possessing the same spatial resolution as functional magnetic resonance imaging (fMRI), quantitative measures of brain electrical signals derived from scalp-recorded electroencephalograms (EEG) provide superb temporal resolution of brain activity. Further, EEG offers many of the outlined practical advantages [e.g., easy-to-administer, low-cost; (23)]. Power spectral measures of resting-state EEG activity have been found to be sensitive to both acute and chronic effects of antidepressant pharmacotherapies in MDD (24, 25). Additionally, members by our own group and others have found that when EEG profiles are assessed before or early in the course of treatment (≤1 week), they are predictive of/associated with antidepressant response (e.g., theta EEG source-localized to the anterior cingulate cortex [ACC] or alpha EEG/frontal alpha asymmetry) (26–29). Alpha power is thought to be inversely related to cortical arousal (30), therefore, excess alpha power may represent decreased cortical arousal (though alpha presence should not be thought of as reflecting an "idle"/inactive brain state). Measures of prefrontal theta cordance, which is a combination of absolute and relative EEG power, have been shown to correlate strongly with cerebral perfusion (31), and have also been associated with treatment response. In other words, theta cordance appears to be an electrocortical proxy of fronto-cortical activity as indexed by cerebral blood flow. Several groups have noted that initial (32) or rapid decreases in prefrontal theta cordance were associated with a positive response to treatment with various antidepressant pharmacotherapies (33, 34). However, these predictive EEG profiles tend to be limited to group-level comparisons, which may obscure potentially useful information at the individual-level. Importantly, individual EEG-based biomarkers would be most useful clinically.

The complexity/dimensionality of EEG data lends itself to the use of machine learning (ML) approaches which, unlike conventional analyses, are designed to deal with multivariate inputs. ML can treat EEG measures as patterns rather than considering each measure in isolation, which could potentially be a more informative analytic approach (35, 36). Further, ML approaches may be more conducive to extracting response prediction data at the individual-level (after we are sufficiently confident that we input appropriate information). While there have been several ML-based studies using EEG data to separate individuals with and without MDD (37), including work from our own group (38), there have only been a handful of studies utilizing ML-based approaches of EEG data for response prediction (see **Supplementary Table 1** for a summary). However, the few that exist have yielded relatively high prediction accuracies of response to SSRI treatment based on pre-treatment EEG features (39), and appear to be more accurate than prediction models based on clinician ratings (40). A recent study of depressed individuals treated with repeated transcranial magnetic stimulation (rTMS) assessed baseline and week 1 EEG

profiles, including theta and alpha power and connectivity, frontal theta cordance and alpha peak frequency (41). A ML approach was used to differentiate responders/non-responders using these measures, coupled with depression change scores. The 12 eventual rTMS responders were separated from non-responders ($N = 30$) based on elevated theta connectivity at baseline and week 1 (sensitivity: 0.84; specificity: 0.89). The same group also found that a ML model consisting of 30 features, collected during a working memory task (including baseline/week 1/changes in theta, upper alpha & gamma power, connectivity, theta-gamma coupling), could distinguish rTMS responders/non-responders [sensitivity: 0.90; specificity: 0.92; (42)]. In addition to the SSRI and rTMS findings, frontal EEG sites have been shown to be most predictive of clinical and cognitive outcome in MDD patients following transcranial direct current stimulation (tDCS) treatment using ML approaches (43).

Despite the promise shown by the application of ML for predicting antidepressant treatment response, logistical obstacles exist (e.g., ethics/privacy concerns, technical expertise). Other challenges which impede ML from being used in predictive psychiatry include the relatively small sizes (though that is not an inherent limitation *per se*) of many clinical/biological datasets. A further challenge is that that data may be lacking/are incomplete, or datasets require considerable processing prior to analyses (therefore, the preparation of the data for analysis can be onerous). Further, ML sometimes focuses on the most efficient use of data rather than the most valuable, which leads to variability in ML approaches (including biases), and the tendency to overfit data (44). Additionally, many studies using ML on EEG data in antidepressant response prediction tend to be based on unequal responder/non-responder samples, which requires over/under sampling techniques (e.g., Synthetic Minority Oversampling Technique [SMOTE] or weighting subjects by their inverse proportion of being responders or non-responders); whether this is applied is generally not stated in the methodology. Under-sampling may lead to discarding potentially useful data while over-sampling duplicates samples, which could greatly increase the possibility of overfitting. As a result, ML-derived results can sometimes be difficult to replicate, and comparisons between various ML approaches in one study are rare. We are also not aware of any studies that have assessed whether source-localized EEG activity using approaches such as low-resolution brain electromagnetic tomography [LORETA; (45)] contribute to antidepressant response prediction with ML, despite the fact that this has shown predictive promise using non-ML analyses. Finally, the contribution of specific depression symptom scores [e.g., items of the Montgomery-Asberg Depression Rating Scale [MADRS]; (46)] and demographic features (e.g., age, sex), are generally not included in ML approaches utilizing EEG data for predicting response. This is despite the fact that specific symptom profiles and demographic variables have been shown to be predictive of response (47).

As such, in the present study we carried out several experiments addressing the outlined gaps. First, we explored the utility of exact low-resolution electromagnetic tomography software (eLORETA)-localized EEG data at baseline and at week 1 of antidepressant pharmacotherapy in predicting responder ($N = 27$)/non-responder ($N = 24$) status (i.e., balanced sample) in depressed adults at week 12 of treatment using several ML approaches, with a focus on Random Forest. In other words, we extracted predictive features of response from eLORETA data (**Experiment A**). Second, the same ML approaches were applied to scalp-level EEG power in order to extract pertinent predictive features from this dataset (**Experiment B**). Third, ML was applied to clinical and demographic data to extract predictive features of response (i.e., sex, age, individual/total MADRS score items at baseline/week 1, score changes; **Experiment C**). Subsequently, all of the relevant predictive features were put into a combined ML model, and prefrontal theta cordance data was included (**Experiment D**), this was followed by a final analysis that identified the *most* relevant predictive features of antidepressant response (**Experiment E**). We expected that ML approaches would be useful for predicating antidepressant response, and that the combined model would yield superior prediction values as compared to each individual model.

METHODS

Participants

In total, 51 adults (18–60 years) with a primary diagnosis of MDD, and enrolled in a clinical trial assessing antidepressant pharmacotherapies [details below; (48)], participated in this EEG study. As previously outlined (49), psychiatrists ascertained the diagnosis with the Structured Clinical Interview for DSM (Diagnostic & Statistical Manual of Mental Disorders) IV-TR Diagnoses, Axis I, Patient Version [SCID-I/P; (50)]. Symptom severity was evaluated using the MADRS (46), with scores ≥22 at enrollment. A secondary diagnosis of an anxiety disorder was permitted. Patients with Bipolar Disorder (BP I/II or NOS), psychosis history, current (<6 months) drug/alcohol abuse or dependence, history of seizures, unstable (≥3 months) medical condition(s) and history of anorexia/bulimia were excluded. Patients were not taking psychoactive drugs at the time of randomization, and appropriate drug washout periods were applied prior to enrollment. EEG testing occurred pre- and 1-week post-treatment. Participants provided written informed consent, and the study was approved by the Royal Ottawa Health Care Group Research Ethics Board.

Clinical Trial Design

As part of a larger clinical trial (48), patients were randomized (double-blind) to one of three antidepressant regimens: escitalopram + bupropion (ESC+BUP), escitalopram (ESC) + placebo or bupropion (BUP) + placebo. Treatments were initiated at recommended starting doses, and raised only if tolerated. MADRS assessments were conducted prior to treatment, weekly for the first 4 weeks, and then bi-weekly until week 12. Change in MADRS scores from baseline to week 12 were used to categorize patients as responders ($N = 27$; ≥50% MADRS score reduction) or non-responders ($N = 24$; <49% MADRS score reduction). Responder groups were similar on demographic and clinical parameters at baseline (**Table 1**).

TABLE 1 | Clinical characteristics and demographics of participants (Means ± S.D.).

	All participants (*N* = 51)	Treatment responders (*N* = 27)	Treatment non-responders (*N* = 24)
Sex (M/F)	24/27	12/15	11/13
Age	40.2 (±11.8)	35.9 (±11.3)	45 (±10.6)
Total baseline MADRS scores	30.6 (±5.2)	29.6 (±4.5)	31.6 (±5.8)
Total week 1 MADRS scores	26.2 (8.7)	22.7 (7.9)	30.2 (7.9)
Total week 12 MADRS scores	15.9 (12.5)	6.2 (5.2)	26.8 (8.6)
% Change (week 1-baseline)	−13.7 (27.5)	±23.2 (24.6)	−3.1 (27.1)
% Change (week 12-baseline)	−48.8 (37.9)	−79.0 (17.0)	−14.9 (22.9)

MADRS, Montgomery-Asberg Depression Rating Scale.

EEG Recordings and EEG Data Processing

As described elsewhere (49), prior to each EEG session, participants abstained for >3 h from caffeine and/or nicotine, as well as from alcohol/drugs (excluding prescribed drugs) as of midnight. Using an average scalp reference, AF_z ground, and a sampling rate of 500 Hz, EEG recordings were obtained from 32 sites using the 10–20 system (see **Supplementary Figure 1**) by way of Ag/AgCl electrodes embedded in a cap (EasyCap, Inning A. Ammersee, Germany). Additional electrodes were used to monitor vertical and horizontal electrooculographic (EOG) activity. Amplifier filters were 0.1–80 Hz, and electrode impedance was ≤5 kΩ during recordings (Brain Vision Quickamp®; Brain Products, Gilching, Germany). Vigilance-controlled resting-state EEG activity was recorded for 3 min during the eyes-closed (EC) condition (BrainVision Recorder®, Brain Products, Gilching, Germany).

EEG processing included re-referencing with averaged mastoid electrodes ($TP_{9/10}$), filtering (0.1–30 Hz) and segmentation (2 s; Brain Vision Analyzer® Software, Brain Products, Gilching, Germany). Ocular-corrected [Gratton & Coles method; (51)] epochs were excluded if voltages exceeded ±75 μV. A minimum of 100 s of artifact-free data were subjected to a Fast Fourier Transform algorithm (Hanning Window; 5% cosine taper) for computation of absolute (μV^2) power in frequency bands of interest (delta: 1–4 Hz, theta: 4–8 Hz, $alpha_1$: 8–10.5 Hz, $alpha_2$: 10.5–13 Hz, beta: 13–30 Hz) at 28 sites (mastoids, ground, reference electrode sites excluded). EEG data was ln-transformed prior to analyses to ensure normality. The ln-transformation minimizes the influence of extreme values (i.e., skewness) within the dataset.

eLORETA Analyses

EC ln-normalized EEG data in each frequency band (mastoid-referenced) was subjected to analysis with eLORETA (45) software (v. 2081104). eLORETA analysis estimates neural activity as current density based on the Montreal Neurological Institute-152 template creating a low-resolution activation image. The solution space consists of 6,239 voxels (5 mm^3 voxel) restricted to gray matter. Current source density is calculated from a linear, weighted sum of scalp potentials. This value is then squared per voxel, yielding current density power measures (A/m^2). Its validation has been independently replicated (52), and cross-validated (53, 54). Current source density measures from 84 Brodmann areas (BA; 42/hemisphere), available through the eLORETA software, were extracted (single voxel at the centroid of each BA).

Theta Cordance Analyses

Theta EEG cordance values were calculated using an algorithm provided by the UCLA Laboratory of Brain, Behavior, and Pharmacology (55). Briefly, values were computed by normalizing theta power across electrode sites (calculated using 19 electrodes, 30 bipolar pairings) and then combining absolute and relative theta power values. Average cordance values from prefrontal electrodes (Fp_1, Fp_2) at baseline and week 1 were extracted, as these two sites have been shown to be most predictive of response outcome in the past (33, 34).

Machine Learning (ML) Methodology

As outlined, patients were classified into responders ($N = 27$) and non-responders ($N = 24$, i.e., this was the dependent/outcome feature) based on their clinical outcome by week 12 (**Table 1**), thus, this ML problem was a binary classification problem. To achieve our objective, which was to assess the utility of the datasets for predicting week 12 response, we started by pre-processing the data.

Data Preprocessing

Initially, we prepared and structured the raw data in order to obtain the final datasets that could be used to build predictive ML models. The preprocessing of data consisted of the following steps:

a) Construction of Analytical Base Tables
   We constructed the following analytical base tables (ABT): (1). ln-normalized absolute EEG power from 28 electrodes (mastoid-referenced/EC data) at baseline and after week 1 of treatment for each of delta, theta, $alpha_1$, $alpha_2$, and beta bands. (2). eLORETA-localized power values (ln-normalized mastoid-referenced/EC data) at 84 BAs at baseline and week 1 for each band. (3). theta cordance data (EC data from left and right prefrontal sites) at baseline and week 1. (4). clinical/demographic data consisting of age, sex, each item of the MADRS (10 items) as well as total MADRS scores at baseline and week 1 as well as change scores for each MADRS item (i.e., difference from baseline to week 1).
b) Data Clean Up
   Individuals with missing data (i.e.. those without week 1 data) were removed from the final ATB tables ($N = 2$). As such, the final sample per ATB table included $N = 27$ responders and $N = 24$ non-responders ($N = 51$ total).
c) Data/Feature Scaling & Normalization
   Subsequently a scaling technique, called Min-Max scaling (56), was applied to normalize the data (also referred to as data

features or attributes) to a fixed range between minimum and maximum values. Given a feature/attribute "A," the Min-Max scaling value x_{norm} of a value x in "A" is done via the following equation:

$$x_{norm} = \left(\frac{x - x_{min}}{x_{max} - x_{min}}\right) \times \left(\acute{x}_{max} - \acute{x}_{min}\right) + \acute{x}_{min}$$

Where x_{min} and x_{max} are the minimum and maximum values in features "A" respectively, and $\acute{x}_{min}$ and $\acute{x}_{max}$ are the new minimum and maximum values of "A" after scaling. As such, if $\acute{x}_{min} = 0$ and $\acute{x}_{max} = 1$, then the maximum absolute value of "A" is scaled to unit size. In practice, scaling plays an important role for improving predictive models' performance (57, 58). The motivation to use this form of data standardization is due to its robustness to small standard deviations of features, and preserving zero (or near zero) entries in relatively sparse datasets. Further, standardization brings all features into the same range, allowing for scale-invariant features. Generally, ML algorithms benefit from data standardization to efficiently reduce data dimensionality, which aids with learning algorithms and prediction.

Machine Learning Strategy: Three Stages

Although there are numerous ML approaches we could have adopted, we focused on results obtained using Random Forests (RF). As such, all of the steps are described in relation to RF.

a) Stage 1: Tree-Based Feature Selection
As the large number of features involved in the structured ABTs (i.e., datasets) can represent a bottleneck for building efficient predictive models, we applied the extremely randomized trees (ERT) algorithm (59) to simplify the ABTs by discarding irrelevant features. Irrelevant features frequently capture unnecessary/redundant and noisy data. ERT is a tree-based feature selection algorithm that can be used to rank features using an importance measure (e.g., average Gini impurity reduction score). Relevant features are obtained by discarding irrelevant features that have an importance score less than a certain threshold (e.g., average impurity reduction $\geq$0.01). Strictly speaking, ERT builds an ensemble of unpruned decision trees and aggregates their outputs for prediction. When building each decision tree in ERT, every node uses Gini impurity measures (60) as a locally optimal condition on a single feature to split the ABT into two subsets such that the samples with identical classes (i.e., target value – in this case, responders and non-responders) end up being in the same subset. Gini impurity measure can be computed as follows:

$$Gini\_impurity\,(ABT) = \sum_{i=1}^{K} p_i \times \left(1 - p_i\right)$$

Where K is the number of class labels (target values: responders/non-responders), and p_i is the probability of a certain classification i in K. Thus, $G\,(ABT)$ measures the likelihood of an incorrect classification of a new sample of a random feature, if that new instance were randomly classified according to the distribution of class labels from the dataset. Thus, during the training of each tree, we can quantify how each feature decreases the weighted impurity in this tree (i.e., with every split made of a node on a feature, the Gini impurity measures of the two descendent nodes should be less than the parent node). Thus, averaging the Gini impurity reduction for each single feature over all trees in the forest provides its importance, which allows us to rank the features based on their relevance. It is worth noting that using only a relatively small number of important features can dramatically enhance the generalization of the constructed predictive models (i.e., classifiers) by reducing overfitting. This is why we ran feature selection per dataset (vs. on all of the data).

b) Stage 2: Feature Mapping
We then applied kernel principal component analysis (KPCA) on the purified ABTs after removing irrelevant features to map relevant features and reduce dimensionality (61, 62). That is, KPCA is a method that uses a kernel function κ to project the important features data onto a new space. This space often contains a small number of features (compared to the original datasets) and where the samples in the purified ABTs become linearly separable and can be discriminated by finding a decision between the given classes (i.e., responders/non-responders) in the newly mapped space that best maximizes class separation.
In KPCA, the kernel is a nonlinear function κ such that for all samples $x_i, x_j \in$ purified ABT, we have that $\kappa\left\langle\varphi\left(x_i\right), \varphi\left(x_j\right)\right\rangle$, where φ is a mapping from purified ABTs to an inner product feature space (e.g., dot product space). While several kernel functions can be used, such as polynomial and sigmoid, we frequently obtained our best results when applying the radial basis function (RBF) Gaussian kernel, which can be calculated on two samples as follows: $\left\langle x_i, x_j\right\rangle = exp\left\{-\frac{\left\|x_i - x_j\right\|^2}{2\,\sigma^2}\right\}$, where σ is a free parameter. Thus, in the current work, we used the RBF.
Other commonly used feature mapping methods, such as Linear Discriminant Analysis (LDA) and standard PCA, often only allows linear dimensionality reduction. Thus, if the data has more complicated structures, which normally cannot be represented in a linear subspace, such methods will produce poor mapping. Thus, one key advantage of using KPCA in the current work is that it allows us to generalize standard PCA to nonlinear dimensionality reduction (63) and could therefore provide efficient mapping of complicated data.

c) Stage 3: Predictive Data Modeling
At this point, the purified mapped ABT datasets could be classified by building classification models, such as Random Forest [RF; (64, 65)]. RF is a tree-based ensemble learning method that operates by constructing a forest of decision trees at the training phase. That is, we used the mapped purified ABTs to create a number of decision trees. For each sample, RF aggregates the predicted class labels (responder or non-responder) of the individual trees. It then performs a mode vote among all trees to produce the final class prediction. In RF, we created a number of decision trees (i.e., estimators)

in the forest in the domain of {10, 50, 100, 500}. Since we obtained slightly better classification error rates when using approximately 100 estimators this was what was used in the current study [i.e., where the asymptote in the error rate reduction occurred; (66)]. In other words, the classification error rates stabilized with ∼100 decision trees (no notable improvement was noted with 500 decision trees), which is consistent with what others have suggested for RF (67). Since we have a forest of decision trees to be trained, we considered the best random split using the Gini measurement. We chose a minimum impurity split of zero for early stopping of the tree growth (64). We conducted a 10-fold cross validation to increase the accuracy of the classification process and applied regularization methods within our classification models. Summary of the best parameter values that were obtained for each ML method and dimensionality of data matrices can be found in **Supplementary Tables 5** and **6**, respectively.

Experimental Evaluation

We ran three experiments for RF classifier on each of the following purified mapped ABTs: (a) eLORETA dataset (**Experiment A**); (b) EEG dataset (**Experiment B**); (c) clinical/demographic dataset (**Experiment C**). Subsequently, all relevant features were combined into one predictive ML model that also included the cordance dataset, which, given the low-dimensionality of the data, did not undergo feature selection (**Experiment D**). This was followed by a model extracting the *most* predictive features of response/non-response (**Experiment E**).

In order to guarantee a robust study/compare other ML approaches, we additionally explored the following prominent ML predictive models: (1). Classification and Regression Tree [CART; (40, 68, 69)]; (2). Support Vector Machine [SVM; (70)]; (3). Adaboost (71); (4). Multilayer Perceptron (MLP) (72); and (5). Gaussian Naïve Bayes (73). Please see **Supplementary Information** for further details on these methodologies.

All ML predictive models were implemented, learned and tested using Python programming language and Scikit-learn toolkit package on an Intel(R) Core(TM) i7-2600 CPU @ 3.20 GHz computer with 16 GB of memory running on Windows 10.

Experimental Setting

We trained RF models using a 10-folds cross-validation for predicting response (responder/non-responder) on the underlying sub-datasets. That is, in the training phase, we iteratively learned the parameters of models using nine out of the 10-folds in the sub-dataset. Additionally, and to avoid overfitting, we applied regularization methods within our classification models by adding penalty terms for extreme parameters in their objective functions. Specifically, we pruned the tree in RF (and also CART classifier) by penalizing the selection of features and limiting the maximum allowable tree depth (we used L2-norm regularization for SVM).

Evaluation Metrics

In order to judge the performance of the ML classification algorithms, during the testing phase, and using the learned models, we carried out response prediction on all patients by conducting the following: We ran all algorithms until convergence, and then recorded their confusion matrices on the leave-out fold by calculating: (1). The proportion of responders that were correctly classified (i.e., true positives [TP]); (2). The proportion of non-responders that were correctly classified (i.e., true negatives [TN]); (3). The proportion of responders that were misclassified as non-responders (i.e., false positive [FP]); and (4). The portion of non-responders that were misclassified as responders (i.e., false negative [FN]). Based on such confusion matrices, we compared the accuracy of all tested ML predictive models by computing the following evaluation metrics: (a) *Receiver Operating Characteristic (ROC) Curves* (74)—These plot the true positive rate (TPR or sensitivity/recall $= \frac{TP}{TP+FN}$: the probability of the correct identification of the presence of a disorder) against the true negative rate (TNR or specificity $= \frac{TN}{TN+FP}$: the probability of the correct identification of the absence of a disorder) at various thresholds. The closer the ROC curve is to the diagonal, the less accurate the prediction. Thus, a ROC is commonly used as a robust metric to compare diagnostic accuracy of classification methods. (b) *Average F1-Score* (75)—This score measures the harmonic mean of recall and positive predictive value (PPV or precision $= \frac{TP}{TP+FP}$: the probability that the presence of a disorder in a given patient is correctly identified). In this case, if the prediction probability is >0.5, then the person is predicted to be a responder, otherwise, the person is predicted to be a non-responder. F1-scores are insensitive to FN, and therefore, it quantifies the quality of an algorithm for predicting the true positives. (c) *Area Under the Curve (AUC)*-In the context of the current study, AUC quantifies the overall ability of the classification model to discriminate responders/non-responders (76). The greater the area under the curve (i.e., closer it is to 1), the more accurate the prediction (chance is 0.5). Additional evaluation metrics which were computed were the negative predictive value (NPV) $= \frac{TN}{FN+TN}$: the probability that the absence of a disorder in a given patient is correctly identified, as well as overall accuracy $= \frac{TP+TN}{TP+TN+FP+FN}$.

RESULTS

Characteristics of the entire patient sample as well as responders/non-responders are summarized in **Table 1**.

Experiment A: eLORETA ML Predictive Results

Feature selection indicated that the most predictive features of week 12 response/non-response using eLORETA data were: 6 features wherein source-localization was specific to delta, 10 to theta, 13 to alpha$_1$, 9 to alpha$_2$, and 12 beta. The average impurity reduction score of ≥0.01 was used to determine the importance of a feature (for all bands) in the eLORETA dataset. *Delta*: With respect to predictive delta eLORETA features, they were largely at baseline, right-localized and diffuse (though

TABLE 2 | F1 scores of classifiers of source-localized (eLORETA) electroencephalographic (EEG) band power and associated area under the curve values (M ± S.D.) for random forest.

	$Alpha_1$	$Alpha_2$	Beta	Delta	Theta
Random forest	0.752	0.803	0.674	0.682	0.692
(AUC values)	(0.75 ± 0.22)	(0.74 ± 0.20)	(0.62 ± 0.19)	(0.69 ± 0.25)	(0.77 ± 0.21)
Adaboost	0.694	0.748	0.648	0.661	0.725
SVM	0.757	0.695	0.507	0.659	0.690
CART	0.635	0.638	0.591	0.569	0.659
MLP	0.749	0.585	0.536	0.672	0.771
Gaussian naive bayes	0.756	0.619	0.497	0.718	0.629

AUC, area under the curve; CART, classification and regression trees; MLP, multilayer perceptron; SVM, support vector machine.

TABLE 3 | F1-scores of classifiers of electroencephalographic (EEG) band power and associated area under the curve values for random forest (M ± S.D.).

	$Alpha_1$	$Alpha_2$	Beta	Delta	Theta
Random forest	0.721	0.783	0.701	0.676	0.727
(AUC values)	(0.70 ± 0.2)	(0.80 ± 0.23)	(0.67 ± 0.29)	(0.72 ± 0.22)	(0.71 ± 0.34)
Adaboost	0.674	0.775	0.643	0.576	0.752
SVM	0.612	0.768	0.521	0.657	0.691
CART	0.624	0.757	0.595	0.560	0.680
MLP	0.653	0.689	0.533	0.589	0.664
Gaussian naive bayes	0.719	0.697	0.599	0.646	0.718

AUC, area under the curve; CART, classification and regression trees; MLP, multilayer perceptron; SVM, support vector machine.

not prefrontal). Baseline delta localized to the right lingual gyrus was the most predictive delta feature. *Theta:* Predictive theta features were from baseline and localized to the occipital cortex (lingual gyri), and week 1 theta localized largely to left-lateralized temporo-parietal regions. The most predictive feature was week 1 theta localized to the left transverse temporal gyrus. *Alpha*$_1$: With respect to alpha$_1$ (alpha$_{1/2}$ was split based on previous research that each band could be associated with response (49, 77), predictive alpha$_1$ features were largely week 1, left-lateralized and relatively diffuse (though largely temporal). Although, baseline prefrontal alpha$_1$ was also found to be a predictive feature. The most predictive feature was week 1 alpha$_1$ localized to the left transverse temporal gyrus. *Alpha*$_2$: Alpha$_2$ predictive features were largely baseline, and localized to the left parahippocampal gyrus, right pre/frontal regions (as well as right subcallosal gyrus and ACC). The most predictive feature was baseline alpha$_2$ localized to the right subcallosal gyrus. *Beta*: Predictive beta features were largely week 1 and localized to the left precuneus and precentral gyrus as well as bilateral posterior cingulate cortex, though baseline left-frontal beta was also a predictive feature. The most predictive feature was week 1 beta localized to the left precuneus (**Supplementary Table 2** and **Supplementary Figure 2**). As evidenced by F1 scores (focus on RF), collectively, eLORETA features in the alpha$_{1/2}$ bands were most predictive (across ML approaches), followed by theta; eLORETA-localized activity in beta/delta were less predictive of week 12 response status (**Table 2**).

Experiment B: EEG ML Predictive Results

Feature selection indicated that the most predictive features of week 12 response/non-response using surface-level EEG power were: 17 delta EEG features, 20 theta EEG features, 14 alpha$_1$ EEG features, 20 alpha$_2$ EEG features, and 17 beta EEG features. The average impurity reduction score of $\geq$0.02 was used to determine the importance of a feature (for all bands) in the EEG dataset. *Delta*: Regarding EEG delta features, those associated with response prediction were largely at week 1, right-localized and diffuse, with a handful of predictive features at baseline (which were also predictive at week 1). The most predictive features were EEG delta power at week 1 at T_8 followed by power at CP_6. *Theta*: Predictive baseline EEG theta features were generally frontal and occipital; week 1 predictive EEG theta features were diffuse, though not occipital. The most predictive features were baseline EEG theta power at Fp_2 and week 1 EEG theta power at FC_2. *Alpha*$_1$: With respect to EEG alpha$_1$, predictive features were predominantly baseline and frontal. The most predictive EEG alpha$_1$ feature was baseline power at $F_{7/8}$. *Alpha*$_2$: Baseline EEG alpha$_2$ predictive features were diffuse, while week 1 alpha$_2$ predictive features were parietal and occipital. The most predictive EEG alpha$_2$ features were baseline power at P_8 and week 1 power at O_1. *Beta*: Finally, predictive EEG beta features existed at both baseline and week 1, and were diffuse. The most predictive features were baseline EEG beta power at T_7 and week 1 power at Fz (**Supplementary Table 3**, **Supplementary Figure 3**, and **Table 3**). As evidenced by F1 scores (focus on RF), overall, features in EEG alpha$_2$ (followed by theta) were most predictive of response (across ML approaches) while beta/delta were least predictive (**Table 3**).

Experiment C: Clinical/Demographic ML Predictive Results

Feature selection indicated that there were 31 predictive features of response/non-response using demographic and clinical data. The average impurity reduction score of $\geq$0.02 was used to determine the importance of a feature. Age and sex were found to be predictive features (with comparable predictive value). Baseline, week 1 and score changes (week 1-baseline) were all predictive features for MADRS items #2 (sadness), #5 (reduced appetite), #6 (concentration difficulty), #8 (inability to feel), #9 (pessimistic thoughts), #10 (suicidal thoughts) and total MADRS score. Change scores were also predictive for items #1 (apparent sadness), #3 (inner tension), #4 (reduced sleep) and #7 (lassitude), as were week 1 scores for #1 and #7, as well as baseline scores for #3 and #4. Interestingly, the strongest feature

TABLE 4 | F1-scores of classifiers of clinical and demographic data as well as associated area under the curve values for random forest (M ± S.D.).

Random forest	0.737
(AUC values)	(0.74 ± 0.23)
Adaboost	0.715
SVM	0.620
CART	0.652
MLP	0.544
Gaussian naive bayes	0.534

AUC, area under the curve; CART, classification and regression trees; MLP, multilayer perceptron; SVM, support vector machine.

TABLE 5 | F1-scores of classifiers of important features extracted from source-localization (eLORETA) and surface-level EEG power in various bands, demographic/clinical as well as cordance data.

Random forest	0.901
(AUC values)	(0.90 ± 0.14)
Adaboost	0.838
SVM	0.716
CART	0.791
MLP	0.687
Gaussian naive bayes	0.775

Associated area under the curve values for random forest (M ± S.D.) are presented. AUC, area under the curve; CART, classification and regression trees; MLP, multilayer perceptron; SVM, support vector machine.

predictive of response, by far, was the "concentration difficulty" score (MADRS #6) at week 1, followed by "sadness" score (#2) changes, and total MADRS score (**Supplementary Table 4**, **Supplementary Figure 4** and **Table 4**).

Experiment D: Combined ML Models

Subsequently, all of the most predictive features from the above sections (**Experiments A–C**) were included in another ML experiment, to which cordance data (theta EC from baseline and week 1) was included, and the F1 values are presented in **Table 5** (**Figure 1**). At a sensitivity of 0.77 and specificity of 0.99, the model has a PPV of 0.99, NPV of 0.81, and overall classification accuracy of 0.88.

Experiment E: ML Model of Most Important Features

Finally, the last step was to combine all of the predictive features from above, and extract the most predictive features of response. The average impurity reduction score of $\geq$0.01 was used to determine the importance of a feature. As is evident from **Table 6,** baseline alpha$_1$ power in frontopolar electrodes, baseline alpha$_2$ in the right parietal electrode as well as lower frequency (delta/theta) power at the right parietal electrode at week 1 were significant features associated with response. With respect to the eLORETA data, baseline alpha$_2$ localized to the ACC as well as week 1 alpha$_1$/theta data localized to left temporal/auditory region were the features which most strongly contributed to response. Finally, concentration difficulties at week 1 and change in reported sadness from baseline to week 1 were the clinical features associated with response. The most predictive feature within this model was baseline theta EEG power at Fp_2, followed closely by baseline EEG alpha alpha$_2$ at P_8, and by baseline EEG alpha$_1$ power at Fp_2. Together, these 12 features strongly predicted response status as exemplified by the F scores (**Tables 6**, **7** and **Figure 2**). At a sensitivity of 0.65 and specificity of 0.99, the model has a PPV of 0.98, NPV of 0.74, and an overall classification accuracy of 0.78.

DISCUSSION

This study aimed to assess the utility of pre-treatment and week 1 clinical information as well as various types of EEG data (source-localized current density, scalp-level power, prefrontal theta cordance), alone and in combination, in predicting antidepressant response at week 12 of pharmacotherapy treatment using ML. In this study, comprised of a balanced sample of eventual antidepressant treatment responders/non-responders, we focused on Random Forest, though six other ML approaches were compared (such comparisons are currently lacking). To our knowledge, this is the first known study assessing the predictive utility of source-localized EEG current density across brain regions using ML. Further, in addition to sex and age, *individual* depression symptom questionnaire item scores were assessed in predicting antidepressant response (alone/in combination with EEG data). Most comparable work generally includes only *total* scores. This work expands on the ever-growing body of research investigating the utility of ML tools in aiding with antidepressant response prediction, with the broader aim of improving clinical care by integrating precision-based and personalized interventions in treating MDD.

Briefly, when considering each dataset separately, we found 50 eLORETA features to be predictive of response. Predictive delta eLORETA features were largely baseline and right-localized; those of theta were mainly baseline occipital and week 1 left temporo-parietal. Predictive eLORETA alpha$_1$ features were mainly week 1 and left temporally-localized, while predictive alpha$_2$ features were baseline and localized to the left parahippocampal and right pre/frontal cortex. Predictive eLORETA beta features were localized to the precentral gyrus and posterior regions at week 1. Overall, fewest predictive eLORETA features exited for delta, and most for alpha$_1$/theta. Regarding scalp EEG power, 88 features were predictive. Predictive EEG delta features were largely week 1 and right-localized; those of EEG theta were generally baseline frontal and occipital, while week 1 were diffuse (not occipital). EEG alpha$_1$ predictive features were generally baseline and frontal, while those of alpha$_2$ were diffuse at baseline, and parieto-occipital at week 1. Diffuse predictive EEG beta features existed at both timepoints. Theta and alpha$_2$ were the most predictive scalp EEG features. Clinical and demographic data consisted of 31 predictive features; the most salient being "concentration difficulty" score at week 1, followed by "sadness" score and total MADRS score changes from baseline to week 1. When all of the features were included

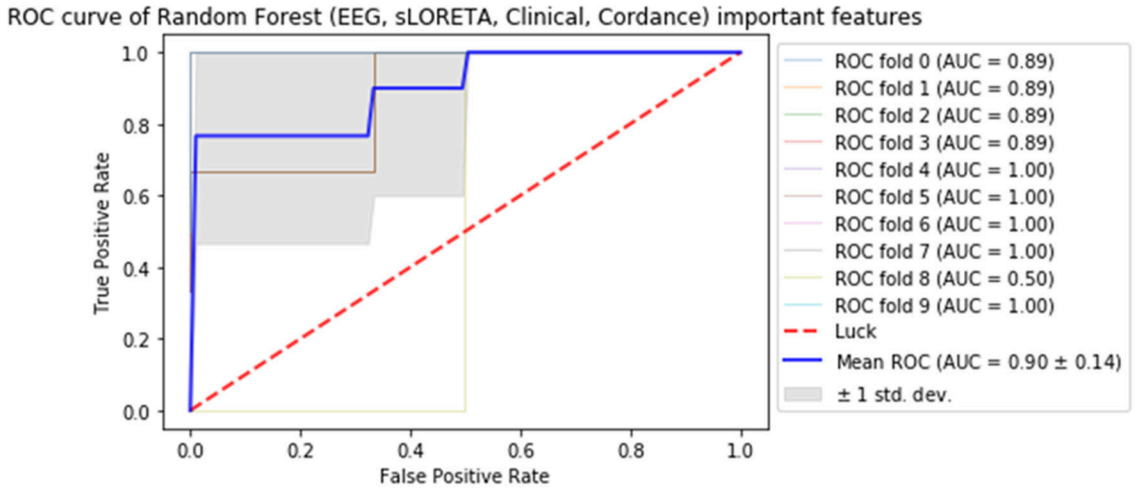

FIGURE 1 | Receiver operator curve (ROC) & area under the curve (AUC) scores for all important features extracted from all datasets (source-localized EEG current density, scalp-level EEG power, clinical/demographic data & theta cordance) random forest.

into one ML experiment, the predictive utility of this model was high (PPV: 0.99; NPV: 0.81). When the *most* important features were identified in the final experiment, 12 predictive features were extracted, with the most predictive being baseline scalp EEG theta at Fp_2, followed by baseline scalp EEG $alpha_2$ at P_8, and baseline scalp EEG $alpha_1$ at Fp_2. We found that a model combining all important features (**Experiment D**) had very high specificity (0.99), i.e., true negative rate, with a modest sensitivity (0.77), i.e., true positive rate, and a classification accuracy of 0.88. The high PPV indicates that the model is able to predict, with a high degree of certainty, that a given patient will truly be an eventual responder by week 12. The model which contains the *most* important features (**Experiment E**) had similar accuracy measures, however, the sensitivity, NPV, and overall accuracy were lower than those in **Experiment D**. However, this can be explained by the feature extraction process (we are reducing the number of features from 171 to 12). These data support the utility of EEG biomarkers in antidepressant response prediction.

The majority of studies using non-ML approaches assessing the predictive utility of EEG data have focused on midline, generally rostral anterior cingulate cortex (ACC)-localized theta current density. The rACC is known to be a region critical in conflict resolution as well as in coordinating the physiological response to conflict. Several studies have shown that higher pre-treatment rACC-theta tends to be associated with a favorable antidepressant response (78–81), though notable exceptions exist (82, 83). There is also work suggesting that early changes in rACC-theta may be associated with antidepressant response (49). Further, pre-treatment rACC theta has been shown to be predictive of placebo response. As such, activity in this region and frequency band may be reflective of "response readiness" (or malleability within a region highly implicated in MDD) rather than solely physiological changes induced by antidepressant drugs (84). In fact, the possibility of a placebo response driving some of the findings reported herein cannot be discounted. Interestingly, one group noted that rACC-delta was predictive of response (85), though a high

TABLE 6 | Features most predictive of antidepressant response laid out in order of importance (as indexed by average impurity reduction scores; with the top-most features having the most impact on the predictive values).

Dataset	Features (in order of importance)
EEG	Baseline Fp_2 theta
	Baseline P_8 $alpha_2$
	Baseline Fp_2 $alpha_1$
eLORETA	Baseline $alpha_1$ localized to the right subcallosal gyrus (BA25)
Clinical	Concentration difficulties at week 1
EEG	Week 1 P_8 theta
eLORETA	Week 1 $alpha_1$ localized to the left middle temporal gyrus (BA21)
eLORETA	Week 1 $alpha_1$ localized to the left transverse temporal gyrus (BA41)
EEG	Week 1 P_8 delta
Clinical	Reported sadness change score (baseline to week 1)
EEG	Baseline Fp_1 $alpha_1$
eLORETA	Week 1 theta localized to the left transverse temporal gyrus (BA41)

TABLE 7 | F1-scores of classifiers of the most important features across all of the datasets and associated area under the curve values for random forest (M ± S.D.).

Random forest	0.827
(AUC values)	(0.83 ± 0.23)
Adaboost	0.815
SVM	0.730
CART	0.762
MLP	0.625
Gaussian naive bayes	0.731

AUC, area under the curve; CART, classification and regression trees; MLP, multilayer perceptron; SVM, support vector machine.

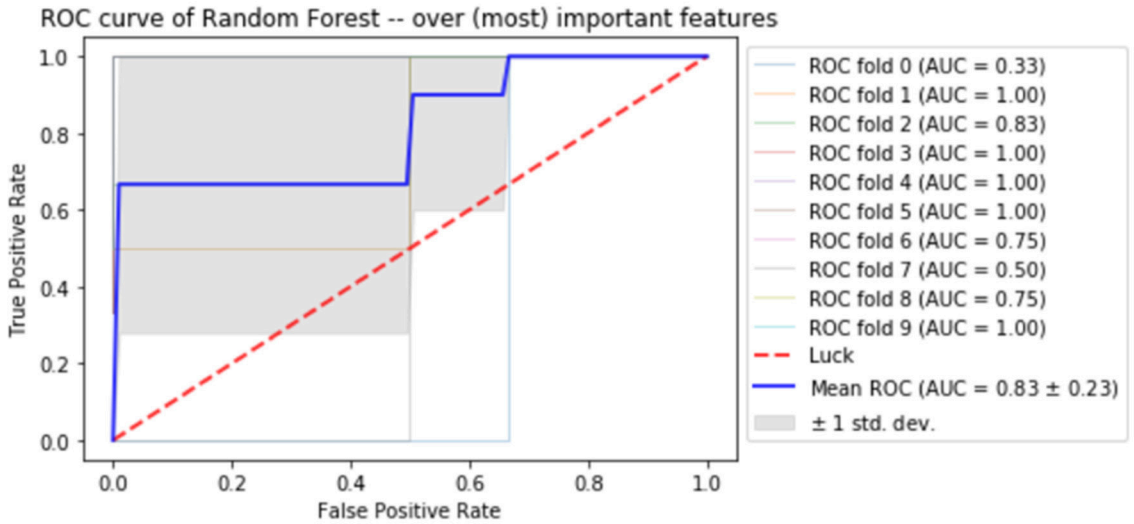

FIGURE 2 | Receiver operator curve (ROC) using random forest when only the most important features are imputed into the model.

correlation existed between delta and theta current density. Thus, although the rACC appears to be an important nexus in antidepressant response prediction, is seems worthwhile to investigate current density across all EEG bands and brain regions in relation to response (as was done in the current study), in order to identify other potentially predictive features of response.

In the eLORETA dataset, we found many features -across all bands, in diffuse brain areas, and at both timepoints- to be associated with response. Further, in this study, rACC-theta was not a predictive feature of response, though baseline alpha$_2$ current density in this region was. Overall, the response predictive regions that alpha$_2$ current density was localized to are regions typically associated with structural and functional alterations in MDD, such as the subgenual ACC [sgACC; (81)], parahippocampal regions (86) and pre/frontal regions (87). In the final model of most relevant features, only baseline sgACC-alpha$_2$, week 1 alpha$_1$ in the left middle temporal gyrus and alpha$_1$/theta localized to the left auditory cortex contributed to response prediction. This is in keeping with the importance of the sgACC in response prediction (though not necessarily theta-localized), while the implication of the auditory cortex may be related to its high innervation by serotonergic fibers (88), though, this interpretation is speculative.

When considering the literature on the utility of resting-state scalp EEG in predicting response using non-ML approaches, the literature -while extensive- is rather inconsistent. In general, pre-treatment alpha power has been shown to differentiate responders and non-responders (49, 78, 89–92). However, in a large sample of depressed patients, the International Study to Predict Optimized Treatment in Depression (iSPOT-D) did not observe this [frontal alpha asymmetry was predictive of response in females; (82)]. Similarly, there has been an association between treatment response and baseline scalp EEG theta activity. Several groups reported that increased frontal/diffuse scalp-level theta was associated with antidepressant non-response (92–94) while others noted the opposite (95, 96) (i.e., increased fronto-midline theta was associated with a favorable outcome). As outlined in the introduction, several studies exist with respect to the predictive abilities of prefrontal theta cordance, wherein decreases in prefrontal theta cordance early in the course of antidepressant treatment tend to be associated with treatment outcome (32–34). Further, combining theta cordance data with clinical scores strengthened response predication (97). Such work underscores the importance of diverse data in improving predictive algorithms.

Assessments of the scalp-level EEG dataset revealed a degree of similarly between the scalp-level and eLORETA predictive features; though the overlap was far from perfect. Further, in the final predictive experiment of most relevant features, more scalp-level EEG features were predictive of response. In the current study, ML indicated that alpha was one of the most predictive bands of antidepressant response outcome, replicating previous work (49, 78, 89–92). Indeed, in the final model, alpha$_{1/2}$ power at frontopolar electrodes and alpha/delta/theta power at P_8 were most predictive scalp-level EEG features. The predictive utility of frontopolar electrodes fits with the findings of Al-Kaysi et al. (43), who found that frontal sites were most predictive of depression symptom and cognitive improvement (vs. non-improvement) following tDCS using ML approaches (i.e., SVM, linear discriminate analysis, extreme learning machine). The importance of frontopolar electrodes is also in keeping with the work on frontal theta cordance, which focuses on pre/frontal sites. The importance of the P_8 site fits with the work associating the parietal region with anxious arousal (76, 98, 99). Most individuals with depression exhibit heighted anxiety, either at sub-clinical or clinical levels (a handful of our participants had

co-morbid anxiety, and many had sub-clinical anxiety features). Finally, alterations in fronto-parietal networks are implicated in MDD (100), thus, predictive scalp-level EEG features at these sites may reflect this. Indeed, future work should investigate the utility of EEG connectivity (particularly between fronto-parietal regions) which may also have predictive value, as shown by others (41, 42).

Early depression symptom changes have been associated with eventual response. A meta-analysis found that a 20% reduction on the Hamilton Rating Scale for Depression (HAMD-17) within 2 weeks post-treatment initiation was predictive of later response and remission with a high sensitivity [81–87%; (101)]. In another meta-analysis, Wagner et al. (102) found that early improvement (>20/25% HAMD/MADRS reductions from baseline to week 1 or 2) predicted later response with high sensitivity (85%), but lower specificity (54%). However, analyses of individual trajectories of symptom change found that both early and delayed improvement are equally common (51% showed a delayed response); thus, eventual response cannot be predicted from early assessments in all patients (103). In terms of specific or individual symptom score changes being predictive of response, one study found that individual symptoms performed better than total improvement scores, though the difference was small (104). Such findings suggest that both total depression symptom scores or individual items cannot be solely relied upon as a predictive tool of response; thus, combining clinical measures with EEG may yield higher predictive accuracy. Interestingly, we found that "concentration difficulty" scores at week 1, as well as "sadness" and total MADRS score changes were most predictive; the "sadness" change scores and "concentration difficulty" scores at week 1 were also included in the most predictive/final feature model. Concentration difficulties and general cognitive dysfunction are potential risk factors for MDD relapse, as well as being associated with psychosocial and impaired daily functioning in the disorder (105). As such, the importance of concentration difficulties in relation to response prediction is noteworthy.

Early prediction of a negative response/non-response is just as important as positive response prediction. For instance, if one could predict -with a high degree of certainty- that a given patient will not respond to a particular treatment shortly after treatment initiation, then an adjunctive or alternative treatment could be offered. Indeed, there are EEG-based ML initiatives that attempt to do just that: characterize the probability of response and non-response to antidepressant interventions, and provide treatment recommendations. For instance, PEER (Psychiatric Electroencephalography Evaluation Registry) is a registry which selects an individual's medication class, independent of diagnosis, based on a pre-treatment EEG indices (the PEER database consists of EEG data from thousands of patients and associated clinical outcomes). The PEER report provides the probability of both response and non-response to medication classes. Preliminary data from PEER trials show promise [e.g., (106)].

There is limited published data to which we can directly compare our findings. Khodayari-Rostamabad et al. (39), who carried out ML using EEG to predict antidepressant treatment outcome at 2-weeks post-treatment initiation, found similar prediction accuracy [though the ML techniques were different and they did not report F1-scores; further, their sample was smaller ($N = 22$)]. However, different features were selected in the two studies, as we did not include coherence measures, which were found to be the most predictive features by Khodayari-Rostamabad et al. Another group that used ML techniques for predicting antidepressant response following rTMS found that elevated theta connectivity (particularly frontal to posterior connectivity) at baseline and week 1 was most predictive, with relatively high sensitivity (0.84) and specificity (0.89). Al-Kaysi et al. (43) used ML approaches to predict which MDD patients would respond to tDCS-induced cognitive and depressive symptom changes (MADRS total score) found that frontal electrodes were most useful (and that fronto-central connectivity was highly predictive). Despite a small sample size, they were able to correctly classify a substantial proportion of their patients correctly with respect to response (frequency bands were considered together).

FUTURE DIRECTIONS AND LIMITATIONS

While existing research, coupled with data from the current study, is difficult to synthesize, certain themes exist. First, the most predictive emergent features of antidepressant response using ML (and non-ML) approaches tend to be either from alpha and/or theta EEG bands. While features from other bands are also valuable, discarding them seems to be helpful in tackling the problem of dimensionality and building generalizable predictive models, and therefore improving response prediction. Thus, focusing on alpha/theta bands may be reasonable. Second, pre/frontal regions tend to be most associated with response, though the contribution of parietal regions is also notable. Third, combining various EEG measures (e.g., connectivity, coherence, power) may yield the most powerful ML predictive models. A recent ML study, which employed a wavelet-based technique for predicting response to SSRI treatment in MDD, found a classification accuracy of 87.5% using pre-treatment wavelet data in delta and theta frequencies in frontal and temporal regions (107). Thus, this method may be another useful contributor of response prediction in comparable future ML work. However, the utility of these measures has to be balanced with practical considerations. For example, the time associated with extracting eLORETA current density is substantial; further, localization of sources using eLORETA is based on several assumptions-when these are violated, source-localization can be flawed. While we found that eLORETA features added predictive value, the contribution of scalp-level EEG power was greater. Moving forward, if ML approaches using EEG data are to be viable tools for antidepressant response prediction, input features should require limited data clean-up and pre-processing. As such, source-localized EEG features (as well as wavelet analyses) may be less practical when considering clinical applications. Our selection of frequency band cut-offs (though not atypical) may also vary from published work (the same is true for our filter parameters). Specifically, with respect to the beta band, we analyzed frequencies ranging for 13–30 Hz (with a 0.1–30 Hz

bandpass filter); as such, the influence of upper beta values (i.e., closer to 30 Hz) may have been attenuated. This, in turn, could have altered the prediction results from the beta band. Similarly, the predictive utility of gamma was not considered. Another methodological consideration is reference choice [e.g., average, linked-mastoid, reference electrode standardization technique [REST]; (108)]. In the current study, linked-mastoids were used as the reference. However, we acknowledge that this may introduce a degree of physiological noise that may have altered the data and thus classification accuracies. Future ML studies may benefit from constructing datasets with different reference montages, and comparing accuracy. This would also aid in potentially standardizing reference choice in the context of ML. In a similar vein, there is a need for combining datasets from a large number of centers/different groups to ensure that ML-identified response prediction features are properly tested on independent cases (i.e., models built on one large dataset but tested on another large dataset). Further, large datasets are required to extract features predictive of response to various antidepressant medication classes, which is critical information for personalized care. Such initiatives are already in place, and the results of such efforts are eagerly awaited. A final point is that the measures included in the current study were based on commonly-employed EEG features (i.e., power, source-localized activity, cordance). However, this approach may be obscuring potentially useful information that we did not think to include. Therefore, future studies may be optimized by employing a more data-driven process of feature extraction (though, there are practical considerations that must be considered).

It is also worth commenting on the value of including clinical features in predictive ML algorithms using EEG data. In the current work, we found that both individual item scores and total scores were predictive of response. Further, it seems that cognitive symptom scores, such as concentration, may be particularly important. Interestingly, in the current study, age and sex did not have as high a predictive value; however, given that this information is not difficult to obtain, it should be included in future predictive ML work (but, is generally not). EEG features at both baseline and week 1 were predictive of response. Thus, if possible, data from before and shortly after treatment commencement should be included in prediction algorithms (though, from a practical point of view, this may not always be possible). We also did not include changes in theta cordance from baseline to week 1, which has been more consistently utilized in differentiating antidepressant responders/non-responders; this may have contributed to improving our final predictive models. Finally, while our prediction accuracy scores for each ML method were derived from ROC curves, it has been suggested that meaningful qualitative conclusions should be drawn from ROC analyses that include >100 cases. This is especially important when differences between categories are subtle (109). As such, in the context of the current and future work, a larger sample size would be optimal for drawing values from ROC curves. In other words, larger samples ($N > 100$ participants) would ostensibly yield more valid sensitivity and specificity values.

There are several considerations that warrant discussion from a ML perspective. First, there is no hard-and-fast rule to determine a specific sample size for building a stable predictive model. Traditionally, this is based on the trade-off between factors such as feature (dimension) space, sample size, distribution of samples across classes, the nature of the data, and whether the problem is a binary or a complex multi-classification problem. Hence, while a larger sample size is always preferable, and is certainly recommended for comparable future work (i.e. multi-center, multi-group data), our predictive models should be considered appropriate in this context for the following reasons: First, the ML approaches we used were based on a simple binary classification problem, and the number of features was generally less than the sample size (which aids with the issue of overfitting; see **Supplementary Information**). Further, the target feature was balanced in terms of the number of responders/non-responders. Finally, we also attempted to avoid overfitting by applying cross-validation and regularization methods.

Additionally, although there are positive aspects to using Random Forests, which was our primary ML focus (e.g., easy to apply), there are caveats which must be considered. Namely, features can be correlated, and any of these correlated features can be used as a predictor in the model. As a result, once a predictive feature is selected, the importance of other correlated features decreases, which means that even strong features can be ranked with a lower importance. While this reduces overfitting, it may lead to the erroneous assumption that the certain predictors are significantly less important (65). We attempted to deal with this by including various other ML approaches in the current paper. Generally, if a specific set of features was ranked as having high predictive utility (e.g., alpha/theta scalp-level EEG) with RF, it tended to be highly predictive using the other approaches.

CONCLUSION

In conclusion, a set of predictive methods in ML applied to our resting-state EEG dataset proved to be a viable approach for extracting salient predictive features of antidepressant treatment efficacy in patients with MDD. Importantly, the combination of datasets seems to provide enhanced predictive ability. A recent meta-analysis found that, as of now, quantitative EEG does not appear to be clinically reliable in the prediction of antidepressant treatment response; one suggested explanation is that depression itself is heterogeneous, therefore, the prediction of response via EEG may be clouded by differences in patient sub-groups or characteristics (110). This supports the combination of both electrophysiological as well as individual depression symptoms to improve predictive ability and future reliability. However, the generalizability of the current findings needs to be assessed in larger populations, and with different pharmacological antidepressant agents and/or other forms of antidepressant interventions. While it is premature to conclude whether this EEG-based technology will be suitable for integration in daily clinical practice, our data, along with those of others,

suggests that the use of ML approaches with scalp-level EEG, clinical/demographic features, and EEG source-localization, may have significant potential in defining optimal predictors which can be used to guide and personalize antidepressant treatment.

DATA AVAILABILITY STATEMENT

The datasets for this manuscript are not publicly available because, in the original study, patients did not consent to public sharing of their data. However, anonymized data can be accessed upon request, by emailing Dr. Natalia Jaworska (Natalia.Jaworska@theroyal.ca).

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Royal Ottawa Health Care Group and University of Ottawa Social Sciences and Humanities Research Ethics Boards.

AUTHOR CONTRIBUTIONS

NJ recruited and tested the patients, processed the EEG files, and wrote the manuscript. SdlS processed and assembled EEG and clinical data files and assisted in writing and editing the manuscript. M-HI was responsible for performing ML experiments, writing the ML methods section, and editing the manuscript. PB the clinical lead on the project, screened, interviewed, and diagnosed all patients and helped to edit the paper. VK was responsible for developing the research questions and overseeing the project as well as editing the manuscript.

FUNDING

NJ was funded by a Canadian Institutes of Mental Health (CIHR) Doctoral award at the time of data collection; the clinical trial described herein was funded by a National Institutes of Health (NIH) grant awarded to PB (5R01MH077285- registered August 22, 2007). The funding bodies played no role and had no influence on the study design, the collection, analysis and interpretation of the data; they also had no influence on the writing of this manuscript. The current study only includes patients who underwent EEG testing.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2018.00768/full#supplementary-material

REFERENCES

1. Collins PY, Patel V, Joestl SS, March D, Insel TR, Daar AS, et al. Grand challenges in global mental health. *Nature* (2011) 475:27–30. doi: 10.1038/475027a
2. Druss BG, Schlesinger M, Allen HM. Depressive symptoms satisfaction with health care and 2-year outcomes in an employed population. *Am J Psychiat*. (2001) 158:731–4. doi: 10.1176/appi.ajp.158.5.731
3. Evans DL, Charney DS. Mood disorders and medical illness: a major public health problem. *Biol Psychiatry* (2003) 54:177–80. doi: 10.1016/S0006-3223(03)00639-5
4. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health:results from the World Health Surveys. *Lancet* (2007) 370:851–8. doi: 10.1016/S0140-6736(07)61415-9
5. Frasure-Smith N, Lespérance F, Gravel G, Masson A, Juneau M, Talajic M, et al. Social support, depression, and mortality during the first year after myocardial infarction. *Circulation* (2000) 101:1919–24. doi: 10.1161/01.CIR.101.16.1919
6. Pigott HE, Leventhal AM, Alter GS, Boren JJ. Efficacy and effectiveness of antidepressants: current status of research. *Psychother Psychosom*. (2010) 79:267–79. doi: 10.1159/000318293
7. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. Evaluation of outcomes with citalopram for depression using measurement-based core in STAR*D:implications for clinical practice. *Am J Psychiatry* (2006) 163:28–40. doi: 10.1176/appi.ajp.163.1.28
8. Malone DC. A budget-impact and cost-effectiveness model for second-line treatment of major depression. *J Manag Care Pharm*. (2007) 13:S8–18. doi: 10.18553/jmcp.2007.13.s6-a.8
9. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment step:a STAR*D report. *Am J Psychiatry* (2006) 163:1905–17. doi: 10.1176/ajp.2006.163.11.1905
10. Wagner JA. Overview of biomarkers and surrogate endpoints in drug development. *Dis Markers* (2002) 18:41–6. doi: 10.1155/2002/929274
11. Labermaier C, Masana M, Muller M. Biomarkers predicting antidepressant treatment response:how can we advance the field? *Dis Markers* (2013) 35:23–31. doi: 10.1155/2013/984845
12. Leuchter AF, Cook IA, Hamilton SP, Narr KL, Toga A, Hunter AM, et al. Biomarkers to predict antidepressant response. *Curr Psychiat Rep*. (2010) 12:553–62. doi: 10.1007/s11920-010-0160-4
13. Insel T, Cuthbert B, Garvey M, Heinssen R, Pine DS, Quinn K, et al. Research Domain Criteria (RDOC):Toward a new classification framework for research or mental disorders. *Am J Psychiatry* (2010) 167:748–51. doi: 10.1176/appi.ajp.2010.09091379
14. de Almeida CJR, Phillips ML. Distinguishing between unipolar depression and bipolar depression:Current and future clinical and neuroimaging perspectives. *Biol Psychiatry* (2013) 73:111–8. doi: 10.1016/j.biopsych.2012.06.010
15. Hasler G, Northoff G. Discovering imaging endophenotypes for major depression. *Mol Psychiatry*. (2011) 16:604–19. doi: 10.1038/mp.2011.23
16. Hasler G, Drevets WC, Manji HK, Charney DS. Discovering endophenotypes for major depression. *Neuropsychopharmacology* (2004) 29:1765–81. doi: 10.1038/sj.npp.1300506
17. McGrath CL, Kelley ME, Holtzheimer PE, Dunlop BW, Craighead WE, Franco AR, et al. Toward a neuroimaging treatment selection biomarker for major depressive disorder. *JAMA Psychiatry* (2013) 70:821–9. doi: 10.1001/jamapsychiatry.2013.143
18. Niciu MJ, Mathews DC, Nugent AC, Ionescu DF, Furey ML, Richards EM, et al. Developing biomarkers in mood disorders research through the use of rapid-acting antidepressants. *Depress Anxiety* (2014) 31:297–307. doi: 10.1002/da.22224
19. Schmidt HD, Shelton RC, Duman RS. Functional biomarkers of depression:Diagnosis, treatment and pathophysiology. *Neuropsychopharmacology* (2011) 36:2375–94. doi: 10.1038/npp.2011.151

20. Schneider B, Prvulovic D. Novel biomarkers in depression. *Curr Opin Psychiatry* (2013) 26:47–53. doi: 10.1097/YCO.0b013e32835a5947
21. Wise T, Cleare AJ, Herane A, Young AH, Arnone D. Diagnostic and therapeutic utility of neuroimaging in depression:an overview. *Neuropsychiatr Dis Treat*. (2014) 10:1509–22. doi: 10.2147/NDT.S50156
22. Ritsner M, Gottesman E. Chapter 1: Where do we stand in the quest for neuropsychiatric biomarkers and what next? In: Ritsne MS, editor. *The Handbook of Neuropsychiatric Biomarkers, Endophenotypes and Genes*. New York, NY: Springer (2009). p. 3–17. doi: 10.1007/978-1-4020-9464-4_1
23. Michel C, Murray M. Towards the utilization of EEG as a brain imaging trial. *Neuroimage* (2012) 61:371–85. doi: 10.1016/j.neuroimage.2011.12.039
24. Alhaj H, Wisniewski G, McAllister-Williams RH. The use of EEG in measuring therapeutic drug action: focus on depression and antidepressants. *J Psychopharmacol.* (2011) 25:1175–91. doi: 10.1177/0269881110388323
25. Knott V, Mahoney C, Kennedy S, Evans K. EEG correlates of acute and chronic paroxetine treatment in depression. *J Affect Dis.* (2002) 69:241–9. doi: 10.1016/S0165-0327(01)00308-1
26. Baskaran A, Milev R, McIntyre RS. The neurobiology of the EEG biomarker as a predictor of treatment response in depression. *Neuropharmacology* (2012) 63:507–13. doi: 10.1016/j.neuropharm.2012.04.021
27. Iosifescu DV. Electroencephalography-derived biomarkers of antidepressant response. *Harv Rev Psychiatry* (2011) 19:144–54. doi: 10.3109/10673229.2011.586549
28. Leuchter AF, Cook IA, Hunter A, Korb A. Use of clinical neurophysiology for the selection of medication in the treatment of major depressive disorder: the state of the evidence. *Clin EEG Neurosci.* (2009) 40:78–83. doi: 10.1177/155005940904000207
29. Olbrich S, Arns M. EEG biomarkers in major depressive disorder:Discriminative power and prediction of treatment response. *Int Rev Psychiatry* (2013) 25:604–18. doi: 10.3109/09540261.2013.816269
30. Neuper C, Pfurtscheller G. Event-related dynamics of cortical rhythms:frequency-specific features and functional correlates. *Int J Psychophysiol.* (2001) 43:41–58. doi: 10.1016/S0167-8760(01)00178-7
31. Leuchter AF, Cook IA, Lufkin RB, Dunkin J, Newton TF, Cummings JL, et al. Cordance:a new method for assessment of cerebral perfusion and metabolism using quantitative electroencephalography. *Neuroimage* (1994) 1:208–19. doi: 10.1006/nimg.1994.1006
32. Cook IA, Leuchter AF, Witte E, Abrams M, Uijtdehaage SH, Stubbeman W, et al. Neurophysiologic predictors of treatment response to fluoxetine in major depression. *Psychiatry Res.* (1999) 85:263–73. doi: 10.1016/S0165-1781(99)00010-4
33. Bares M, Brunovsky M, Kopecek M, Novak T, Stopkova P, Kozeny J, et al. Early reduction in prefrontal theta QEEG cordance value predicts response to venlafaxine treatment in patients with resistant depressive disorder. *Eur Psychiatry* (2008) 23:350–5. doi: 10.1016/j.eurpsy.2008.03.001
34. Cook IA, Leuchter AF, Morgan M, Witte E, Stubbeman WF, Abrams M, et al. Early changes in prefrontal activity characterize clinical responders to antidepressants. *Neuropsychopharmacology* (2002) 27:120–31. doi: 10.1016/S0893-133X(02)00294-4
35. Lemm S, Blankertz B, Dickhaus T, Müller KR. Introduction to machine learning for brain imaging. *Neuroimage* (2011) 56:387–99. doi: 10.1016/j.neuroimage.2010.11.004
36. Pereira F, Mitchell T, Botvinick M. Machine learning classifiers and fMRI:A tutorial overview. *Neuroimage* (2009) 45:5199–209. doi: 10.1016/j.neuroimage.2008.11.007
37. Khodayari-Rostamabad A, Reilly JP, Hasey G, Debruin H, Maccrimmon D. Diagnosis of psychiatric disorders using EEG data and employing a statistical decision model. *Conf Proc IEEE Eng Med Biol Soc*. (2010) 2010:4006–9. doi: 10.1109/IEMBS.2010.5627998
38. Mohammadi M, Al-Azab F, Raahemi B, Richards G, Jaworska N, Smith D, et al. Data mining EEG signals in depression for their diagnostic value. *BMC Med Inform Decis Mak*. (2015) 15:108. doi: 10.1186/s12911-015-0227-6
39. Khodayari-Rostamabad A, Reilly JP, Hasey GM, de Bruin H, Maccrimmon DJ. A machine learning approach using EEG data to predict response to SSRI treatment for major depressive disorder. *Clin Neurophysiol.* (2013) 124:1975–85. doi: 10.1016/j.clinph.2013.04.010
40. Rabinoff M, Kitchen CM, Cook IA, Leuchter AF. Evaluation of quantitative EEG by classification and regression trees to characterize responders to antidepressant and placebo treatment. *Opin Med Info J.* (2011) 5:1–8. doi: 10.2174/1874431101105010001
41. Bailey NW, Hoy KE, Rogasch NC, Thomson RH, McQueen S, Elliot D, et al. Differentiating responders and non-responders to rTMS treatment for depression after one week using resting EEG connectivity measures. *J Affect Disord*. (2019) 242:68–79. doi: 10.1016/j.jad.2018.08.058
42. Bailey NW, Hoy KE, Rogasch NC, Thomson RH, McQueen S, Elliot D, et al. Responders to rTMS for depression show increased fronto-midline theta and theta connectivity compared to non-responders. *Brain Stimul.* (2018) 11:190–203. doi: 10.1016/j.brs.2017.10.015
43. Al-Kaysi AM, Al-Ani A, Loo CK, Breakspear M, Boonstra TW. Predicting brain stimulation treatment outcomes of depressed patients through the classification of EEG oscillations. *Conf Proc IEEE Eng Med Biol Soc*. (2016) 2016:5266–9. doi: 10.1109/EMBC.2016.7591915
44. Crown WH. Potential application of machine learning in health outcomes research and some statistical cautions. *Value Health* (2015) 18:137–40. doi: 10.1016/j.jval.2014.12.005
45. Pascual-Marqui RD, Pascual-Montano AD, Lehmann D, et al. Exact low resolution brain electromagnetic tomography (eLORETA). *Neuroimage* (2006) 31:S86.
46. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry* (1979) 134:382–9. doi: 10.1192/bjp.134.4.382
47. Howland RH, Wilson MG, Kornstein SG, Clayton AH, Trivedi MH, Wohlreich MM, et al. Factors predicting reduced antidepressant response:experience with the SNRI duloxetine in patients with major depression. *Ann Clin Psychiatry* (2008) 20:209–18. doi: 10.1080/10401230802437639
48. Stewart JW, McGrath PJ, Blondeau C, Deliyannides DA, Hellerstein D, Norris S, et al. Combination antidepressant therapy for major depressive disorder: speed and probability of remission. *J Psychiatr Res.* (2014) 52:7–14. doi: 10.1016/j.jpsychires.2013.12.001
49. Jaworska N, Blondeau C, Tessier P, Norris S, Fusee W, Blier P, et al. Examining relations between alpha power as well as anterior cingulate cortex-localized theta activity and response to single or dual antidepressant pharmacotherapies. *J Psychopharmacol.* (2014) 28:587–95. doi: 10.1177/0269881114523862
50. First M, Gibbon M, Spitzer R. *Structured Clinical Interview for DSM-IV Axis II Personality Disorder (SCID-II)*. Washington, DC: American Psychiatric Press Inc. (1997).
51. Gratton G, Coles MG, Donchin E. A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol.* (1983) 55:468–84. doi: 10.1016/0013-4694(83)90135-9
52. Phillips C, Rugg M D, Fristont KJ. Systematic regularization of linear inverse solutions of the EEG source localization problem. *Neuroimage* (2002) 17:287e301. doi: 10.1006/nimg.2002.1175
53. Mulert C, Jäger L, Propp S, Karch S, Störmann S, Pogarell O, et al. Sound level dependence of the primary auditory cortex:simultaneous measurement with 61-channel EEG and fMRI. *Neuroimage* (2005) 28:49e58. doi: 10.1016/j.neuroimage.2005.05.041
54. Pizzagalli D, Oakes T, Fox A, Chung MK, Larson CL, Abercrombie HC, et al. Functional but not structural subgenual prefrontal cortex abnormalities in melancholia. *Mol Psychiatry* (2004) 9:393e405. doi: 10.1038/sj.mp.4001469
55. Leuchter AF, Uijtdehaage SH, Cook IA, O'Hara R, Mandelkern M. Relationship between brain electrical activity and cortical perfusion in normal subjects. *Psychiatry Res.* (1999) 90:125–40. doi: 10.1016/S0925-4927(99)00006-2
56. Grus J. *Data Science from Scratch:First Principles with Python*. Sebastopol, CA: O'Reilly Media (2015).
57. Han J, Pei J, Kamber M. *Data Mining:Concepts and Techniques*. Waltham, MA: Elsevier (2011).
58. Juszczak P, Tax D, Duin RPW. Feature scaling in support vector data description. In: *Proceedings of ASCI*. Citeseer (2002). p. 95–102.
59. Geurts P, Damien E, Louis W. Extremely randomized trees. *Mach Learn.* (2006) 63:3–42. doi: 10.1007/s10994-006-6226-1

60. Lerman R, Yitzhaki S. A note on the calculation and interpretation of the Gini index. *Econ Lett*. (1984) 15:363–8. doi: 10.1016/0165-1765(84)90126-5
61. Heiko H. Kernel PCA for novelty detection. *Pattern Recogn*. (2007) 40:863–74. doi: 10.1016/j.patcog.2006.07.009
62. Schölkopf B, Smola A, Müller K-R. Nonlinear component analysis as a kernel eigenvalue problem. *Neural Comput*. (1998) 10:1299–319. doi: 10.1162/089976698300017467
63. Sidhu GS, Asgarian N, Greiner R, Brown MR. Kernel principal component analysis for dimensionality reduction in fMRI-based diagnosis of ADHD. *Front Syst Neurosci*. (2012) 6:74. doi: 10.3389/fnsys.2012.00074
64. Breiman L. Random forests. *Mach Learn*. (2001) 45:5–32. doi: 10.1023/A:1010933404324
65. Strobl C, Boulesteix AL, Zeileis A, Hothorn T. Bias in random forest variable importance measures:illustrations, sources and a solution. *BMC Bioinformatics* (2007) 8:25. doi: 10.1186/1471-2105-8-25
66. Probst P, Boulesteix A-L. To tune or not to tune the number of trees in random forest. *J Mach Learn Res*. (2018) 18:1–18.
67. Oshiro TM, Perez PS, Baranauskas JA. How many trees in a random forest? Machine. In: *Learning and Data Mining in Pattern Recognition*. Berlin: Springer (2012). p. 154–168. doi: 10.1007/978-3-642-31537-4_13
68. Breiman L. *Classification and Regression Trees*. New York, NY: Routledge (2017). doi: 10.1201/9781315139470
69. Loh W-Y. Classification and regression trees. *Wiley Int Rev*. (2011) 1:14–23. doi: 10.1002/widm.8
70. Cortes C, Vapnik V. Support-vector networks. *Mach Learn*. (1995) 20:273–97. doi: 10.1007/BF00994018
71. Freund Y, Schapire R. A decision-theoretic generalization of on-line learning and an application to boosting. *J Comp Syst Sci*. (1997) 55:119–39. doi: 10.1006/jcss.1997.1504
72. John Lu QZ. The elements of statistical learning:data mining, inference, and prediction. *J R Stat Soc Ser A*. (2010) 173:693–4. doi: 10.1111/j.1467-985X.2010.00646_6.x
73. Mitchell TM. Logistic regression. *Mach Learn*. (2005). 10:701.
74. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots:a fundamental evaluation tool in clinical medicine. *Clin Chem*. (1993) 39:561–77.
75. Powers DM. Evaluation:from precision, recall and F-measure to ROC, informedness, markedness and correlation. *J Mach Learn Technol*. (2011) 2:37–63.
76. Myerson J, Green L, Warusawitharana M. Area under the curve as a measure of discounting. *J Exp Anal Behav*. (2001) 76:235–43. doi: 10.1901/jeab.2001.76-235
77. Hunter AM, Cook IA, Greenwald SD, Tran ML, Miyamoto KN, Leuchter AF. The antidepressant treatment response index and treatment outcomes in a placebo-controlled trial of fluoxetine. *J Clin Neurophysiol*. (2011) 28:478–82. doi: 10.1097/WNP.0b013e318230da8a
78. Korb AS, Hunter AM, Cook IA, Leuchter AF. Rostral anterior cingulate cortex theta current density and response to antidepressants and placebo in major depression. *Clin Neurophysiol*. (2009) 120:1313–9. doi: 10.1016/j.clinph.2009.05.008
79. Mulert C, Juckel G, Brunnmeier M, Karch S, Leicht G, Mergl R, et al. Rostral anterior cingulate cortex activity in the theta band predicts response to antidepressive medication. *Clin EEG Neurosci*. (2007) 38:78–81. doi: 10.1177/155005940703800209
80. Narushima K, McCormick LM, Yamada T, Thatcher RW, Robinson RG. Subgenual cingulate theta activity predicts treatment response of repetitive transcranial magnetic stimulation in participants with vascular depression. *J Neuropsychiatry Clin Neurosci*. (2010) 22:75–84. doi: 10.1176/jnp.2010.22.1.75
81. Pizzagalli D, Pascual-Marqui RD, Nitschke JB, Oakes TR, Larson CL, Abercrombie HC, et al. Anterior cingulate activity as a predictor of degree of treatment response in major depression:evidence from brain electrical tomography analysis. *Am J Psychiatry* (2001) 158:405–15. doi: 10.1176/appi.ajp.158.3.405
82. Arns M, Bruder G, Hegerl U, Spooner C, Palmer DM, Etkin A, et al. EEG alpha asymmetry as a gender-specific predictor of outcome to acute treatment with different antidepressant medications in the randomized iSPOT-D study. *Clin Neurophysiol*. (2016) 127:509–19. doi: 10.1016/j.clinph.2015.05.032
83. Konarski JZ, Kennedy SH, Segal ZV, Lau MA, Bieling PJ, McIntyre RS, et al. Predictors of nonresponse to cognitive behavioural therapy or venlafaxine using glucose metabolism in major depressive disorder. *J Psychiatry Neurosci*. (2009) 34:175–80.
84. Pizzagalli DA, Webb CA, Dillon DG, Tenke CE, Kayser J, Goer F, et al. Pretreatment rostral anterior cingulate cortex theta activity in relation to symptom improvement in depression: a randomized clinical trial. *JAMA Psychiatry* (2018) 75:547–54. doi: 10.1001/jamapsychiatry.2018.0252
85. Rentzsch J, Adli M, Wiethoff K, Gómez-Carrillo de. Castro, A, Gallinat J. Pretreatment anterior cingulate activity predicts antidepressant treatment response in major depressive episodes. *Eur Arch Psychiatry Clin Neurosis*. (2014) 264:213–23. doi: 10.1007/s00406-013-0424-1
86. Vasic N, Wolf ND, Grön G, Sosic-Vasic Z, Connemann BJ, Sambataro F, et al. Baseline brain perfusion and brain structure in patients with major depression:a multimodal magnetic resonance imaging study. *J Psychiatry Neurosci*. (2015) 40:412–21. doi: 10.1503/jpn.140246
87. Rigucci S, Serafini G, Pompili M, Kotzalidis GD, Tatarelli R. Anatomical and functional correlates in major depressive disorder:the contribution of neuroimaging studies. *World J Biol Psychiatry* (2010) 11:165–80. doi: 10.3109/15622970903131571
88. Hegerl U, Gallinat J, Juckel G. Event-related potentials. Do they reflect central serotonergic neurotransmission and do they predict clinical response to serotonin agonists? *J Affect Disord*. (2001) 62:93–100 doi: 10.1016/S0165-0327(00)00353-0
89. Bruder GE, Sedoruk JP, Stewart JW, McGrath PJ, Quitkin FM, Tenke CE. Electroencephalographic alpha measures predict therapeutic response to a selective serotonin reuptake inhibitor antidepressant:Pre- and post-treatment findings. *Biol Psychiatry* (2008) 63:1171–7. doi: 10.1016/j.biopsych.2007.10.009
90. Knott VJ, Telner JI, Lapierre YD, Browne M, Horn ER. Quantitative EEG in the prediction of antidepressant response to imipramine. *J Affect Disord*. (1996) 39:175–84. doi: 10.1016/0165-0327(96)00003-1
91. Tenke CE, Kayser J, Manna CG, Fekri S, Kroppmann CJ, Schaller JD, et al. Current source density measures of electroencephalographic alpha predict antidepressant treatment response. *Biol Psychiatry* (2011) 70:388–94. doi: 10.1016/j.biopsych.2011.02.016
92. Ulrich G, Renfordt E, Zeller G, Frick K. Interrelation between changes in the EEG and psychopathology under pharmacotherapy for endogenous depression: a contribution to the predictor question. *Pharmacopsychiatry* (1984) 17:178–83. doi: 10.1055/s-2007-1017433
93. Arns M, Drinkenburg WH, Fitzgerald PB, Kenemans JL. Neurophysiological predictors of non-response to rTMS in depression. *Brain Stimul*. (2012) 5:569–76. doi: 10.1016/j.brs.2011.12.003
94. Iosifescu DV, Greenwald S, Devlin P, Mischoulon D, Denninger JW, Alpert JE, et al. Frontal EEG predictors of treatment outcome in major depressive disorder. *Eur Neuropsychopharmacol*. (2009) 19:772–7. doi: 10.1016/j.euroneuro.2009.06.001
95. Arns M, Etkin A, Hegerl U, Williams LM, DeBattista C, Palmer DM, et al. Frontal and rostral anterior cingulate (rACC) theta EEG in depression:implications for treatment outcome? *Eur Neuropsychopharmacol*. (2015) 25:1190–200. doi: 10.1016/j.euroneuro.2015.03.007
96. Spronk D, Arns M, Barnett KJ, Cooper NJ, Gordon E. An investigation of EEG, genetic and cognitive markers of treatment response to antidepressant medication in patients with major depressive disorder:a pilot study. *J Affect Disord*. (2011) 128:41–8. doi: 10.1016/j.jad.2010.06.021
97. Bares M, Novak T, Kopecek M, Brunovsky M, Stopkova P, Höschl C. The effectiveness of prefrontal theta cordance and early reduction of depressive symptoms in the prediction of antidepressant treatment outcome in patients with resistant depression: Analysis of naturalistic data. *Eur Arch Psychiatry Clin Neurosci*. (2015) 265:73–82. doi: 10.1007/s00406-014-0506-8
98. Heller W, Nitschke JB, Etienne MA, Miller GA. Patterns of regional brain activity differentiate types of anxiety. *J Abnorm Psychol*. (1997) 106:376–85. doi: 10.1037/0021-843X.106.3.376
99. Manna CB, Tenke CE, Gates NA, Kayser J, Borod JC, Stewart JW, et al. EEG hemispheric asymmetries during cognitive tasks in depressed patients

with high versus low trait anxiety. *Clin EEG Neurosci.* (2010) 41:196–202. doi: 10.1177/155005941004100406

100. Kaiser RH, Andrews-Hanna JR, Wager TD, Pizzagalli DA. Large-scale network dysfunction in major depressive disorder: a meta-analysis of resting-state functional connectivity. *JAMA Psychiatry* (2015) 72:603–11. doi: 10.1001/jamapsychiatry.2015.0071
101. Szegedi A, Jansen WT, van Willigenburg AP, van der Meulen E, Stassen HH, Thase ME. Early improvement in the first 2 weeks as a predictor of treatment outcome in patients with major depressive disorder:a meta-analysis including 6562 patients. *J Clin Psychiatry* (2009) 70:344–53. doi: 10.4088/JCP.07m03780
102. Wagner S, Engel A, Engelmann J, Herzog D, Dreimüller N, Müller MB, et al. Early improvement as a resilience signal predicting later remission to antidepressant treatment in patients with Major Depressive Disorder:Systematic review and meta-analysis. *J Psychiatr Res.* (2017) 94:96–106. doi: 10.1016/j.jpsychires.2017.07.003
103. Uher R, Mors O, Rietschel M, Rajewska-Rager A, Petrovic A, Zobel A, et al. Early and delayed onset of response to antidepressants in individual trajectories of change during treatment of major depression:a secondary analysis of data from the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. *J Clin Psychiatry* (2011) 72:1478–84. doi: 10.4088/JCP.10m06419
104. de Vries Y, Roest A, Bos E, Burgerhof JGM, van Loo HM, de Jonge P. Predicting antidepressant response by monitoring early improvement of individual symptoms of depression: individual patient data meta-analysis. *Br J Psychiatry* (2018) 28:1–7. doi: 10.1192/bjp.2018.122
105. Godard J, Baruch P, Grondin S, Lafleur MF. Psychosocial and neurocognitive functioning in unipolar and bipolar depression: a 12-month prospective study. *Psychiatry Res.* (2012) 196:145–53. doi: 10.1016/j.psychres.2011.09.013
106. Iosifescu DV, Neborsky RJ, Valuck RJ. The use of the psychiatric electroencephalography evaluation registry (PEER) to personalize pharmacotherapy. *Neuropsychiatr Dis Treat.* (2016) 12:2131–42. doi: 10.2147/NDT.S113712
107. Mumtaz W, Xia L, Mohd Yasin MA, Azhar Ali SS, Malik AS. A wavelet-based technique to predict treatment outcome for Major Depressive Disorder. *PLoS ONE* (2017) 12:e0171409. doi: 10.1371/journal.pone.0171409
108. Yao D. A method to standardize a reference of scalp EEG recordings to a point at infinity. *Physiol Meas.* (2001) 22:693–711. doi: 10.1088/0967-3334/22/4/305
109. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med.* (1978) 8:283–98. doi: 10.1016/S0001-2998(78)80014-2
110. Widge AS, Bilge MT, Montana R, Chang W, Rodriguez CI, Deckersbach T, et al. Electroencephalographic biomarkers for treatment response prediction in major depressive illness: a meta-analysis. *Am J Psychiatry* (2018). doi: 10.1176/appi.ajp.2018.17121358. [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 17 January 2019
doi: 10.3389/fpsyt.2018.00735

Heart Rate Variability as Indicator of Clinical State in Depression

Ralf Hartmann, Frank M. Schmidt, Christian Sander and Ulrich Hegerl*

Department of Psychiatry and Psychotherapy, University Hospital Leipzig, Leipzig, Germany

Background: Depression is a severe disease with great burdens for the affected individuals and public health care systems. Autonomic nervous system (ANS) dysfunction indexed by measures of heart rate variability (HRV) has repeatedly been associated with depression. However, HRV parameters are subject to a wide range of multi-factorial influences and underlying mechanisms in depression are still unclear. HRV parameters have been proposed to be promising candidates for diagnostic or predictive bio-markers for depression but necessary longitudinal design studies investigating the relationship between HRV and depression are scarce.

Methods: The sample in this study consisted of 62 depressive individuals without antidepressant medication prior to assessment and 65 healthy controls. Fifteen minute blocks of resting ECGs were recorded 1–2 days before onset of antidepressant treatment and 2 weeks thereafter. The ECGs were pre-processed to extract inter-beat-intervals. Linear and non-linear methods were used to extract HRV parameters. ANOVAS were performed to investigate group differences between depressive patients and healthy controls. Associations between the change in severity of depression and HRV parameters were assessed in a repeated measurements design.

Results: Analyses revealed HRV parameter differences between the groups of depressive patients and healthy controls at baseline. Further results show differences in HRV parameters within subjects after 2 weeks of antidepressant treatment. Change in HRV parameter values correlated with changes in symptom severity of depression.

Discussion: The current results provide further insight into the relationship between HRV parameters and depression. This may help to underpin utilization of HRV parameters are bio-maker for disease state in depression. Results are discussed within a theoretical framework to link arousal and ANS regulation in depression.

Keywords: heart rate variability, depression, biomarker, diagnostics, antidepressants, response parameter, trait marker, state marker

OPEN ACCESS

Edited by:
Michele Fornaro,
New York State Psychiatric Institute (NYSPI), United States

Reviewed by:
Stefano Novello,
University of Naples Federico II, Italy
Annalisa Anastasia,
Villa Camaldoli Foundation, Italy

****Correspondence:***
Ralf Hartmann
ralf.hartmann@medizin.uni-leipzig.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *16 October 2018*
Accepted: *13 December 2018*
Published: *17 January 2019*

Citation:
Hartmann R, Schmidt FM, Sander C and Hegerl U (2019) Heart Rate Variability as Indicator of Clinical State in Depression. Front. Psychiatry 9:735. doi: 10.3389/fpsyt.2018.00735

INTRODUCTION

Depression is a severe disease with high prevalence (1–3) and an elevated risk for recurrence and chronicity (4). It is a major source of burden of disease and contributes significantly to years lived with disability (5). Repeated findings of increased occurrence of cardio-vascular disease among patients with Major Depressive Disorder [MDD, (6–11)] have drawn attention to autonomous regulation of the heart rate as a potential pathophysiological mechanism in depression (12–19). Consequently, altered Autonomous Nervous System (ANS) regulation of the heart rate indicated

by measures of heart rate variability (HRV) has repeatedly been observed in depression across the life span (15, 20–22). Heart rate is typically assessed by electrocardiogram (ECG) and different procedures have been established to describe HRV and derive parameters from different time-length ECG signals (23–27). A compelling body of research indicates a reduced HRV indexed by lower values of SDNN, RMSSD and HF power and increased values of LF power for patients with depression in comparison to healthy controls (15, 28–32). LF/HF Ratio has repeatedly been found to be decreased in depression (15) but recent research leaves doubts about interpretation of the LF/HF ratio (33–35). Some HRV parameters have been used to separate patients with MDD and healthy controls (31, 36). Moreover, reduced HRV has been associated with severity of depression and parameters derived from HRV have been applied to delineate the severity of depression or even changes in symptom severity (13, 28, 37). Inconsistent results exist concerning the association between HRV and the response to antidepressant treatment (38–41). Hitherto, research on longitudinal monitoring of HRV and symptom severity of depression is scarce. Though some studies found changes in HRV to normalize along with improvement in severity of depression during antidepressant treatment (15, 38, 42) findings for specific HRV parameters remain inconsistent. Studies investigating changes in HRV parameters and severity of depression over the course of time are of importance for a better understanding of the relationship between HRV and disease state when HRV is supposed to be used as a diagnostic or predictive bio-marker for depression. Bio-markers for depression might be helpful in clinical treatment and disease management (43). Previous attempts to utilize HRV parameters as bio-markers for depression are promising (44–46). To underpin these attempts we aim to examine in a repeated measurements design the relationship between HRV and symptom severity of depression.

This study investigates the relationship between HRV parameters and the severity of depression in a repeated measurements design. First aim was to identify HRV parameters which could serve as diagnostic markers to separate patients with MDD from healthy controls at baseline. A further aim of this study was to examine if patients with MDD show normalization of HRV parameters parallel to an improvement in symptom severity of depression and if changes in HRV correlate with changes in symptom severity of depression over the course 2 weeks of antidepressant treatment. This may augment understanding if HRV parameters may serve as indicators for the presence of MDD or change in symptom severity of depression over the course of time.

MATERIALS AND METHODS

Sample

All data presented in this work were gathered alongside a study on brain arousal regulation as response predictor for antidepressant therapy in Major Depressive Disorder (MDD) using Electroencephalography (EEG) signals (47). ECG was obtained in parallel to the EEG recording at two time points: prior to antidepressant treatment (T1) and 14 ± 1 days following onset of antidepressant treatment (T2). The total sample of the aforementioned study (47) comprised 65 unmedicated patients with a diagnosis of MDD and 65 healthy controls. The patient subsample consisted of depressed in- and outpatients consecutively recruited between 2012/02 and 2015/01 from the Department of Psychiatry and Psychotherapy of the University Hospital Leipzig. Individuals had to meet the inclusion criteria: age $\geq$ 18 years; a diagnosis of MDD with a current depressive episode with a base-line score $\geq$ 10 in the HDRS-17. Exclusion criteria were defined to meet the EEG study requirements: use of a centrally active medication (including anti-depressants) within 14 days prior to the T1 assessment. Other exclusion criteria were acute risk of suicidal tendencies; somatic mental disorders; illegal drugs and/or alcohol abuse within the past 6 months; schizophrenia, schizotypal and delusional disorders; a history of head injury with loss of consciousness exceeding 1 h; seizure disorder; acute or chronic infection and major somatic disorders. The diagnosis of a unipolar depressive disorder was given by a clinical professional and was substantiated by the Structured Clinical Interview for DSM-IV Axis I (SCID-I) prior to the T1 assessment. The subsample of healthy controls consisted of 65 sex- and age-matched non-depressed controls selected from a database consisting of EEG-recordings from community volunteers recruited via announcements in the local newspapers, University's intranet and internet. For detailed information upon sample characteristics see Schmidt et al. (47).

For the present analyses, three individuals of the original patient subsample had to be excluded because of missing ECG recordings at T1. Therefore, the total sample of the present study comprised 62 unmedicated patients with a diagnosis of MDD (depressive sample, DEP) and 65 healthy controls (HC). The HC sample was only assessed at T1 therefore ECG signals of healthy controls were not available at T2 for direct comparisons between groups. To evaluate normalization of HRV parameters of the DEP sample we used the T1 HRV parameters of the HC sample. Within the DEP sample, 8 individuals had to be excluded from T1/T2 comparisons due to missing ECG recordings at T2.

The study was performed according to the Helsinki Declaration and approved by Leipzig University Ethics Committee (#278-11-22082011).

Procedures

Assessments of symptoms and severity of MDD and measurements of ECG signals took place before the onset of antidepressant treatment (T1) and 2 weeks following the onset of medication (T2). Standard antidepressant treatment was carried out according to an in-house treatment algorithm at the Department of Psychiatry and Psychotherapy of the University Hospital Leipzig which includes antidepressant medication as first line treatment. At both assessments symptom severity of depression was assessed with the 17 Item version of the Hamilton Depression Rating Scale [HDRS-17, (48)], and Inventory of Depressive Symptomatology [IDS-C, (49)] by a trained rater using a structured interview guideline (50). The Beck Depression Inventory II [BDI-II, (51)] was issued for self-rating of symptoms and severity of MDD. All ECG measurements were carried out as additional measurements

alongside EEG recordings. EEG and ECG signals were measured in the same room between 8:00 a.m. and 2:00 p.m. Within patients, time of recording was not allowed to vary more than± 1 h between T1 and T2. For each time point individuals were instructed to relax with eyes closed in a semi-recumbent position and not to fight a possibly occurring urge to fall asleep. ECG signals were recorded with a 40 channel QuickAmp amplifier (Brain Products GmbH, Gilching, Germany) with 2 leads placed on the left forearm at a sampling rate of 1 kHz.

HRV Analyses

BrainVision Analyzer 2 (Brain Products GmbH, Gilching, Germany) was used to analyse all ECGs for interbeat intervals (IBIs). After automated R-wave peak detection of the QRS complex (for details see 26) all data sets were manually scanned for ectopic heartbeats and artifacts were removed. After this procedure RR distances in milli seconds were calculated. Artiifact (52) and Kubios (53) were used to obtain HRV parameters from the resulting tachograms. Both applications were used to double check results for accuracy and consistency. Time domain HRV parameters were calculated by linear methods, frequency domain HRV parameters were calculated by using Spectral Analyses (e.g., Fast Fourier Transformation) and non-linear HRV parameters were calculated by using Poincare plots. All HRV parameters were selected based on literature (23, 25, 27) and are presented in **Table 1**.

Statistical Analyses

ANOVAs were performed to investigate group differences at baseline. Simple regressions and binary logistic regressions were performed to predict severity of depression or group assignment (DEP vs. HC) according to data level. To investigate differences between time points repeated measures ANOVAs were performed with HRV parameters as within subject factors and age, sex and heart rate as between subject factors. Effect sizes were calculated using Eta square. For approximation of normalization of HRV parameter the T1 values of HC were carried forward to T2. Change in symptom severity between time points was defined as reduction in sum score of HDRS-17, BDI-II and IDS-C of 30 and 50% at T2 compared to T1. To estimate change in HRV parameters we calculated absolute value differences between T1 and T2. Principle component analyses were performed to assess subtypes of depression (atypical subtype, melancholic subtype) based on respective single item ratings of BDI-II and HDRS-17 separately [see (54)]. Sample was stratified for severity of depression (a) based on cut-offs for BDI-II with values from 14 to 19 for mild depression, values from 20 to 28 for moderate depression and values above 29 for severe depression (51) and (b) HDRS-17 [9–16 = mild depression, 17–24 = moderate depression, ≥ 25 = severe depression (55)]. All Statistical analyses were performed using SPSS (SPSS 24, IBM, Chicago, IL, United States) and the significance level was set to a $p < 0.05$.

RESULTS

General

Total sample mean age was 36.99 ($SD = 12.108$) ranging from 19 to 61 years. The total sample comprised of 62 males and 65 females. We found no violation of normal distribution for age within groups. Furthermore, we found no significant differences between groups for age [$t_{(125)} = -0.066$, $p = 0.948$] and sex ($X^2 = 0.378$, $df = 1$, $p = 0.538$). We collapsed data across sex and age for subsequent analyses. There were no differences between groups for heart rate [$F_{(1, 125)} = 1.087$, $p = 0.299$] and Mean RR interval [$F_{(1, 125)} = 0.770$, $p = 0.382$].

Group Differences in HRV at Baseline

To investigate which HRV parameters separate between depressive patients (DEP) and healthy controls (HC) we conducted an ANOVA for mean differences between both groups at T1. Group differences in HF power in % [$F_{(1, 125)} = 15.029$, $p = 0.000$, $Eta^2 = 0.115$] were highly significant. Group differences in SD1 [$F_{(1, 125)} = 4.909$, $p = 0.029$, $Eta^2 = 0.034$], RMSSD [$F_{(1, 125)} = 4.909$, $p = 0.029$, $Eta^2 = 0.038$] and LF power in % [$F_{(1, 125)} = 4.605$, $p = 0.23$, $Eta^2 = 0.04$] were significant. Age and sex as confounding variables were not significant. Means and Standard Errors for time domain, frequency domain and non-linear parameters for DEP vs. HC at T1 and for repeated measurements (DEP_{T1} vs. DEP_{T2}) are presented in **Table 2**. Furthermore, we performed binary logistic regressions analyses to predict group assignment to HC and DEP from HRV parameters. HF power in % predicted group assignment to the HC vs. DEP sample ($X^2 = 15.517$, $df = 1$, $p = 0.000$) explaining 62.2% of all variance. Also LF power in % ($X^2 = 5.137$, $df = 1$, $p = 0.023$, explained variance = 51.2%) and SD1 ($X^2 = 6.154$, $df = 1$, $p = 0.013$, explained variance = 51.2%) significantly predicted group assignment. The simple regression analyses to predict symptom severity from HRV parameters within the depressive sample did not show significant results.

Changes in HRV and Depression Severity

Assessing changes in symptom severity of depression between T1 and T2 within the depressive sample (DEP), the Repeated Measurements ANOVAs revealed highly significant differences with moderate to high effect sizes in self-rated (BDI-II) as well as observer-rated (HDRS, IDS-C) instruments. Results are displayed in **Table 3**. Repeated Measurements ANOVAs revealed changes in HRV parameters within the depressive sample (DEP, for results see **Table 2**). Differences between time points for HF power in % [$F_{(1, 52)} = 33{,}180$, $p = 0.000$, $Eta^2 = 0.39$] and SD1/SD2 Ratio [$F_{(1, 52)} = 14{,}773$, $p = 0.001$, $Eta^2 = 0.221$] were highly significant with moderate effect size. Differences between time points for HF power in n.u. [$F_{(1, 52)} = 12{,}375$, $p = 0.001$, $Eta^2 = 0.192$] and SD2 [$F_{(1, 52)} = 13{,}019$, $p = 0.001$, $Eta^2 = 0.2$] were significant with moderate effect size. In addition differences between time points for LF power in n.u. [$F_{(1, 52)} = 14{,}773$, $p = 0.001$, $Eta^2 = 0.19$] were significant with moderate effect size. Age and heart rate were not identified as confounders. We found a significant interaction of sex and HF power in % [$F_{(1, 51)} = 5.075$, $p = 0.029$, $Eta^2 = 0.091$]. The difference in HF

TABLE 1 | HRV parameters derived from time-domain, frequency-domain and non-linear procedures, abbreviations and descriptions.

Parameter	Description	Unit	ANS branch
TIME DOMAIN			
SDNN	Standard deviation of NN intervals	ms	SNS
RMSSD	Root mean square of successive RR interval differences	ms	PNS
pNN50	Percentage of successive RR intervals that differ by more than 50 ms	%	SNS, PNS
FREQUENCY DOMAIN			
LF power	Relative power of the low-frequency band (0.04–0.15 Hz)	ms^2, %, n.u.	SNS
HF power	Relative power of the high-frequency band (0.15–0.4 Hz)	ms^2, %, n.u.	PNS
LF/HF Ratio	Ratio of LF-to-HF power	%	SNS, PNS
NON-LINEAR			
SD1	Poincaré plot standard deviation vertical the line of identity	ms	Short term flexibility of ANS
SD2	Poincaré plot standard deviation along the line of identity	ms	Long term flexibility of ANS
SD1/SD2 Ratio	Ratio of SD1 and SD2	ms	

ANS, Autonomous Nervous System; SNS, Sympathetic Nervous System; PNS, Parasympathetic Nervous System; Hz, Hertz; ms, milli seconds; ms^2, milli seconds squared; n.u., normalized units; NN Intervals, interval between two normal R-peaks; RR intervals, interval between two R-peaks; for further information see Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology (23).

TABLE 2 | Heart Rate Variability in healthy controls (HC) and MDD patients (DEP) and MDD patients before (T1) and after 2 weeks of antidepressant treatment (T2).

	HC	DEP		DEP		
	T1/N = 65	T1/N = 62		T1/N = 53	T2/N = 53	
	Mean (SE)	Mean (SE)	*p*	Mean (SE)	Mean (SE)	*p*
TIME DOMAIN						
SDNN in ms	50.464 (3.831)	48.030 (6.059)	0.732	41.489 (2.168)	40.525 (2.611)	0.666
RMSSD in ms	41.547 (5.323)	28.872 (1.778)	**0.029**	28.859 (1.952)	32.318 (3.413)	0.264
pNN50 in %	16.133 (2.390)	10.301 (1.688)	0.050	10.202 (1.865)	13.206 (2.617)	0.203
FREQUENCY DOMAIN						
LF in Hz	0.075 (0.003)	0.074 (0.003)	0.761	0.074 (0.003)	0.078 (0.003)	0.394
LF power in %	51.283 (2.055)	43.511 (2.716)	**0.023**	44.385 (2.910)	46.076 (2.533)	0.513
LF power in n.u.	56.173 (2.330)	59.932 (2.205)	0.244	59.038 (2.443)	49.943 (2.889)	**0.001**
HF in Hz	0.250 (0.007)	0.244 (0.007)	0.519	0.241 (0.008)	0.250 (0.008)	0.291
HF power in %	40.782 (2.305)	28.593 (1.939)	**0.000**	29.695 (2.115)	47.396 (2.932)	**0.000**
HF power in n.u.	43.721 (2.323)	39.989 (2.197)	0.246	40.877 (2.434)	50.001 (2.885)	**0.001**
LF/HF Ratio	2.093 (0.350)	2.071 (0.197)	0.955	2.008 (0.209)	1.561 (0.243)	0.052
NON-LINEAR						
SD1 in ms	29.394 (3.766)	20.426 (1.258)	**0.029**	20.417 (1.381)	22.864 (2.415)	0.264
SD2 in ms	46.274 (3.793)	57.509 (10.038)	0.289	47.399 (3.380)	38.293 (3.003)	**0.001**
SD1/SD2 Ratio	1.935 (0.092)	3.135 (0.679)	0.075	2.466 (0.137)	1.901 (0.090)	**0.000**

Hz, Hertz; ms, milli seconds; ms^2, milli seconds squared; n.u., normalized units; SDNN, Standard deviation of NN intervals; RMSSD, Root mean square of successive RR interval differences; pNN50% = Percentage of successive RR intervals that differ by more than 50 ms; LF power = Relative power of the low-frequency band (0.04–0.15 Hz); HF power = Relative power of the high-frequency band (0.15–0.4 Hz); SD1 = Poincaré plot standard deviation vertical the line of identity; SD2 = Poincaré plot standard deviation along the line of identity. Significant p-values are highlighted in bold.

power values between sexes at T1 [$M_{males} = 26.975(SE = 2.885)$, $M_{females} = 32.742$ $(SE = 3.053)$] was more pronounced at T2 [$M_{males} = 38.376$ $(SE = 3.634)$, $M_{females} = 57.498$ $(SE = 3.846)$]. To appraise normalization of HRV parameters we conducted ANOVA for group differences between T1 HC and T2 DEP. No significant group differences were found. Furthermore, we aimed to test our hypothesis that change in HRV parameters correlate with change in symptom severity of depression after 2 weeks of antidepressant treatment. Point-Biserial correlations were run to determine the relationship between changes in HRV parameters and changes in symptom severity of depression indexed by reduction in sum scores of HDRS, BDI-II and IDS-C. Only results for Delta HF power were significant, age and sex as confounding variables were not significant (for results see **Table 4**).

Furthermore, we conducted exploratory analyses to investigate relationships between HRV parameters and other variables, such as severity of depression and subtypes

TABLE 3 | Change in Symptom severity of MDD patients before (T1) and after 2 weeks of antidepressant treatment (T2) as indexed by HDRS, BDI-II and IDS-C.

	T1 (before medication)		T2 (after medication)			
	Mean	**SE**	**Mean**	**SE**	***p***	**Eta^2**
BDI-II	29.579	1.514	24.140	1.855	**0.000**	0.289
HDRS	21.934	0.798	15.541	0.961	**0.000**	0.471
IDS-C	36.317	1.408	27.450	1.689	**0.000**	0.346

BDI-II, Beck Depression Inventory II; HDRS, Hamilton Depression Scale; IDS-C, Inventory of Depressive Symptomatology Clinician Version. Significant p-values are highlighted in bold.

TABLE 4 | Change in HF power (Delta HF power in %) and change symptom severity of MDD before and after antidepressant treatment, correlations r_{pb} and *p*-values, $N = 53$.

Variables	**Delta HF power**	***p***
50% [a] HDRS	−0.337	**0.021**
50% [a] BDI-II	−0.133	0.388
50% [a] IDS-C	−0.218	0.146
30% [b] HDRS	−0.273	0.063
30% [b] BDI-II	−0.115	0.458
30% [b] IDS-C	−0.301	**0.042**

[a]*Reduction in values between T1 (before medication) and T2 (after 14 days of medication) ≤ 50%.*
[b]*Reduction in values between T1 and T2 ≤ 30%. Significant p-values are highlighted in bold.*

of depression (atypical vs. melancholic subtype), which did not reveal significant results.

DISCUSSION

Heart rate variability has been in the focus of research for bio-markers of depression for a long time (46). Promising attempts to utilize HRV parameters as diagnostic or predictive bio-markers of depression have been presented. However, there is still a lack of studies investigating the relationship between HRV and severity of depression in longitudinal designs which is important if HRV is supposed to be used as bio-marker for depression. The purpose of this study was to provide further insight into the relationship of HRV and severity of depression in a repeated-measurements design.

Group Differences in HRV at Baseline

Aim of this study was to identify HRV parameters that may serve as diagnostic maker to separate between depressive patients and healthy controls prior to antidepressant treatment. Our results show significant differences between groups of patients with depression and healthy controls. The depressive sample displays lower HF power values compared to healthy controls. This result can be interpreted as a reduced ability of the para-sympathetic nervous system to regulate the heart rate via vagal activity [e.g., (56, 57)]. SD1 results show significantly reduced values in the depressive patients sample compared to healthy controls. This result may be interpreted as diminished short-term flexibility of the ANS to adapt to changing environments and tasks [i.e., (58–60)]. These findings present further evidence to previous findings of reduced HRV in depressive patients (15, 31). HF power was able to predict group assignment (MDD patients and healthy controls) with acceptable accuracy but regression analyses revealed no statistically significant relationship between HRV and symptom severity. Depressive individuals may be limited in their ability to adapt their ANS activity to challenging environments and fail to evoke adaptive resources (58, 61). This may result in phenotypical depressive symptoms like exhaustion, fatigue, stress-reactivity and disrupted sleep patterns (62). We also found significant results for RMSSD, LF power and SD1. Those parameters may serve as diagnostic bio-marker for pathophysiologic differences between MDD and healthy controls. But since underlying mechanisms that motor those parameters remain unclear interpretation should be treated with caution.

Changes in HRV and Depression Severity

Another aim of this study was to investigate if patients with MDD show normalization of HRV parameters parallel to an improvement in symptom severity of depression after 2 weeks of antidepressant treatment. Differences between time points for parameters reflecting severity of depression (HDRS-17, BDI-II and IDS-C) were significant. Individuals had lower levels of symptom severity after 2 weeks of antidepressant treatment assessed with both self-rated and observer-rated instrument even though the period between time points was very short and we did not expect individuals to remit fully from MDD symptom severity. We interpreted results as a reduction (i.e., improvement) in symptom severity of depression over the course of 2 weeks of antidepressant treatment, even though individuals partly remained diseased. We expected HRV parameters to normalize over the course of 2 weeks of antidepressant treatment. Within the group of depressive patients the differences between time points for HF power, LF power, SD2 and SD1/SD2 Ratio were significant. Values of HF power and Non-linear parameters increased over time and were even more pronounced in women than man and may be interpreted as an increase in vagal activity [see (25)]. Although LF power n.u., SD2 and SD1/SD2 Ratio differences between time points were significant, we refused to interpret these parameters due to heterogeneous findings at baseline. Furthermore, HRV parameter values at T2 did not differ from HRV parameter values of healthy controls at T1. This may be interpreted as a normalization of HRV parameter values in correspondence with improvement in symptom severity. To

further investigate the relationship between HRV parameters and symptom severity we aimed to test the hypothesis that changes in HRV parameters correlate with changes in symptom severity of depression. We found reciprocal correlations for observer-rated instruments HDRS-17 (at 50% reduction) and IDS-C (at 30% reduction) for HF power but with a small effect size. We interpret these findings so that increase of HF power values correlates with reduction in symptom severity.

Our results support our hypothesis of normalization of HRV parameters and improvement of symptom severity of depression in correspondence to an increase in HRV parameters values. This may be due to a link between both, nonetheless the exact mechanism behind the link remains unclear. A possible mechanism could be a regulation deficit between the two branches of the Autonomous Nervous System. A similar dysregulation has been proposed as explanation for findings of hyperstable vigilance found in MDD (63), as well as changes in the arousal regulation during therapy to relate to changes in depression severities (47). However, explained variance is still small and we suggest further research to unveil relationship between HRV and depression and the influence of confounding variables if HRV parameters shall be utilized as diagnostic or predictive bi-markers for depression.

LIMITATIONS

Our study faces some limitations which may impeach our interpretations. First of all our data shown here is based on ECG signals that were obtained parallel to EEG recordings and therefore the ECG signals were not suited to extract reliable time-domain measurements (e.g., RMSSD) and we did not control for in/expiration (23). Since overall data acquisition was laid out with accurate and ecologically valid and sample size was good we decided to investigate the ECG data set. Furthermore, our samples were not randomized and we did not have ECG signals for controls at T2. Since we only had a 2 weeks period between measurements long term effects may not be delineated. Our sample size of depressive patients at T1 was probably too small to reveal significant correlations of HRV parameters and measurements indexing symptom severity of depression. At last, though results for change in HRV parameters values and change in symptom severity were significant the amount of explained variance was small. Therefore, interpretation und clinical relevance may be limited.

CONCLUSION

The aim of this study was investigate the relationship between HRV parameters and symptom severity of depression in a repeated measurements design to provide further insight into this relationship which is of importance if HRV parameters shall be utilized as diagnostic or predictive bio-markers of depression. We interpret our results as confirmation of our hypothesis. Patients with MDD show decreased HRV compared to healthy controls before antidepressant treatment. Furthermore, patients with MDD show normalization of HRV parameters parallel to an improvement in symptom severity of depression over the course 2 weeks of antidepressant treatment and change in HRV parameter values correlates with change in symptom severity of depression after 2 weeks of antidepressant treatment. Though HF power seems to be a promising indicator for disease state in depression further research is needed to investigate the relationship between HRV parameters and symptom severity of depression and confounding variables in longitudinal study designs.

DATA AVAILABILITY STATEMENT

Original data is available upon request.

ETHICS STATEMENT

The study was performed according to the Helsinki Declaration and approved by Leipzig University Ethics Committee (#278-11-22082011). Ethical Committee at the Medical Faculty, Leipzig University, Käthe-Kollwitz-Straße 82, 04109 Leipzig.

AUTHOR CONTRIBUTIONS

FMS and UH were responsible for the planning and conduct of the original study (47). FMS recruited the study participants, supervised the EEG/ECG recordings and was responsible for the collection of clinical data. CS and FMS were responsible for data management and the pre-processing of the EEG files. RH performed the HRV analyses as well as the statistical analyses and was the main author of the manuscript. All authors participated substantially in writing the manuscript.

FUNDING

FMS received a junior research grant by the Medical Faculty, University of Leipzig for the conduct of the study. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

ACKNOWLEDGMENTS

We thank Ines Thomas and Mandy Siebert for conducting the EEG-recordings as well as Claudia Nowak for conducting the structured HDRS-17/IDS-C interviews.

REFERENCES

1. Maske UE, Buttery AK, Beesdo-Baum K, Riedel-Heller S, Hapke U, Busch MA. Prevalence and correlates of DSM-IV-TR major depressive disorder, self-reported diagnosed depression and current depressive symptoms among adults in Germany. *J Affect Disord*. (2016) 190:167–77. doi: 10.1016/j.jad.2015.10.006
2. Jacobi F, Höfler M, Siegert J, Mack S, Gerschler A, Scholl L, et al. Twelve-month prevalence, comorbidity and correlates of mental disorders in Germany: the Mental Health Module of the German Health Interview and

Examination Survey for Adults (DEGS1-MH). *Int J Methods Psychiatr Res*. (2014) 23:304–19. doi: 10.1002/mpr.1439
3. Busch MA, Maske UE, Ryl L, Schlack R, Hapke U. Prevalence of depressive symptoms and diagnosed depression among adults in Germany: results of the German Health Interview and Examination Survey for Adults (DEGS1). *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* (2013) 56:733–9. doi: 10.1007/s00103-013-1688-3
4. Ten Have M, de Graaf R, van Dorsselaer S, Tuithof M, Kleinjan M, Penninx BWJH. Recurrence and chronicity of major depressive disorder and their risk indicators in a population cohort. *Acta Psychiatr Scand*. (2018) 137:503–15. doi: 10.1111/acps.12874
5. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* (2015) 386:743–800. doi: 10.1016/S0140-6736(15)60692-4
6. Kemp AH, Brunoni AR, Nunes MA, Santos IS, Goulart AC, Ribeiro AL, et al. The association between mood and anxiety disorders, and coronary heart disease in Brazil: a cross-sectional analysis on the Brazilian longitudinal study of adult health (ELSA-Brasil). *Front Psych*. (2015) 6:187. doi: 10.3389/fpsyg.2015.00187
7. Hare DL, Toukhsati SR, Johansson P, and Jaarsma, T. Depression and cardiovascular disease: a clinical review. *Euro Heart J*. (2014) 35:1365–72. doi: 10.1093/eurheartj/eht462
8. Carney RM, Freedland KE. Depression in patients with coronary heart disease. *Am J Med*. (2008) 121:20–7. doi: 10.1016/j.amjmed.2008.08.006
9. Carney RM, Rich MW, Tevelde A, Saini J, Clark K, Jaffe AS. Major depressive disorder in coronary artery disease. *Am J Cardiol*. (1987) 60:1273–5. doi: 10.1016/0002-9149(87)90607-2
10. Carney RM., Freedland KE, Rich MW, Jaffe AS. Depression as a risk factor for cardiac events in established coronary heart disease: a review of possible mechanisms. *Ann Behav Med*. (1995) 17:142–9. doi: 10.1007/BF02895063
11. Stapelberg NJ, Hamilton-Craig I, Neumann DL, Shum DH, McConnell H. Mind and heart: heart rate variability in major depressive disorder and coronary heart disease-a review and recommendations. *Aust N Z J Psychiatry* (2012) 46:946–57. doi: 10.1177/0004867412444624
12. Carney RM, Freedland KE. Depression and coronary heart disease. *Nat Rev Cardiol*. (2017) 14:145–55. doi: 10.1038/nrcardio.2016.181
13. Bassett, D. A literature review of heart rate variability in depressive and bipolar disorders. *Aust N Z J Psychiatry* (2016) 50:511–9. doi: 10.1177/0004867415622689
14. Sgoifo A, Carnevali, L, Pico Alfonso MD, Amore M. Autonomic dysfunction and heart rate variability in depression. *Stress* (2015) 18:343–52. doi: 10.3109/10253890.2015.1045868
15. Kemp AH, Quintana DS, Gray MA, Felmingham KL, Brown K, Gatt JM. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol Psychiatry* (2010) 67:1067–74. doi: 10.1016/j.biopsych.2009.12.012
16. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol*. (2010) 141:122–31. doi: 10.1016/j.ijcard.2009.09.543
17. Davydov DM, Shapiro D, Cook IA, Goldstein I. Baroreflex mechanisms in major depression. *Prog Psychopharmacol Biol Psychiatry* (2007) 31:164–77. doi: 10.1016/j.pnpbp.2006.08.015
18. Carney RM, Freedland KE, Miller GE, Jaffe AS. Depression as a risk factor for cardiac mortality and morbidity: a review of potential mechanisms. *J Psychosom Res*. (2002) 53:897–902. doi: 10.1016/S0022-3999(02)00311-2
19. Jandackova VK, Britton A, Malik M, Steptoe A. Heart rate variability and depressive symptoms: a cross-lagged analysis over a 10 year period in the Whitehall II study. *Psychol Med*. (2016) 46:2121–31. doi: 10.1017/S003329171600060X
20. Brown L, Karmakar C, Gray R, Jindal R, Lim T, Bryant C. Heart rate variability alterations in late life depression: a meta-analysis. *J Affect Disord*. (2018) 235:456–66. doi: 10.1016/j.jad.2018.04.071
21. Koenig J, Kemp AH, Beauchaine TP, Thayer JF, Kaess M. Depression and resting state heart rate variability in children and adolescents—A systematic review and meta-analysis. *Clin Psychol Rev*. (2016) 46:136–50. doi: 10.1016/j.cpr.2016.04.013
22. Paniccia M, Paniccia D, Thomas S, Taha T, Reed N. Clinical and non-clinical depression and anxiety in young people: a scoping review on heart rate variability. *Auton Neurosci*. (2017) 208:1–14. doi: 10.1016/j.autneu.2017.08.008
23. Task Force of the European Society of Cardiology, and the North American Society of Pacing and Electrophysiology. Heart rate variability—standards of measurement, physiological interpretation, and clinical use. *Circulation* (1996) 93:1043–65. doi: 10.1161/01.CIR.93.5.1043
24. Shaffer F, Venner J. Heart rate variability anatomy and physiology. *Biofeedback* (2013) 41:13–25. doi: 10.5298/1081-5937-41.1.05
25. Shaffer F, Ginsberg JP. An overview of heart rate variability metrics and norms. *Front Public Health* (2017) 5:258. doi: 10.3389/fpubh.2017.00258
26. Bauer A, Camm AJ, Cerutti S, Guzik P, Huikuri H, Lombardi F, et al. Reference values of heart rate variability. *Heart Rhythm* (2017) 14:302–3. doi: 10.1016/j.hrthm.2016.12.015
27. Stapelberg NJ, Neumann DL, Shum BHK, McConnell H, Hamilton-Craig I. The sensitivity of 38 heart rate variability measures to the addition of artifact in human and artificial 24-hr cardiac recordings. *Ann Noninvasive Electrocardiol*. (2018) 23:e12483. doi: 10.1111/anec.12483
28. Brunoni AR, Kemp AH., Dantas EM, Goulart AC, Nunes MA, Boggio P, et al. Heart rate variability is a trait marker of major depressive disorder: evidence from the sertraline vs electric current therapy to treat depression clinical study. *Int J Neuropsychopharmacol*. (2013) 16:1937–49. doi: 10.1017/S1461145713000497
29. Bassett D, Bear N, Nutt D, Hood S, Bassett S, Hans D. Reduced heart rate variability in remitted bipolar disorder and recurrent depression. *Aust N Z J Psychiatry* (2016) 50:793–804. doi: 10.1177/0004867416652734
30. Alvares GA, Quintana DS, Hickie IB, Guastella AJ. Autonomic nervous system dysfunction in psychiatric disorders and the impact of psychotropic medications: a systematic review and meta-analysis. *J Psychiatry Neurosci*. (2016) 41:89. doi: 10.1503/jpn.140217
31. Jangpangi D, Mondal S, Bandhu R, Kataria D, Gandhi A. Alteration of heart rate variability in patients of depression. *J Clin Diagn Res*. (2016) 10:CM04–6. doi: 10.7860/JCDR/2016/22882.9063
32. Nahshoni E, Aravot D, Aizenberg D, Sigler M, Zalsman G, Strasberg B, et al. Heart rate variability in patients with major depression. *Psychosomatics* (2004) 45:129–34. doi: 10.1176/appi.psy.45.2.129
33. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol*. (2013) 4:26. doi: 10.3389/fphys.2013.00026
34. von Rosenberg W, Chanwimalueang T, Adjei T, Jaffer U, Goverdovsky V, Mandic DP. Resolving ambiguities in the LF/HF ratio: LF-HF scatter plots for the categorization of mental and physical stress from HRV. *Front Physiol*. (2017) 8:360. doi: 10.3389/fphys.2017.00360
35. Reyes del Paso GA, Langewitz W, Mulder LJ, Roon A. Duschek S. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology* (2013) 50:477–87. doi: 10.1111/psyp.12027
36. Schumann A, Andrack C, Bär KJ. Differences of sympathetic and parasympathetic modulation in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2017) 79:324–31. doi: 10.1016/j.pnpbp.2017.07.009
37. Carnevalli L, Thayer JF, Brosschot JF, Ottaviani C. Heart rate variability mediates the link between rumination and depressive symptoms: a longitudinal study. *Int J Psychophysiol*. (2018) 131:131–8. doi: 10.1016/j.ijpsycho.2017.11.002
38. Hage B, Sinacore J, Heilman K, Porges SW, Halaris A. Heart rate variability predicts treatment outcome in major depression. *JPBS* (2017) 2:1. doi: 10.20900/jpbs.20170017
39. Kemp AH, Quintana DS, Felmingham KL, Matthews S, Jelinek HF. Depression, comorbid anxiety disorders, and heart rate variability in physically healthy, unmedicated patients: implications for cardiovascular risk. *PLoS ONE* (2012) 7:e30777. doi: 10.1371/journal.pone.0030777
40. Rottenberg J, Wilhelm FH, Gross JJ, Gotlib IH. Respiratory sinus arrhythmia as a predictor of outcome in major depressive disorder. *J Affect Disord* (2002) 71:265–72. doi: 10.1016/S0165-0327(01)00406-2
41. Park HY, Oh S, Noh Y, Kim JY, Kim JH. Heart rate variability as a marker of distress and recovery: the effect of brief supportive expressive group therapy

with mindfulness in cancer patients. *Integr Cancer Ther*. (2018) 17:825–31. doi: 10.1177/1534735418756192
42. Park SW, Lee JH, Kim J, Suh S, Lee MS. Changes in heart rate variability in first-episode drug-naïve adolescents with major depressive disorder: a 12-week prospective study. *J Affect Disord*. (2018) 238:250–5. doi: 10.1016/j.jad.2018.05.068.
43. Strawbridge R, Young AH, Cleare AJ. Biomarkers for depression: recent insights, current challenges and future prospects. *Neuropsychiatr Dis Treat*. (2017) 13:1245–62. doi: 10.2147/NDT.S114542
44. Kim EY, Lee MY, Kim SH, Ha K, Kim KP, Ahn YM. Diagnosis of major depressive disorder by combining multimodal information from heart rate dynamics and serum proteomics using machine-learning algorithm. *Prog Neuropsychopharmacol Biol Psychiatry* (2017) 76:65–71. doi: 10.1016/j.pnpbp.2017.02.014
45. Kobayashi M, Sun G, Shinba T, Matsui T, Kirimoto T. Simple and objective screening of major depressive disorder by heart rate variability analysis during paced respiration and mental task conditions. *Conf Proc IEEE Eng Med Biol Soc*. (2017) 2017:1316–9. doi: 10.1109/EMBC.2017.8037074
46. Beauchaine TP, Thayer JF. Heart rate variability as a transdiagnostic biomarker of psychopathology. *Int J Psychophysiol*. (2015) 98:338–50. doi: 10.1016/j.ijpsycho.2015.08.004
47. Schmidt FM, Sander C, Dietz MA, Nowak C, Schröder T, Mergl R, et al. Brain arousal regulation as response predictor for antidepressant therapy in major depression. *Sci Rep*. (2017) 7:45187. doi: 10.1038/srep45187
48. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* (1960) 23:56–62.
49. Rush AJ, Gullion CM, Basco MR, Jarrett RB, Trivedi MH. The inventory of depressive symptomatology (IDS): psychometric properties. *Psychol Med*. (1996) 26:477–86. doi: 10.1017/S0033291700035558
50. Kobak KA, Williams JBW, Rush AJ. *Structured Interview guide for the Hamilton Depression Scale (SIGH-D) and the Inventory of depressive symptomatology (IDS_C) (SIGHD-IDS)* (2007). Available online at: http://narr.bmap.ucla.edu/docs/StructuredinterviewHAMd.pdf
51. Beck AT, Steer RA, Brown GK. *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation (1996).
52. Kaufmann T, Sütterlin S, Schulz SM, Vögele C. ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. *Behav Res Methods* (2011) 43:1161–70. doi: 10.3758/s13428-011-0107-7
53. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-ahoa PO, Karjalainen PA. Kubios HRV–Heart rate variability analysis software. *Comput Methods Programs Biomed*. (2014) 113:210–20. doi: 10.1016/j.cmpb.2013.07.024
54. van Loo HM, de Jonge P, Romeijn JW, Kessler RC, Schoevers RA. Data-driven subtypes of major depressive disorder: a systematic review. *BMC Med*. (2012) 10:156. doi: 10.1186/1741-7015-10-156
55. DGPPN, BÄK, KBV, AWMF, AkdÄ, BPtK, et al. *(Hrsg) für die Leitliniengruppe Unipolare Depression. S3-Leitlinie/Nationale VersorgungsLeitlinie Unipolare Depression-Langfassung, 1. Auflage*. Version 5. 2009, last update: Juni 2015. Available online at: www.depression.versorgungsleitlinien.de
56. Carreno FR, Frazer A. Vagal nerve stimulation for treatment-resistant depression. *Neurotherapeutics* (2017) 14:716–27. doi: 10.1007/s13311-017-0537-8
57. Aaronson ST, Sears P, Ruvuna F, Bunker M, Conway CR, Dougherty DD, et al. A 5-Year observational study of patients with treatment-resistant depression treated with vagus nerve stimulation or treatment as usual: comparison of response, remission, and suicidality. *Am J Psychiatry* (2017) 174:640–8. doi: 10.1176/appi.ajp.2017.16010034
58. Williams DP, Cash C, Rankin C, Bernardi A, Koenig J, Thayer JF. Resting heart rate variability predicts self-reported difficulties in emotion regulation: a focus on different facets of emotion regulation. *Front Psychol*. (2015) 6:261. doi: 10.3389/fpsyg.2015.00261
59. Appelhans BM, Luecken LJ. Heart rate variability as an index of regulated emotional responding. *Rev Gen Psychol*. (2006) 10:229–40. doi: 10.1037/1089-2680.10.3.229
60. Sakaki M, Yoo HJ, Nga L, Lee TH, Thayer JF, Mather M. Heart rate variability is associateed with amygdala functional connectivity with MPFC across younger and older adults. *NeuroImage* (2016) 139:44–52. doi: 10.1016/j.neuroimage.2016.05.076
61. Grippo AJ, Johnson AK. Stress, depression and cardiovascular dysregulation: a review of neurobiological mechanisms and the integration of research from preclinical disease models. *Stress* (2009) 12:1–21. doi: 10.1080/10253890802046281
62. Kwon H, Yoon H, Jung D, Choi S, Choi J, Lee Y, et al. Heart rate variability in patients with major depressive disorder and healthy controls during non-REM sleep and REM sleep. *Conf Proc IEEE Eng Med Biol Soc*. (2017) 2017:2312–5. doi: 10.1109/EMBC.2017.8037318
63. Hegerl U, Hensch T. The vigilance regulation model of affective disorders and ADHD. *Neurosci Biobehav Rev*. (2014) 44:45–57. doi: 10.1016/j.neubiorev.2012.10.008

Conflict of Interest Statement: The authors have the following conflicts to declare: Within the last 3 years, UH was an advisory board member for Lundbeck, Takeda Pharmaceuticals, Servier and Otsuka Pharma; a consultant for Bayer Pharma; and a speaker for Bristol-Myers Squibb, Medice Arzneimittel, Novartis and Roche Pharma.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 23 January 2019
doi: 10.3389/fpsyt.2018.00779

Quantitative Electroencephalography in Guiding Treatment of Major Depression

Mark J. Schiller[1,2]*

[1] Mind Therapy Clinic, San Francisco, CA, United States, [2] MYnd Analytics, Inc., Mission Viejo, CA, United States

This paper reviews significant contributions to the evidence for the use of quantitative electroencephalography features as biomarkers of depression treatment and examines the potential of such technology to guide pharmacotherapy. Frequency band abnormalities such as alpha and theta band abnormalities have shown promise as have combinatorial measures such as cordance (a measure combining alpha and theta power) and the Antidepressant Treatment Response Index in predicting medication treatment response. Nevertheless, studies have been hampered by methodological problems and inconsistencies, and these approaches have ultimately failed to elicit any significant interest in actual clinical practice. More recent machine learning approaches such as the Psychiatric Encephalography Evaluation Registry (PEER) technology and other efforts analyze large datasets to develop variables that may best predict response rather than test a priori hypotheses. PEER is a technology that may go beyond predicting response to a particular antidepressant and help to guide pharmacotherapy.

Keywords: electroencephalography, quantitative EEG, biomarkers, depression, machine learning, PEER

OPEN ACCESS

Edited by:
Frank M. Schmidt, Universitätsklinikum Leipzig, Germany

Reviewed by:
Philippe Jawinski, Humboldt-Universität zu Berlin, Germany
Natalia Jaworska, Univeristy of Ottawa, Canada

***Correspondence:**
Mark J. Schiller
drschiller@mindtherapyclinic.com

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *03 October 2018*
Accepted: *27 December 2018*
Published: *23 January 2019*

Citation:
Schiller MJ (2019) Quantitative Electroencephalography in Guiding Treatment of Major Depression. Front. Psychiatry 9:779. doi: 10.3389/fpsyt.2018.00779

INTRODUCTION

Psychiatry largely remains unique in the field of medicine in the lack of physiologically based diagnostic tools to diagnose a specific disorder. There are no objective physiology-based tests in psychiatry equivalent to those commonly used in other areas of medicine such as x-ray, ultra sound, or blood tests. There is also no physiological test to guide treatment, comparable, for example, to assaying malignant breast tissue for the presence of estrogen receptors to support treatment with tamoxifen.

This lack of physiological tools to diagnose and guide treatment is of particular concern in the treatment of Major Depressive Disorder (MDD)—a leading cause of disability worldwide. The 2016 National Survey on Drug Use and Health found that 16.2 million adults in the United States had experienced at least one major depressive episode. This number represented 6.7% of all U.S. adults (1). Worldwide the WHO estimated that 4.4% of the population suffered with depression (2). The financial costs of depression are tremendous with the global costs per year of depression and anxiety estimated to be $1.15 trillion (3).

The financial and personal burdens of depression could be reduced by more effective treatment. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, a very large, NIMH-funded study of depression treatment algorithms, reported remission rates of 36.8% in Step 1 which dropped to 13% by Step 4 (4). However, a variety of factors, including lack of consideration of dropout rates, may have actually inflated these relatively poor STAR*D outcomes (5).

Identifying effective biomarkers to support more effective treatment of depression is extremely important. There has long been interest in the potential for electroencephalography to develop clinically useful biomarkers given the relatively low cost and widespread availability of the technology. Indeed, there have been efforts to identify EEG biomarkers for depression for over four decades.

There are already excellent, comprehensive reviews of this literature (6–8). While some of the reviewed studies examine Event-Related Potentials, the majority of the studies utilized resting state EEGs, evaluating both pre and post-treatment changes and the differences between antidepressant treatment responders and non-responders. These studies identified a number of interesting findings including alpha band changes, theta QEEG cordance, and EEG source localization. More recently, machine learning approaches to identify the most relevant potential biomarkers have shown promise. This paper will first present a few of the most significant studies related to EEG biomarkers in depression and contrast this with a potentially more useful approach utilizing machine learning.

ALPHA BAND ACTIVITY

Alpha waves have a frequency of ∼8–12 Hz, varying slightly based on different definitions. They are generally thought to reflect a relaxed state and are more prominent with closed eyes. A number of studies have generally found elevated alpha power in depressed patients though others found decreased frontal alpha power in comparison to controls and others didn't find any correlations at all (8–10). Some studies found a correlation between alpha excess occipitally and on the right side and antidepressant response (11) though a later study failed to replicate these asymmetry findings (12). This latter study had examined alpha differences between responders and non-responders to treatment with SSRIs and dual-action SSRI/SNRI antidepressants and found that a classifier based upon the median alpha for healthy controls demonstrated good positive predictive value (93.3) and specificity (92.3). However, sensitivity was low (50%) so that half of responders had alpha below the control median and were not expected to be responders.

The studies looking at alpha band activity were hampered by being small, non-randomized treatment of different medications that were not designed to examine medication from non-medication treatment effects (7). However, a more recent study did at least address the small size and non-randomized medication treatment of prior studies in the large, 1,008 subject International Study to Predict Optimized Treatment of Depression (iSPOT-D) in which study subjects were randomized to treatment with either escitalopram, sertraline, or venlafaxine XR. One component of the study was analysis of the predictive effects of alpha band power (13). Neither occipital nor frontal alpha was associated with treatment outcomes at 8 weeks nor did patients and healthy controls differ in occipital or frontal alpha or alpha asymmetry. The study did find a sex difference with relatively greater right frontal alpha in women associated with a good response to the selective serotonin reuptake inhibitors of escitalopram and sertraline, while finding no such effect for the dual action selective serotonin and norepinephrine reuptake inhibitor venlafaxine XR.

THETA BAND ACTIVITY

Theta waves have a frequency from 4 to 8 Hz and have also been examined in relation to depression and medication response. Theta activity is related to the activity of the anterior cingulate cortex (ACC). Its affective division in the rostral ACC (rACC) has been found to play roles in assigning emotional valence to internal and external stimuli and emotional expression (14). In his meta-analysis, Pizzagalli found that 19 of 23 studies reviewed suggested that higher pretreatment rACC activity was associated with better treatment response. This link was demonstrated in a variety of different medication and biological treatments suggesting potential in differentiating responders from non-responders in general and not in guiding which particular treatment to use. Pizzagalli and his collaborators examined the role of theta activity in the rACC as part of the Establishing Moderators and Biosignatures of Antidepressant Response for Clinical Care (EMBARC) study in which 248 subjects with usable pretreatment EEG data were randomized to treatment with sertraline or placebo over 8 weeks (15). High rACC activity was found to be a significant predictor of lower Hamilton Rating Scale of Depression (HAM-D) scores at 8 weeks. Baseline rACC activity was responsible for 8.5% of the unique variance in outcome outside of that deriving from clinical and demographic covariates. There was no difference between treatment groups, so that rACC was found to be a general predictor of treatment response rather than a means to guide choice of treatment. This contrasts with the analysis of theta activity from the above-mentioned, large i-SPOTD study which found that both pretreatment high frontal and rACC theta activity were associated with treatment non-response (16). The authors hypothesized that their contrasting results to other studies demonstrating greater response with high rACC theta might be related to different medications studied, differences in degree of treatment resistance, and differences in electrode montages.

The role of theta activity has also been evaluated using cordance, which is a measure reflecting cortical perfusion using a formula that combines absolute and relative power (7). Cordance was investigated in a randomized, double-blinded, placebo controlled study of response to fluoxetine and venlafaxine (17). Decreased frontal theta cordance as measured 1 week after the start of medication treatment correlated with response to these antidepressants at 8 weeks a pattern not seen in placebo responders. Decrease in cordance to predict response and lack of decrease to predict non-response was found to have an accuracy of 72% with sensitivity 69%, specificity 75%, positive predictive value 75%, and negative predictive value 69%.

COMBINED QEEG PARAMETERS: ATR

The Antidepressant Treatment Response Index (ATR) is a measure that combines prefrontal theta and alpha power at baseline and Week 1 (7). Specifically it is a non-linear

combination of relative alpha and theta power, alpha power in high and low alpha bands, and change in alpha power from baseline to Week 1. ATR is then presented as a probability score from 0 (low probability) to 100 (high probability). In an initial study of ATR, 82 subjects with MDD were treated with either an SSRI or venlafaxine (18). 54.9% of subjects responded based on a ≥50% reduction in HAM-D scores. Retrospective analysis indicated that ATR predicted response with 70% accuracy [82% sensitivity, 54% specificity ($p = 0.001$)].

A second study examined ATR as part of the Biomarkers for Rapid Identification of Treatment Effectiveness in Major Depression (BRITE-MD) study (19). Subjects were treated prospectively with escitalopram 10 mg daily. Response was defined as a ≥50% reduction in HAM-D scores and remission was defined as a HAM-D score of ≤7 after 7 weeks of treatment. The 73 evaluable subjects had an overall response rate of 52.1% and a remission rate of 38.4%. ROC analysis was used to determine a threshold value to maximize classification of responders vs. non-responders. Using this, the ATR predicted response with 74% accuracy, 58% sensitivity, 91% specificity, 88% positive predictive accuracy, and 67% negative predictive accuracy and predicted remission with 74% accuracy, 61% sensitivity, 82% specificity, 68% positive predictive accuracy, and 77% negative predictive accuracy. Of a variety of markers of response investigated, only ATR ($p = 0.001$) and the change in HAM-D at Week 1 ($p = 0.034$) significantly predicted response. The latter measure, it should be noted, is certainly easier to obtain, though at the same time only ATR predicted remission ($p = 0.002$).

A further examination of ATR in a larger subset of the BRITE-MD study was based upon a randomization of subjects at Week 1 to either continued treatment with escitalopram, switch to bupropion XL 300 mg daily, or the combination of escitalopram and bupropion (20). Two hundred and twenty subjects were evaluated at Week 7. Overall the ATR showed 74% accuracy in predicting response and remission ($p = 0.001$ for both). Response rates of those with high ATR values compared to low values were 68 v. 28% ($p = 0.001$). Interestingly, those with low ATR values who were switched to bupropion were 1.9 times more likely to respond than those who remained on escitalopram (53 v. 28%, $p = 0.034$). This is important in demonstrating some potential to differentiate between antidepressant treatments albeit not until 1 week after the initiation of SSRI treatment. The ATR index did not prove useful in predicting response to combination treatment.

MACHINE LEARNING APPROACHES

The approaches taken above are all based upon a priori hypotheses of specific frequency band variables or combinations thereof. A different approach is to use intensive computational analyses of large datasets to derive predictive biomarkers, a method that has become increasingly common in biomedical research. One pilot study examined responders to SSRIs (noted to generally be sertraline hydrochloride) in 22 subjects with MDD (21). All but one had comorbid diagnoses, and all had failed at least two previous adequate trials of various antidepressants. Pre-treatment EEGs were collected after 10 days of medication withdrawal. Responders were defined as those having ≥30% reduction in HAM-D scores rather than the more common 50% bar. A machine learning prediction method was used to identify candidate features with a Fisher discriminant ratio being used to identify the most relevant features. The most relevant features were primarily found to be a variety of frontotemporal coherence measures in frequencies lying in the low beta frequency band (12–20 Hz). A multi-dimensional model demonstrated an average prediction rate of 87.9% (80.93% specificity and 94.86% sensitivity). There was considerable sex imbalance between responders and non-responders in this study with 6 of 11 females but only 1 of 11 males found to be responders. The model has not yet been tested on a larger dataset and provides no aid in predicting response to non-SSRI antidepressants. The potential for greater applicability would require examining larger datasets and assessing the predictive model for other antidepressant types.

Bailey et al. (22) also used machine learning techniques in attempting to identify responders from non-responders to rTMS treatment. Fifty subjects with treatment resistant MDD and 21 healthy controls had baseline QEEGs. Forty two MDD subjects also had EEG testing at Week 1 and at the end of 5–8 weeks of rTMS treatment. Responders showed lower Montgomery-Asberg Depression Rating Scale (MADRS) scores at Week 1 and endpoint ($p < 0.01$ for both) and lower Beck Depression Inventory (BDI) scores at Week 1 ($p = 0.03$) and endpoint ($p < 0.01$). In an a priori analysis, theta connectivity averaged across baseline and Week 1 was higher in responders than non-responders ($p = 0.0216$). Subsequently, 54 EEG features were chosen to design a predictive model using a linear support vector machine (SVM). The algorithm was evaluated using 5,000 repeats of five-fold cross validation. Using the resulting model, responders could be distinguished from non-responders with mean sensitivity of 0.84 ($p = 0.001$) and mean specificity of 0.89 ($p = 0.002$). However, the relative success of the easily collected MADRS and BDI makes one question the clinical utility of the more difficult to obtain EEG data. In addition, the study was limited by the low response rate of 12 of 50 subjects.

Mumtaz et al. (23) also developed a predictive model of response using a machine learning approach. Thirty four patients diagnosed with MDD were washed out of medications for 2 weeks and then had baseline and weekly EEGs during 6 weeks of treatment with an SSRI. Seventeen subjects were found to be responders based upon a >50% improvement in the BDI. The authors used a wavelet transform analysis to develop an EEG data matrix. This matrix dimensionality was reduced using rank based feature selection. Resulting training and testing led to the development of a logistic regression model which was then validated with 100 iterations of 10-fold cross validation. Frontal and temporal delta and theta frequency variables were found to be the most accurate predictors of response. The model's sensitivity was 95% (±4.3) with a specificity of 80% (±8.8). As in the other machine learning studies the potential for greater applicability would require examining larger datasets

and determining utility in predicting response to other classes of antidepressants.

META-ANALYSIS OF EEG PREDICTION OF TREATMENT RESPONSE

Widge et al. (24) sought to quantify the reliability of QEEG in predicting response to depression treatment in a recent meta-analysis. The authors analyzed articles of interest published between January 2000 and November 2017. Seventy six studies comprising 81 EEG features were identified that merited inclusion for descriptive analysis, while 53 studies comprising 57 EEG features reported sufficient information for inclusion in the meta-analysis. The studies were heterogeneous in degree of treatment resistance, inclusion/exclusion criteria, statistical analysis, EEG methodology, EEG feature studied, and treatment. 57/81 studies looked at medication response while 14/81 investigated rTMS treatment response. Studies tended be small. Quality measures were generally not met with 24 /36 studies testing multiple features failing to make statistical corrections for multiple comparisons and with only 6 of 71 biomarkers reported to have statistical predictive validity subjected to cross-validation. The meta-analysis found an overall sensitivity of 0.72 (95% CI = 0.67–0.76), specificity of 0.68 (95% CI = 0.63–0.73), and log (diagnostic odds ratio) 1.89 (95% CI = 1.56–2.21) indicating that predictive power was greater than chance, though without any difference between biomarkers or treatment type. Funnel analysis suggested a strong publication bias, while further analysis suggested that the predictive power was fueled by small studies with strong positive results. The authors stated that their "results do not imply QEEG findings were not real" but that greater rigor and replication of prior positive studies were necessary for QEEG to be a reliable tool ready for clinical practice. Of note, the meta-analysis did not include results from the EMBARC study, presumably as that study publication fell outside of the stated time parameters, though there was also a mention that insufficient information was provided for inclusion in a meta-analysis.

PEER

The Psychiatric Encephalography Evaluation Registry (PEER) previously known as referenced-EEG (rEEG) is another machine learning approach which is applied to a large dataset. Currently the PEER database comprises ∼11,000 baseline EEGs and 39,000 medication treatment outcome points (25). There are a number of characteristics of PEER that set it apart from other approaches that have been reviewed here. One is that PEER is not a diagnostic tool to predict response to treatment but a tool to guide medication selection. Medication guidance is not restricted strictly to antidepressant treatment; the technology provides indications for a broad range of psychiatric medications. Of note, PEER was specifically not included in the meta-analysis by Widge and his colleagues, as it is not a diagnostic test predicting treatment response but a tool to inform pharmacotherapy (24). PEER has been applied to a range of psychiatric diagnoses in addition to depression. This is based upon a primary assumption that patients with similar EEG biomarkers should respond to the same medications in a consistent manner regardless of diagnosis. This assumption was derived from the observation that psychiatric diagnoses are defined by symptoms rather than objective physiological measures. This contrasts with non-psychiatric medicine, where for example the presence of chest pain and other associated symptoms may point to the presence of a myocardial infarction, but its definition relies on the physiological definition of cardiac cell damage or death, and the diagnosis is demonstrated by physiological tests such as an electrocardiogram or cardiac enzyme assay. Thus, psychiatric patients within a diagnostic category may demonstrate phenomenological similarities but be physiologically heterogeneous and not responsive to the same medications. At the same time patients receiving different diagnoses may have important physiological similarities that would potentially respond to similar medications. A further unusual aspect of PEER is that a patient may simultaneously have different biomarkers that suggest different types of medications resulting in treatment with medication combinations. This seems consistent with general clinical practice in which multiple medications are often prescribed. The use of medication combinations complicates evaluating PEER in comparison to evaluating how well a single electrophysiological measure predicts response to a specific type of medication.

PEER Interactive, the term describing the report generator, provides a statistical analysis comparing electrophysiological abnormalities (identified by comparison to a normal database of subjects screened for psychiatric and neurological disorders) of a patient to abnormalities found in known responders to a medication in the PEER outcome database (25). PEER Interactive uses two response/non-response classifiers so a scatter plot can be utilized to best represent if a certain subject will be a responder or non-responder to specific medications in the PEER Interactive database (**Figure 1**). The first classifier (C1) is based upon "net hits" and the second classifier (C2) is based upon a logistical regression.

Responsiveness to classifiers for a specific medication can be presented graphically. The graph illustrates this for classifier I (C1) and classifier II (C2) for the population of either responders or non-responders to the medication. Each point represents: (score on C2, score on C1, response (blue), non-response (red).

Peer interactive uses machine learning techniques to develop combinatorial algorithms with the best predictive power from the full range of available QEEG variables. The individual medication models are tested through multiple cross validations which test the performance of the model against a set of cases not used to develop the model itself. The true positive (complement of type I error) and true negative (complement of type II error) are reported. A validation sample is developed by querying the outcomes database for medication responses not used in constructing the model. The validation sample is further subdivided into a tuning sample and the final validation sample. The tuning sample helps to refine the model by running the scoring model and comparing the score distribution to known responses. The final validation sample is used to further validate

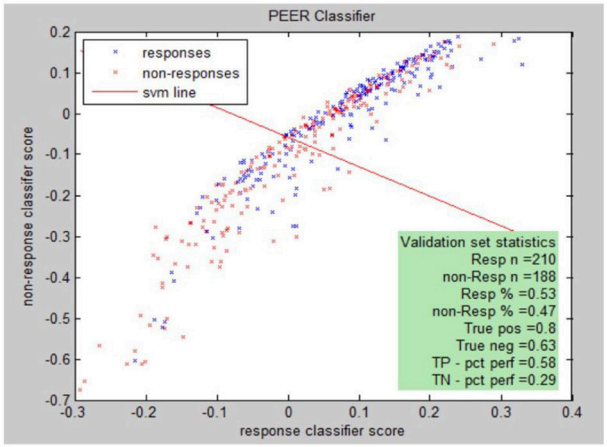

FIGURE 1 | PEER medication response scatter plot.

the model by running the model without any adjustment of parameters or thresholds. The model is ready for use if the results of the final validation meet the specifications of the previous clinical correlations. The specific machine learning algorithms are proprietary, though drug/class models are reported. In addition, the model is periodically refined as the PEER outcome database is expanded and additional medications are added to this database.

There have been four randomized, controlled trials of PEER technology or its earlier iteration of rEEG. Suffin and colleagues conducted a pilot study on 13 subjects with treatment-resistant major depression who were randomized to a usual care, control group based on the clinical decisions of treating psychiatrists in a naturalistic setting or the experimental EEG-guided medication group (26). Subjects and independent rating physicians were blinded to treatment group. After 6 weeks the decline in HAM-D scale and BDI scores in the experimental group were significantly greater ($p < 0.009$). A larger percentage of subjects in the experimental group had good to excellent outcomes compared to the control group based on Clinical Global Impression-Improvement scores ($p = 0.02$).

Debattista et al. (27) conducted a randomized, blinded, controlled pilot study of treatment-resistant depression in which subjects were treated based upon the Texas Medication Algorithm Project (TMAP) or guided by referenced-EEG. Eighteen subjects completed the pilot study. Outcomes were compared regardless of assigned treatment group based on whether TMAP and rEEG treatments were equivalent after 10 weeks. In comparing the 12 subjects with treatment consistent with the rEEG report vs. the 6 subjects with TMAP guided treatment inconsistent with the rEEG report there was a significantly greater mean change in Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) and Quick Inventory of Depressive Symptomatology (QIDS) scores ($p < 0.0094$ and $p < 0.0066$).

This pilot study helped refine the design of a larger, multicenter, randomized, controlled, single-blinded study in relatively treatment-resistant subjects with major depression (28). One hundred and fourteen subjects were randomized to rEEG-guided treatment or to a control group treated based upon a modified STAR*D protocol. Eighty two subjects completed the 12 week study. In a per protocol analysis, rEEG-guided subjects had significantly greater mean change improvements in QIDS-SR16 and Q-LES-Q scores ($p < 0.0002$ for both; **Table 1**). In addition, there was increasing separation on these measures between groups over the course of the study. The rEEG-guided group also showed superiority in 9 of 12 secondary endpoints.

TABLE 1 | Least square means.

	rEEG-guided	Control	*p*-value	90% CI
PER PROTOCOL				
QIDS-SR16	−6.8 (SE 0.35)	−4.5 (SE 0.38)	<0.0002	1.52 to 2.99
Q-LES-Q-SF	18.0 (SE 1.06)	8.9 (SE 1.14)	<0.0002	−11.21 to −6.81
MODIFIED ITT				
QIDS-SR16	−5.7 (SE 0.30)	−4.2 (SE 0.34)	<0.0002	0.84 to 2.17
Q-LES-Q-SF	14.1 (SE 0.92)	8.0 (SE 1.05)	<0.0002	−8.17 to −4.07

Adapted from DeBattista et al. (28).
Repeated measures, two tailed, mixed procedure with covariance structure including stratification and study sites in the model.

TABLE 2 | *P*-values obtained by ANOVA.

Endpoint	RF %change	RNF %change	Difference	*n*	*p*-value
QIDS-SR16	−30.0	−12	−18%	39	0.029
CHRT	−24	−14	−10.0%	150	0.002
PTSD	−9	−4	−5%	91	0.035
CGS	−23	−13	−10.0%	145	0.017
CGI-physician	−34	−22	−12%	150	0.002
CGI-patient	−40.0	−22	−18%	150	0.0001

Adapted from Iosifescu et al. (29).
QIDS-SR, Quick Inventory of Depressive Symptomatology Self-Report; CHRT, Concise Health Risk Tracking Scale; PTSD, Post-Traumatic Stress Disorder; CGS, Clinical Goal Setting; CGI, Clinical Global Impressions.

Iosifescu et al. (29) reported on the use of PEER Interactive in a study at two military hospitals (Walter Reed National Military Medical Center and Fort Belvoir Community Hospital). This was a prospective, randomized, controlled, single-blinded study of subjects with a primary depressive disorder diagnosis who were not required to be treatment-resistant. One hundred and fifty subjects were enrolled and were randomized to a control group treated per VA/DOD Clinical Guidelines or PEER-guided treatment over 6 months. A *post hoc* analysis analyzed subjects based upon the PEER report being followed (RF) or not being followed (RNF). The percentage reduction in QIDS-SR16 was 144% greater for the RF vs. RNF groups ($p = 0.029$). Reduction in suicidal ideation was 75% greater for the RF vs. the RNF group on the Concise Health Risk Tracking Scale—Self Report (CHRT-7SR) ($p = 0.0017$). There was also a 139% greater improvement in the PTSD Checklist Military/Civilian (PCL/MC) in the RF vs. RNF group ($p = 0.0348$; **Table 2**).

DISCUSSION

The studies reviewed here suggest that there are aspects of quantitative EEG that do correlate with response to pharmacologic treatment of depression. While these findings may be of interest in helping us to better investigate the biological underpinnings of depression and provide direction into future avenues of research, ultimately the important question is whether these potential biomarkers have utility in guiding treatment in actual clinical practice. In that the clinical marketplace has primarily indicated that the answer is no. Despite decades of interest in electrophysiological biomarkers, none of the single variable measures reviewed above has generally become accepted on a clinical basis. The combinatorial ATR measure has also been of research interest but has not made its way to general clinical use despite prior significant attempts to commercialize the technology. This lack of success may have resulted from inadequate marketing resources or other purely business factors but may also point to the medical community not finding sufficient value to change clinical practice. Testing to predict whether an antidepressant would ultimately be effective 1 week after already beginning treatment may not be a worthwhile cost without greater predictive accuracy since the important outcome—actual clinical response—will be available after a few more weeks of continued treatment.

Machine learning approaches, however, may prove key in bringing the use of EEG biomarkers from a state of research interest to clinical relevance. PEER technology in particular is being commercialized and is currently used in an expanding number of clinical settings, suggesting clinical value to the effectiveness so far demonstrated in the research projects reviewed. The commercialization model is that of a reference laboratory. Clinicians arrange QEEG testing following standard procedures with any available, local EEG equipment. The resulting data is electronically sent in a secure manner to be analyzed by PEER Interactive. Clinicians are then sent a PEER Report to inform their prescribing decisions in conjunction with clinical factors. In addition to the value of machine learning to derive the most useful predictive markers from large datasets, PEER technology has further advantages in being a pretreatment rather than mid-treatment test and in not being a predictor of response to a single medication or single medication class. Thus, it is a potential tool for broadly guiding effective psychiatric pharmacotherapy of depression. Because of the underlying assumptions, PEER technology is intended to be useful in disorders other than depression, which is the focus of this review. In addition to further research support for the effectiveness of this technology that would benefit from larger studies, further expansion of its current clinical use will be telling.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

The author's activities with MYnd Analytics, Inc. are supported by the company.

REFERENCES

1. National Institute of Mental Health. *Major Depression Among Adults*. Available online at: https://nimh.nih.gov/health/statistics/prevalence/major-depression-among-adults.shtml
2. Global Health Estimates 2016. *Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016*. Geneva: World Health Organization (2018).
3. Chisholm D, Sweeny K, Sheehan P, Rasmussen B, Smit F, Cuijpers P, et al. Scaling-up treatment of depression and anxiety: a global return on investment analysis. *Lancet Psychiatry* (2016) 3:415–24. doi: 10.1016/S2215-0366(16)30024-4
4. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* (2006) 163:1905–17. doi: 10.1176/appi.focus.12.2.146
5. Pigott HE, Leventhal AM, Alter GS, Boren JJ. Efficacy and effectiveness of antidepressants: current status of research. *Psychother Psychosom*. (2010) 79:267–79. doi: 10.1159/000318293
6. Iosifescu DV. Electroencephalography-derived biomarkers of antidepressant response. *Harv Rev Psychiatry* (2011) 19:144–54. doi: 10.3109/10673229.2011.586549
7. Wade EC, Iosifescu DV. Using electroencephalography for treatment guidance in major depressive disorder. *Biol Psychiatry Cogn Neurosci Neuroimaging* (2016) 1:411–22. doi: 10.1016
8. Olbrich S, van Dinteren R, Arns M. Personalized medicine: review and perspectives of promising baseline EEG biomarkers in major depressive disorder and attention deficit hyperactivity disorder. *Neuropsychobiology* (2015) 72:229–40. doi: 10.1159/000437435
9. Begić D, Popović-Knapić V, Grubišin, J, Kosanović-Rajačić B, Filipcic I, Telarović, et al. Quantitative electroencephalography in schizophrenia and depression. *Psychiatr Danub*. (2011) 23:355–62.
10. Knott V, Mahoney C, Kennedy S, Evans K. Pre-treatment EEG and it's relationship to depression severity and paroxetine treatment outcome. *Pharmacopsychiatry* (2000) 33:201–5. doi: 10.1055/s-2000-8356
11. Bruder, GE, Stewart J, Tenke C, McGrath PJ, Leite P, Bhattacharya N, et al. Electroencephalographic and perceptual asymmetry differences between responders and nonresponders to an SSRI antidepressant. *Biol Psychiatry* (2001) 49:416–25. 10.1016/S0006-3223(00)01016-7
12. Tenke C, Kayser J, Manna C, Fekri S, Kroppmann CJ, Schaller JD, et al. Current source density measures of electroencephalographic alpha predict antidepressant treatment response. *Biol Psychiatry* (2011) 70:388–94. 10.1016/j.biopsych.2011.02.016
13. Arns M, Bruder G, Hegerl U, Spooner C, Palmer DM, Etkin A, et al. EEG alpha asymmetry as a gender-specific predictor of outcome to acute treatment with different antidepressant medications in the randomized iSPOT-D study. *Clin Neurophysiol.* (2016) 127:509–19. doi: 10.1016/j.clinph.2015.05.032
14. Pizzagalli D. Frontocingulate dysfunction in depression: toward biomarkers of treatment response. *Neuropsychopharmacology* (2011) 36:183–206. doi: 10.1038/npp.2010.166
15. Pizzagalli D, Webb C, Dillon, D, Tenke, C, Kayser J, Goer F, et al. Pretreatment rostral anterior cingulate cortex theta activity in relation to symptom improvement in depression: a randomized clinical trial. *JAMA Psychiatry* (2018) 75:252. doi: 10.1001/jamapsychiatry.2018.0252
16. Arns M, Etkin M, Hegerl U, Williams L, DeBattista C. Frontal and rostral anterior cingulate (rACC) theta EEG in depression: implications for treatment outcome? *Eur Neuropsychopharmacol.* (2015) 25:7. doi: 10.1016/j.euroneuro.2015.03.007

17. Cook I, Leuchter A, Morgan M, Witte E, Stubbeman WF, Abrams M, et al. Early changes in prefrontal activity characterize clinical responders to antidepressants. *Neuropsychopharmacology* (2002) 27:120–31. doi: 10.1016/S0893-133X(02)00294-4
18. Iosifescu D, Greenwald S, Devlin P, Mischoulon D, Denninger JW, Alpert J, et al. Frontal EEG predictors of treatment outcome in major depressive disorder. *European* (2009) 19:772–7. doi: 10.1016/j.euroneuro.2009.06.001
19. Leuchter A, Cook I, Gilmer W, Marangell L, Burgoyne K, Howland RV, et al. Effectiveness of a quantitative electroencephalographic biomarker for predicting differential response or remission with escitalopram and bupropion in major depressive disorder. *Psychiatry Res.* (2009) 169:132–8. doi: 10.1016/j.psychres.2009.04.004
20. Leuchter A, Cook I, Marangell L, Gilmer W, Burgoyne K, Howland RH, et al. Comparative effectiveness of biomarkers and clinical indicators for predicting outcomes of SSRI treatment in Major Depressive Disorder: results of the BRITE-MD study. *Psychiatry Res.* (2009)169:124–31. doi: 10.1016/j.psychres.2009.06.004
21. Khodayari-Rostamabad A, Reilly J, Hasey G, Debruin H, Maccrimmon D. A machine learning approach using EEG data to predict response to SSRI treatment for major depressive disorder. *Clin Neurophysiol.* (2013) 124:10. doi: 10.1016/j.clinph.2013.04.010
22. Bailey NW, Hoy KE, Rogasch NC, Thomson RH, McQueen S, Elliot D, et al. Differentiating responders and non-responders of rTMS treatment for depression one week using resting EEG connectivity measures. *J Affect Disord.* (2019) 242: 68–79. doi: 10.1016/j.jad.2018.08.058
23. Mumtaz W, Xia L, Yasin MAM, Ali SSA, Malik AS. A wavelet-based technique to predict treatment outcome for major depressive disorder. *PLoS ONE* (2017) 12:2. doi: 10.1371/journal.pone.0171409
24. Widge AS, Bilge MT, Montana R, Chang W, Rodriguez CI, Deckersbach T, et al. Electroencephalographic biomarkers for treatment response prediction in major depressive illness: a meta-analysis. *Am J Psychiatry* (2018) 3:appiajp201817121358. doi: 10.1176/appi.ajp.2018.17121358
25. Carpenter G, Harbin HT, Smith RL, Hornberger J, David DB. A promising new strategy to improve treatment outcomes for patients with depression. *Popul Health Manag.* (2018) 29:101. doi: 10.1089/pop.2018.0101
26. Suffin, SC, Emory WH, Gutierrez G, Arora G, Mark S, Kling A. A QEEG database method for predicting pharmacotherapeutic outcome in refractory major depressive disorders. *J Am Physicians Surg.* (2007) 12:104–8.
27. Debattista C, Hoffman D, Schiller M. *Review of Current Results in the Use of Referenced-EEG in the Guidance of Psychotropic Medication Selection for Treatment-Resistant Patients*. National Clinical Drug Evaluation Unit (2009).
28. DeBattista C, Kinrys, G, Hoffman D, Goldstein C, Zajecka J, Kocsis, et al. The use of referenced-EEG (rEEG) in assisting medication selection for the treatment of depression. *J Psychiatr Res.* (2011) 45. 64–75. doi: 10.1016/j.jpsychires.2010.05.009
29. Iosifescu D, Neborsky R, Valuck RJ. The use of the Psychiatric Electroencephalography Evaluation Registry (PEER) to personalize pharmacotherapy. *Neuropsychiatr Dis Treat.* (2016) 12:2131–42. doi: 10.2147/NDT.S113712

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 07 February 2019
doi: 10.3389/fpsyt.2019.00030

Cytokine Research in Depression: Principles, Challenges, and Open Questions

Hubertus Himmerich[1,2]*, *Olivia Patsalos*[1], *Nicole Lichtblau*[3], *Mohammad A. A. Ibrahim*[4] *and Bethan Dalton*[1]

[1] *Department of Psychological Medicine, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom,* [2] *South London and Maudsley NHS Foundation Trust, London, United Kingdom,* [3] *Maidstone and Tunbridge Wells NHS Trust, Maidstone, United Kingdom,* [4] *Department of Clinical Immunological Medicine and Allergy, King's Health Partners, King's College Hospital, London, United Kingdom*

Cytokines have been implicated in the pathology of depression. Currently, the evidence is based on cross-sectional studies and meta-analytic research comparing blood concentrations of T helper type 1 (T_H1), T helper type 2 (T_H2), pro-inflammatory or anti-inflammatory cytokines of patients with a depressive disorder to those of healthy controls. Additionally, multiple longitudinal studies have investigated cytokine levels during antidepressant treatment. According to the current literature, it seems that peripheral levels of interleukin (IL)-6, IL-10, IL-12, IL-13, and tumor necrosis factor (TNF)-α are elevated and that interferon (IFN)-γ levels are lower in patients with depression compared to healthy controls. However, the overlap of cytokine values between acutely depressed patients, remitted and recovered patients and healthy controls is considerable. Thus, the discriminative power of cytokine concentrations between depressed and non-depressed people is likely weak. Treatment with certain antidepressants appears to decrease peripheral levels of IL-6, IL-10, and TNF-α. However, weight gain-inducing psychopharmacological substances, such as the antidepressant mirtazapine, have been reported to potentially increase the production of pro-inflammatory cytokines. Even though cytokines are often discussed as biomarkers for depression, they have also been shown to be altered in other psychiatric disorders. Moreover, many environmental, social, psychological, biological, and medical factors are also associated with cytokine changes. Thus, cytokine alterations seem extremely unspecific. The interpretation of the results of these studies remains a challenge because it is unknown which type of cells are most responsible for cytokine changes measured in the blood nor have the main target cells or target tissues been identified. The same cytokine can be produced by multiple cell types, and the same cell can produce various cytokines. Additionally, redundancy, synergy, antagonism, and signaling cascades of cytokine signaling must be considered. Cytokines might not be associated with the diagnosis of depression according to the currently used diagnostic manuals, but rather with specific subtypes of depression, or with depressive symptoms across different psychiatric diagnoses. Therefore, the currently available diagnostic systems may not be the ideal starting point for psychiatric cytokine research.

Keywords: cytokine, interleukin, interferon, tumor necrosis factor, depression

OPEN ACCESS

Edited by:
Martin Walter,
University of Tübingen, Germany

Reviewed by:
Eva Brandl,
Charité Medical University of Berlin, Germany
Carsten Culmsee,
University of Marburg, Germany

***Correspondence:**
Hubertus Himmerich
hubertus.himmerich@kcl.ac.uk

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *04 September 2018*
Accepted: *18 January 2019*
Published: *07 February 2019*

Citation:
Himmerich H, Patsalos O, Lichtblau N, Ibrahim MAA and Dalton B (2019) Cytokine Research in Depression: Principles, Challenges, and Open Questions. Front. Psychiatry 10:30. doi: 10.3389/fpsyt.2019.00030

INTRODUCTION

Aim of This Review Article

The aim of this narrative review is to explain the fundamentals, implications, challenges, and limitations of cytokine research in depression. This comprises of:

- a brief explanation of what cytokines are,
- a short illustration of the historical developments leading to where we currently are in psychiatric cytokine research,
- an explanation of the physiological fundamentals of cytokine signaling within the immune system and the brain,
- a summary of how cytokines have been linked to depression, its risk factors and antidepressant therapy,
- a critical perspective on the limitations that researchers currently face while interpreting findings of cytokine research in depression.

Several recent meta-analyses have already summarized data on peripheral cytokine alterations in depression [e.g., (1, 2)] and during antidepressant therapy [e.g., (2)]. Therefore, this article intends to help to explain and interpret such findings in the current scientific literature. Results of animal studies, *in vitro* studies and research on serum or plasma levels of cytokines in patients with other psychiatric disorders will be mentioned, as this is necessary to understand the advantages and limitations of cytokine research in depression.

Cytokines

The term "cytokine" is a compound word derived from the ancient Greek language. Its first component "κύτος" means "cell," and its second part "κίνησις" means "movement." Cytokines are a broad and loose category of secreted proteins that are important in cell signaling. This group of messenger molecules includes chemokines, interferons (IFN), interleukins (IL), lymphokines, and tumor necrosis factors (TNF). Cytokines are produced by immune cells such as macrophages, B lymphocytes, T lymphocytes, and mast cells which are mobile within the body, as well as parenchymal cells. Thus, cytokine production is not bound to a specific organ, but can happen everywhere in the human organism. Apart from immune cells, other cells that release cytokines include endothelial cells, fibroblasts, and epithelial and stromal cells within the body's periphery, and microglia and astrocytes in the brain (3–6).

Cytokines are distinct from hormones and neurotransmitters (7, 8) which are other important signaling molecules in the body. Hormones are measured in less variable concentrations in the blood circulation and are usually produced and secreted by specific cells within endocrine glands. Neurotransmitters generally transmit signals across a chemical synapse, such as a neuromuscular junction, from one nerve cell to another, or from a nerve cell to gland cell (9). Most cytokines act in their immediate micro-environment. A few exert a hormone-like effect by being released in to the blood to act on distant organs, e.g., the cytokine mediators of the acute phase response, IL-1, IL-6, and TNF-α (10).

For further information about the differences and similarities between cytokines, hormones and neurotransmitters see **Table 1**.

Historical Background of the Discovery of Cytokines and Their Importance for the Brain

From the 1950s to the 1980s, the first cytokines and their functions were discovered. Among those important cytokines were IFN-α (12), IFN-γ (13), the macrophage migration inhibitory factor (MIF) (14, 15), and TNF-α (16). Shortly after the discovery of the first cytokine, IFN-α, it became clear that cytokines from the body's periphery can influence inflammatory processes in the brain (17, 18), that cytokines can be produced in the brain (18), and that immune cells are not the only cells that release cytokines (19). In addition to their role in steering the immune system to defend the body from pathogens (12) and tumors (16), their modifying effect on neurotransmission was discovered (20, 21). In the 1970s, scientists started to understand that cytokines act via specific cytokine receptors on the surface of cells (22).

The first chemical analyses to measure cytokines were complicated by the low concentrations of cytokines in serum, plasma and tissue. For example, cytokines like TNF-α usually circulate in picomolar concentrations in the serum [e.g., (23)]. In contrast, classic hormones, for example cortisol, circulate in nanomolar concentrations [e.g., (24)]. The lack of assay systems was overcome by monoclonal antibody technology and the invention of the radio-immuno-assay (RIA) (25) and the enzyme-linked immunosorbant assays (ELISA) (26, 27). The RIA and ELISA allowed a highly sensitive measurement of cytokine concentrations. Further technical advancements have led to an abundance of methods to measure cytokines including bioassays, protein microarrays, high-performance liquid chromatography (HPLC), sandwich enzyme-linked immunosorbent assay (ELISA), Meso Scale Discovery (MSD) electrochemiluminescence and bead-based multiplex immunoassays (MIA) (28).

In the 1970s and 1980s, the first recombinant DNA molecules were generated (29) which allowed molecular cloning of a gene and the development of organisms that produce a protein product on the basis of such a cloned gene. Gene cloning allowed for the production of large amounts of recombinant cytokines (30).

Difficulties of Cytokine Research in Psychiatry

Cytokine research in psychiatry faces several difficulties, such as conflicting results and high variance of cytokine values within samples. Most of the studies measure serum or plasma cytokine levels, but it has often been discussed whether these results reflect the situation in the brain (31).

Cytokine research in affective disorders reflects these difficulties in an exemplary way. Even though most studies have found, for example, elevated levels of TNF-α in the serum or plasma of depressed patients (32, 33), such positive results have not been obtained by all studies, and heterogeneity of the results between studies is large (2). Results of cytokine research which are partly contradicting and often difficult to interpret have also been obtained in post-traumatic stress disorder (PTSD) (34–36)

TABLE 1 | Synopsis of similarities and differences in characteristics of cytokines, hormones and neurotransmitters (7–9, 11).

	Cytokines	Hormones	Neurotransmitters
Chemical characteristics	• Secreted proteins	• Derivatives of cholesterol • Derivatives of amino acids • Peptides • Proteins	• Gasotransmitters • Amino acids • Monoamines and trace amines • Peptides • Purines • Fatty acids • Acetylcholine
Cells of origin	• Immune cells • Endothelial & epithelial cells • Fibroblasts • Stromal cells • Microglia, Astrocytes	• Cells of endocrine glands	• Nerve cells
Target cells	• Immune cells • Nerve cells • Principally all somatic cells	• Cells of distant target organs • Principally all somatic cells	• Nerve cells • Muscle cells • Gland cells
Prototypical signaling way	• Autocrine • Paracrine • Endocrine	• Endocrine	• Synaptic
Concentration in the circulation	• Picomolar • Increase up to 1,000 times during trauma or infection	• Nanomolar • Little variation	• Spillover of neurotransmitters into the circulation only under certain circumstances
Receptors	• Transmembrane receptors linked to – JAK-STAT pathway – G-proteins – NFκB	• Transmembrane receptors linked to – G-proteins – Enzymes • Intracellular receptors linked to – DNA promotors	• Transmembrane ionotropic receptors: – Neurotransmitter-gated ion channel • Transmembrane metabotropic receptors linked to – G-proteins

and eating disorders (37). In order to understand the challenges and difficulties of cytokine research in psychiatry, one has to consider several characteristics of cytokine signaling and their role within the immune system. Therefore, the next sections will explain these mechanisms.

Methods of This Review Article

This is a narrative review; therefore, we did not apply strict selection criteria for the inclusion of certain articles. We rather selected articles based on how comprehensive, innovative, and clearly written they were and how much information they provided for an in-depth understanding and a critical debate of the topic. We included not only original research, but also reviews, book chapters and case reports. Regarding the section on cytokine alterations in depression and other psychiatric disorders, we strictly included only the latest meta-analysis on cytokine levels for each diagnosis in the text and in **Table 2**.

CHARACTERISTICS OF CYTOKINE SIGNALING

Cytokine Release

Cytokines mediate and regulate the immune system. Their secretion is brief and self-limited.

There are three main types of cytokine signaling: autocrine, paracrine and endocrine (7, 8, 41). An example for autocrine signaling is a T-helper type 2 (T_H2) cell which can stimulate its own growth by producing IL-4 (42). The way in which T_H2 cells can also stimulate nearby B lymphocytes by releasing IL-4 is an example of paracrine signaling (43) and endocrine signaling can be illustrated by TNF-α which is produced by macrophages in the adipose tissue and secreted into circulation. This pro-inflammatory signal can contribute to inflammatory processes within the artery walls and eventually lead to arteriosclerosis (44). In the cases of trauma, infection, stress, neoplasia, and inflammation, the body activates a complex systemic early-defense system. This process is called acute phase response which is a specialized systemic innate immune response in which the cytokines IL-1, IL-6, and TNF-α act like hormones. They are released by innate immune cells at the sites of infection or inflammation and released into circulation to act on distant organs e.g., the liver, to mediate the release of acute phase reactants, e.g., C-reactive protein (CRP) (45).

The same cytokine can be produced by multiple cell types. For instance, TNF-α is released by white blood cells, the endothelium, fat cells and other cells (46). Furthermore, one single cell can produce different cytokines. For example, T_H2 cells can produce IL-3, IL-4, IL-5, IL-6, and IL-13 (47).

TABLE 2 | Summary of cytokine blood concentrations in the context of psychiatric disorders according to relevant meta-analyses (2, 36–40).

Diagnoses	Cytokines										References
	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12	IFN-γ	TNF-α	TGF-β	
AFFECTIVE DISORDERS											
Depression	↔	↔	↔	↑	↔	↑	↑	↓	↑	↔	Köhler et al. (2)
BAD	↑	↔	↑	↔	↔	↑		↔	↑		Modabbernia et al. (39)
- *Manic*				↑		↔			↑		Goldsmith et al. (38)
- *Depressed*			↑	↔		↔			↔		Goldsmith et al. (38)
- *Euthymic*		↑				↑		↑	↑		Goldsmith et al. (38)
SCHIZOPHRENIA											
First episode	↑	↔		↑	↑	↑	↑	↑		↑	Goldsmith et al. (38)
Acute relapse	↑	↔	↓	↑	↑	↓	↑	↑	↑	↑	Goldsmith et al. (38)
TRAUMA- AND STRESSOR-RELATED DISORDERS											
PTSD	↑	↔	↔	↑	↔	↔		↑	↔		Passos et al. (36)
OBSESSIVE-COMPULSIVE DISORDERS											
OCD	↓			↔					↔		Gray and Bloch (40)
EATING DISORDERS											
AN	↔			↑					↑	↔	Dalton et al. (37)
BN				↔					↔		Dalton et al. (37)

↑, increase; ↔, no difference; ↓, decrease in cytokine levels; IL, interleukin; TNF-α, tumor necrosis factor; TGF, transforming growth factor; BAD, bipolar affective disorder; PTSD, post-traumatic stress disorder; OCD, obsessive-compulsive disorder; AN, anorexia nervosa; BN, bulimia nervosa.

Cytokine Effects

One cytokine can act on multiple cell types and have different effects on these cells (48). For example, IL-4 can induce activation of B lymphocytes but inhibit T helper type 1 (T_H1) cells (49). It can further lead to differentiation of cytotoxic T cell precursors and proliferation in mast cells (50). Regarding TNF-α, all cell types of the body express TNF-α receptor type 1 (TNF-R1) (51). When bound to TNF-α, these TNF receptors transduce growth regulatory signals. TNF-α is able to initiate apoptosis in some cells causing DNA fragmentation and cytolysis, but also cell growth and differentiation in others. Whether TNF-α induces cell differentiation or apoptosis depends on the signaling pathway activated within the cell. The NF-κB signaling pathway will lead to cell differentiation, whereas cells in which caspases are activated are more likely to undergo apoptosis in response to TNF-α (24). IL-10 can be inhibitory to macrophages and T_H1 cells, yet activating for T_H2 cells and B cells and can thus be immunosuppressive as well as immunostimulatory (52). The phenomenon that one cytokine can have diverse effects on different cells is called pleiotropy.

Cytokine signaling shows redundancy, because two or more cytokines can have a similar function. IL-2 and IL-4 both enhance T cell proliferation (53). All three IFNs, IFN-α, IFN-β, and IFN-γ, increase the activity of natural killer lymphocytes and stimulate the synthesis of arachidonic acid products (54). IFN-γ, IL-2, and TNF-α promote cellular immunity and the activation of cytotoxic cell contacts (55).

Another typical phenomenon in cytokine signaling is synergy which is a strong combined effect of 2 cytokines when acting together e.g., IL-3 and IL-4 amplify each other to induce growth, differentiation, and activation of mast cells in a synergistic way (47). Cytokines can also antagonize each other's effects. For example, IL-12 which activates T_H1 cells can be blocked by IL-4 (56–58). Another example of cytokine antagonism is that cytokines of the IL-1 superfamily can antagonize IL-18 effects (59). Additionally, cytokine cascades play a significant role in cytokine signaling which means that an activation of one cytokine produced by one cell type induces cytokine production by other cell types. For example, IL-4 induces the expression of IL-3, IL-5, and IL-13 (60).

CYTOKINES WITHIN THE IMMUNE SYSTEM

The Role of Cytokines Possibly Relevant for Research in Affective Disorders Within the Immune System

Many cytokines with vital roles for the regulation of the immune system have been investigated in affective disorders. Examples of such immunologically important cytokines measured in the serum of patients with depression are IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN-α, IFN-γ, TNF-α, and transforming growth factor (TGF)-β (2, 33, 61–64). Other cytokines with immunologically important functions are IL-21 (65) and IL-22 (66), though both of which have only recently gained the interest of the depression research field. IL-21 has been studied regarding its role in response to treatment with the antipsychotic aripiprazole (67), which can also be used as an augmentation strategy in treatment-resistant depression (68), and IL-22 production has been shown to increase during exposure to antidepressants like citalopram or mirtazapine (69).

Figure 1 is an attempt to create a simplified schematic figure that depicts the role of these cytokines within the immune system. This figure tries to bring some order to the different cytokines measured in psychiatric research, which are often discussed without explaining the immunological context of these molecules.

The immune system is divided into the innate and the adaptive immune response. The innate immune system can fight pathogens without a previous contact with them. The tasks of the innate immune system are performed by astrocytes (A), microglia (MG), dendritic cells (DC), granulocytes (G), natural killer cells (NKZ), and macrophages (MΦ) (upper part of **Figure 1**). These cells modulate the further immune response by the production of cytokines like IL-1, IL-4, IL-6, IL-12, and TNF-α.

To understand the adaptive immune system, we will assume two different scenarios that lead either to specific cytotoxic cell contacts or the production of specific antibodies (see left middle and lower part of **Figure 1**). One scenario within the immune system could be a viral infection. This can lead to the production of IL-12 by cells of the innate immune system which activates T_H1 cells. Consequently, they produce IFN-γ, IL-2, and TNF-α which, in turn, activate cytotoxic T cells. Cytotoxic T cells can destroy the virus-infected cells (70). Another potential scenario would be that cells of the innate immune system release IL-4 following a bacterial infection. IL-4 stimulates $T_H2$cells. T_H2 cells, in turn, will produce IL-4, IL-5 and IL-13 and thereby induce the production of antibodies which can help to mark and eliminate the pathogens (70).

If the cells of the innate immune system produce IL-1, IL-6 or TNF-α, the consequence is an activation of the so-called T helper 17 (T_H17) cells which produce cytokines like IL-17, IL-21, and IL-22. Together with IL-1, IL-6, IL-8, TNF-α and IFN-α, IL-17, IL-21, and IL-22 are cytokines which promote inflammation. Therefore, they are called pro-inflammatory cytokines (71, 72). Inflammatory processes, in turn, are bidirectionally linked to cytotoxic and antibody-driven mechanisms. An anti-inflammatory effect, in contrast, occurs when naïve T helper (T_H0) cells produce TGF-β. TGF-β activates regulatory T (T_{reg}) cells which produce IL-10 and further TGF-β. These cytokines have an anti-inflammatory effect (73) (see right middle and lower part of **Figure 1**).

Types of Cytokines According to Their Immunological Function

With this knowledge, we can distinguish between four categories of cytokines which are often talked about in the psychoimmunological literature:

- T_H1 cytokines (IL-2, IL-12, IFN-γ) which fuel the T_H1 branch of the immune system and lead to cytotoxic cell contacts.
- T_H2 cytokines (IL-4, IL-5, IL-13) which stimulate the T_H2 branch of the immune system and induce the production of antibodies.
- Pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-17, IL-21, IL-22, IFN-α, TNF-α) which promote inflammation.
- Anti-inflammatory cytokines (IL-10, TGF-β) which are influenced by regulatory T cells and prevent inflammatory processes from escalating.

Even though these categories are often referred to in the psychoimmunological literature, these are neither distinct nor generally accepted categories for the classification of cytokines. However, this functional classification might help to understand recent research papers on pro-inflammatory, anti-inflammatory, T_H1 and T_H2 cytokines in depression [e.g., (74–77)]. One must also take into account that cytokines can have different effects on different cells and thus may have pro- but also anti-inflammatory properties. Moreover, cytokines produced by certain T helper cells, for example IL-13 which is produced by T_H2 cells, have anti-inflammatory properties (78). Thus, they can be T_H2 as well as anti-inflammatory cytokines. Even though IFN-α has been listed in this figure as a pro-inflammatory cytokine, IFN-α also has several anti-inflammatory properties (79).

THE INFLUENCE OF CYTOKINES ON THE BRAIN

How Cytokines Enter the Brain

As previously mentioned, cytokines can be produced by neurons, astrocytes and microglia within the brain (see **Figure 1**). Additionally, peripherally produced cytokines can access and affect the brain through three pathways: humoral, neural, and cellular (31).

The humoral pathway describes when cytokines access the brain through leaky sections of the blood-brain barrier such as the choroid plexus and the circumventricular organs. The neural pathway involves the stimulation of primary afferent nerve fibers in the vagus nerve by pro-inflammatory cytokines. The cellular pathway describes how pro-inflammatory cytokines stimulate microglia to produce monocyte chemoattractant protein-1 (MCP-1), which subsequently recruits monocytes to the meninges and brain parenchyma (31, 80).

Influence on Neurotransmitter Metabolism and Signaling

Given that peripheral production of cytokines has been associated with depression (2, 81), and that peripherally produced cytokines can influence the central nervous system (CNS), the impact of cytokines on neurotransmitters such as serotonin has been studied extensively. For example, animal (82–84) and clinical (85, 86) studies have found an altered metabolism of serotonin resulting from cytokine exposure. Cytokines can decrease serotonin synthesis by activating the enzyme indoleamine 2,3 dioxygenase (IDO) which breaks down tryptophan, the amino acid precursor of serotonin, to kynurenin (KYN) instead of metabolizing tryptophan to serotonin. This process of serotonin depletion has been postulated to be associated with major depression (87, 88). Cytokines also influence the synthesis of monoamines through disruption of tetrahydrobiopterin, an essential enzyme co-factor to hydroxylases involved in monoamine synthesis. Furthermore, they can modulate serotonin signaling

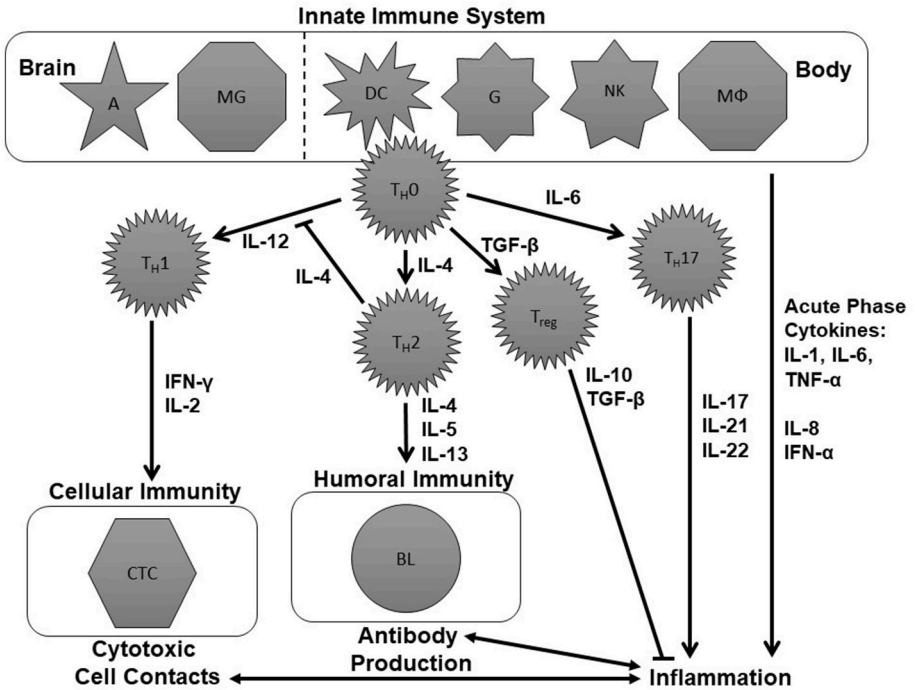

FIGURE 1 | Schematic figure of the immune system. This is an over-simplified representation of the immune system, deliberately so, to focus on aspects that have already been investigated in the immune-psychiatry literature. The tasks of the innate immune system are performed by astrocytes (A), microglia (MG), dendritic cells (DC), naïve granulocytes (G), natural killer cells (NK), and macrophages (MΦ). These cells modulate the further immune response involving naïve T-helper (T_H0), T helper 1 (T_H1), T helper 2 (T_H2), and T helper 17 (T_H17) cells, regulatory T cells (T_{reg}) as well as cytotoxic T cells and B lymphocytes (BL) by the production of cytokines including interleukins (IL), interferons (IFN), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β). For further details see text.

by increasing the expression and function of monoamine transporters which perform the re-uptake of synaptic serotonin (89–92). Moreover, pro-inflammatory cytokines can affect the release of neurotransmitters (93). For example, glutamate release by astrocytes has been shown to be affected by cytokines, potentially leading to excitotoxicity (94, 95). In addition, pro-inflammatory cytokines have been implicated in the stimulation of N-methyl-D-aspartate (NMDA) receptors, and the inhibition of γ-aminobutyric acid (GABA) and acetylcholine signaling (96).

Furthermore, cytokines are involved in autoimmunity, including the production of autoantibodies. For example, autoantibodies against dopamine-2 receptors have been found to contribute to the development of pediatric neuropsychiatric disorders associated with streptococcal infection and subjects with Tourette's syndrome (97). Autoantibodies against serotonergic and cholinergic receptors are thought to induce depressive syndromes (98).

Influence on Neuroendocrine Signaling

Cytokines can also affect neuroendocrine function by increasing hypothalamic-pituitary-adrenal (HPA) axis activity. Acute cytokine administration has been shown to increase corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol release, all of which have been found to be elevated in patients with major depression (99–101). In contrast, chronic cytokine administration is associated with a flattening of the diurnal cortisol curve, which has been linked to adverse behavioral effects and poor outcomes in several illnesses such as cardiovascular disorders and cancer (102–104). Inflammatory cytokines are hypothesized to exert these effects through the disruption of the cortisol receptor's (glucocorticoid receptor) expression and function (101).

Influence on Neurogenesis and Autoimmune Destruction of Nerve Cells

Neurogenesis is another aspect of brain activity influenced by cytokines. As mentioned previously, certain pro-inflammatory cytokines stimulate glutamate release by astrocytes. Glutamate can have a detrimental effect on neurogenesis by binding to extra-synaptic NMDA receptors, leading to a decrease in brain-derived neurotrophic factor (BDNF), which is pivotal for neurogenesis (105). Additionally, an activation of TNF-α signaling has been suggested to contribute to the destruction of hypocretin neurons in patients with narcolepsy (23, 106).

Influence on Specific Brain Regions

Cytokines' effects on specific brain regions have been demonstrated through imaging techniques. Among those brain regions influenced by cytokines are the basal ganglia, which are involved in motor activity and motivation, the dorsal anterior cingulate cortex (ACC), which is a central area for the generation of anxiety, and the subgenual ACC, which has

been reported to be involved in the development of depression (107, 108).

Positron emission tomography (PET) studies have revealed that the application of IFN-α leads to increases in basal ganglia glucose metabolism (109, 110). This is of specific interest for cytokine research in depression because depression and fatigue are the main side effects of treatment with IFN-α (111, 112). IFN-α has also been implicated in increased ACC activation as evidenced by fMRI studies (113, 114), and such ACC activation is associated with increased anxiety, arousal, obsessive-compulsive disorder and bipolar affective disorder (108).

CYTOKINE ALTERATIONS IN DEPRESSION AND OTHER PSYCHIATRIC DISORDERS

Cytokine Alterations in Patients With Affective Disorders

Regarding the main cytokines examined in affective disorders, meta-analytic research has revealed that patients with depression have elevated serum or plasma concentrations of IL-6, IL-10, IL-12, IL-13, IL-18, TNF-α, and its receptor soluble TNFR2 compared to healthy controls, whereas IFN-γ levels are lower in patients with depression than healthy controls (2, 33, 61–63). Similarly, production of IL-6, IL-10, and TNF-α levels have been shown to be increased in animal models for depression using acute or restraint stress, whereas IFN-γ production was significantly decreased by restraint stress in rats (115). In bipolar disorder, Munkholm et al. (116) found significantly higher serum or plasma levels of TNF-α, its receptor soluble TNFR1 and IL-4. However, these cytokines have not been found to be elevated by all studies in patients with depression and bipolar patients (2, 116).

Cytokine Alterations in Patients With Psychiatric Disorders in General

The aforementioned cytokine alterations in affective disorders are not specific to major depression or bipolar disorder. For example, elevated levels of TNF-α have also been found in PTSD studies (34), in patients with anorexia nervosa (37) and in patients with acute relapse of schizophrenia (31, 38). An overview of meta-analytic studies on cytokine research in selected psychiatric disorders is shown in **Table 2**.

If we take a closer look at the raw data of specific studies, we see high variation in cytokine levels within groups of patients or healthy controls. For example, in a paper published by Schmidt et al. (33), mean TNF-α serum concentrations of depressed patients were 50.35 pg/ml with a standard deviation of 78.01 pg/ml, whereas mean TNF-α levels of healthy controls were 33.80 ± 46.17 pg/ml. This range indicates that, despite the significant difference between the means of both groups, depressed participants and healthy controls did not have distinct TNF-α levels, but rather overlapping ranges of TNF-α levels.

POSSIBLE REASONS FOR CYTOKINE ALTERATIONS

Direction of Causality

Even though there is considerable evidence for an involvement of cytokines in the pathophysiology of many psychiatric disorders, the directionality of this relationship has not yet been elucidated with certainty. However, evidence is increasing that inflammation and specifically cytokine signaling play a role in the pathophysiology of psychiatric disorders. Such evidence derives from genetic studies, long-term cohort studies and from studies investigating people with inflammatory diseases. Nevertheless, cytokine changes might also be the consequence of a psychiatric disorder, for example they can be a consequence of treatment with psychopharmacological agents or of weight changes that appear during acute episodes of the disorder or during recovery (117).

There is considerable evidence that genetic risk factors for psychiatric disorders are closely related to cytokines or other functions of the immune system. Genome-wide association studies (GWAS) have identified immune pathway genes that significantly contribute to the risk of psychiatric disorders (118). Psychiatrically relevant genes include, for example, those in the human leukocyte antigen (HLA) gene complex or rare copy number variants important for immune function (119).

The results of the Whitehall II cohort study in which British civil workers were monitored for CRP, IL-6 and cognitive symptoms of depression from 1991 to 2004 (baseline and follow-up) suggest that the inflammatory markers measured predicted symptoms of depression at follow-up, but not the other way around (120).

Studies have shown that CNS inflammation caused by infection can lead to the development of psychiatric symptoms (121) or full-blown syndromes such as depressive or manic episodes (122). However, it must be mentioned that the observation that inflammatory diseases can lead to psychiatric disorders, such as depression, is not an achievement of recent decades of research: a meta-analytic scientific approach to such observations had already been published by Emil Kraepelin in 1881 and 1882 (123).

Risk Factors for Psychiatric Disorders Associated With Changes in Cytokine Production

If we consider the risk factors of depressive disorder, we will find familial and developmental risk factors, factors related to the natural and social environment, psychological and medical risk factors, as well as molecular factors related to genetics, epigenetics, gene expression and the brain, and the endocrine and the immune system (124–126). **Table 3** provides evidence from the scientific literature that all of these risk factors for depression have been shown or suggested to be associated with alterations in cytokine production or cytokine signaling. Thus, the cytokine system seems to be involved in almost all possible predisposing or precipitating risk factors for depression that contribute to the presentation of the disorder. A risk factor

TABLE 3 | Possible risk factors for the development of depression which have also been linked to alterations in cytokine production and signaling.

Adolescent age which goes along with
- Neurodevelopmental changes (127)
- Hormonal changes (127)

Environmental factors
- Air pollution (128, 129)

Social risk factors
- Poverty (129)
- Low socioeconomic status disadvantage (130)
- Unemployment (131)

Oxidative stress (132)

Nutrition and the gut microbiome
- Vitamin deficiency, e.g., vitamin D deficiency (133, 134)
- ω3/ω6 fatty acids ratio (135)
- Changes in the gut microbiome (136, 137)

Psychological risk factors
- Poor attachment in childhood and adolescence (130)
- Stress (138–140)
- Bereavement (141)
- Loneliness (142)
- Psychological Trauma or PTSD (34, 35, 129)
- Critical life events
- Predisposing temperament and personality traits (143–145)
- Subclinical depression (142)

Family history of depression (130)

Psychiatric disorders
- Anxiety disorders (146, 147)
- Substance abuse disorders (148, 149)
- Post-traumatic stress disorder (34–36)

Brain diseases
- Stroke (150)
- Epilepsy (151, 152)
- Parkinson's disease (153, 154)
- Multiple Sclerosis (155)

Physical diseases
- Infectious diseases (62, 156, 157)
- Autoimmune diseases (158, 159)
- Endocrine and hormonal diseases (160)
- Cancer (161)
- Obesity (162, 163)
- Diabetes (164)
- Myocardial infarction (165)
- Physical trauma (166)

Therapies for physical diseases
- Medications (167)
- Chemotherapy (168)
- Surgery (169)
- Transplantation (170)

Disturbed sleep-wake-rhythm
- Disturbed sleep (171)
- Disturbed wakefulness (172)

Hormonal changes
- Pregnancy (173)
- Birth (174)
- Menopause (175)

Functional and structural changes in brain areas:
- Hippocampus (128, 176)
- Amygdala (177)
- Prefrontal cortex (178)

(Continued)

TABLE 3 | Continued

Genetic risk factors
- Susceptibility and candidate genes (179)
- Gene expression (140)
- Epigenetics (180)

Neurochemical risk factors in
- Neurotransmitter systems (31, 81, 88, 91)
- Endocrine systems (99–101)
- Immune system (181)

The cited literature refers to articles that report, review, or hypothesize an association of the risk factor in question with changes in cytokine production or cytokine signaling.

like acute or chronic stress could, for example, lead to an increased production of pro-inflammatory cytokines (115, 138–140), and these cytokines could then enter the brain (31) and lead to changes in neurotransmitter systems involved in the development of depression, such as the serotonin system (82, 83, 85, 86, 182).

Confounding Factors

There are a number of confounding factors that influence cytokine serum or plasma levels. Important confounders include aging, body weight, smoking, excessive alcohol consumption, and medication (183).

Normal aging is marked by chronic low-level inflammation characterized by over-expression of circulating pro-inflammatory factors (184, 185). Chronic inflammation with increased circulating levels of cytokines is also characteristic of obesity (162, 186, 187). Adipose tissue is known to accumulate and activate macrophages and lymphocytes which secrete inflammatory factors (186, 187). Both the obese and the elderly exhibit behavioral symptoms such as depression and cognitive dysfunction at an increased rate compared to the general population. Several studies have shown that the elevated levels of inflammation may contribute to the prevalence of neuropsychiatric disorders in these populations (188–192).

Smoking (193), excessive alcohol consumption (148), and drug abuse (149) have been associated with inflammatory changes in the immune system, as reflected by an increase of pro-inflammatory cytokines. Also, psychopharmacological medication, specifically those agents which lead to weight gain, have been reported to activate cytokine production (117, 194). It is not clear, however, whether the activation of cytokines by psychotropic drugs is the cause or a consequence of weight gain in the course of psychopharmacological treatment. As growing white adipose tissue becomes infiltrated by macrophages, this fatty tissue could be a major source of pro-inflammatory cytokines in the context of increasing body weight during psychopharmacological treatment (195).

One has to keep in mind, however, that alcohol misuse (148), obesity (163) are not only confounding factors, but also risk factors for the development of depression. Therefore, they should not be dismissed as mere confounders whilst performing research on causative factors of affective disorders.

Physical Disorders and Their Therapy

Many physical disorders have been reported to increase the risk for developing a depressive disorder (125) and to activate the production of pro-inflammatory cytokines. Examples are autoimmune and infectious diseases (156–159), endocrine and hormonal diseases (160), cancer (161), diabetes (164), myocardial infarction (165, 196), and physical trauma (166). The treatment of physical disorders, e.g., interferon-based or virostatic treatments for hepatitis C (167, 197), chemotherapy for the treatment of cancer (168), surgery (169) or transplantation (170), can lead to additional cytokine release.

Food and the Microbiome

Vitamin deficiency, e.g., vitamin D deficiency, has also been shown to increase pro-inflammatory cytokine production and to lead to depressive symptoms (133, 134). Another example of how food can influence the immune system is the anti-inflammatory effect of ω3 fatty acids, which can reduce the production of IL-1β, IL-6, and TNF-α. ω6 fatty acids, in contrast, promote the production of these pro-inflammatory cytokines. Therefore, foods with a high ratio of ω3/ω6 fatty acids can dampen inflammatory processes and thus potentially prevent or ameliorate depressive symptoms (135).

There is an increasing body of evidence for an influence of the gut microbiome on cytokine signaling and on the development of depression. Gut microbes have, for example, been found to be capable of producing certain neurotransmitters and of influencing the central neurochemistry of the brain and thus human behavior (136). Molecules produced by microbiota, e.g. LPS, can induce cytokine production (198), and lead to depressive symptoms (199). The microbiome is linked to food intake and diet. However, the relationship is complex (200).

CYTOKINE CHANGES DURING TREATMENT FOR DEPRESSION

The Influence of Antidepressants on Cytokine Production

Some studies have investigated whether cytokine serum or plasma concentrations *in vivo* change during treatment with antidepressants. However, the results are conflicting. For example, in a study by Kraus et al. (201), TNF-α levels were measured longitudinally during treatment with mirtazapine or venlafaxine. Whereas, mirtazapine induced a significant increase in the plasma levels of TNF-α and both soluble TNF receptors, venlafaxine did not alter plasma levels of TNF-α, or soluble TNF receptors significantly (201). These findings that mirtazapine increases circulating TNF-α levels were supported by Kast et al. (202). In contrast, however, Gupta et al. (203) found that successful treatment with mirtazapine led to a decrease in serum TNF-α levels. There is currently not enough scientific literature available to draw firm conclusions about the influence of certain antidepressants on plasma or serum levels of cytokines *in vivo*. However, a recent meta-analysis which included data derived from 45 longitudinal studies and more than 1,500 patients found that antidepressant treatment, overall, decreases peripheral levels of IL-6, IL-10, and TNF-α (2).

Certain subgroups of depressed patients, for example those with psychotic depression, are usually treated with a combination of an antidepressant and an antipsychotic drug (204). Among antipsychotics, it has been shown that those with the highest risk of weight gain, for example clozapine and olanzapine (117), lead to a significant increase in pro-inflammatory cytokine levels in the blood (194). For patients with recurrent episodes of depression or bipolar depression, the treatment with mood stabilizers is recommended (205). Some of these mood stabilizers, for example lithium and carbamazepine, have also been shown to lead to weight gain as well as an increase in pro-inflammatory cytokine levels (206).

The *in vitro* literature on antidepressants suggest that some antidepressants, such as clomipramine and fluoxetine, decrease IL-6, IFN-γ, and TNF-α, whilst others like mirtazapine and venlafaxine tend to increase their levels (207). From these results, one is tempted to draw the conclusion that serotonin reuptake inhibitors (SSRIs) or serotonin and noradrenalin reuptake inhibitors (SNRI) generally decrease IL-6, IFN-γ, and TNF-α levels. However, the SSRI citalopram increased the production of IL-1β, IL-6, and TNF-α in another *in vitro* study (69). What *in vitro* studies clearly show, however, is that antidepressants (69), antipsychotics (208) and mood stabilizers (209) have a direct influence on cytokine production within the blood.

Cytokine Levels and Antidepressant Response

Occasionally, studies have reported that baseline levels of certain cytokines or cytokine changes during treatment were associated with antidepressant treatment response during treatment with specific antidepressants or a certain combination of antidepressants. For example, Jha et al. (210) found that higher baseline levels of IL-17 were associated with greater symptomatic reduction in depressed patients treated with a bupropion-SSRI combination. However, the research in this area is sparse, and therefore, it is too early to draw far reaching conclusions from such observations. Regarding changes of cytokine levels during antidepressant treatment, the aforementioned recent meta-analysis of Köhler et al. (2) did not provide evidence that reductions in peripheral inflammation are associated with antidepressant treatment response.

Cytokine Levels and Psychotherapy

Not only antidepressants, but also psychotherapy has been reported to be associated with cytokine changes. For example, Del Grande da Silva et al. (211) reported a clinical study showing that successful brief psychodynamic psychotherapy leads to a reduction of pro-inflammatory cytokine serum levels.

DISCUSSION

Historical Considerations

The close relationship between inflammatory processes and psychiatric symptoms has been scientifically investigated since the 19th century (123). Shortly after the discovery of the first cytokine, IFN-α (12), it became clear that this cytokine was able to influence immunological processes in the brain

even when peripherally administered (17, 18) and that it can be produced by cells within the brain (18). Therefore, even though cytokines were discovered as messenger molecules with important immunological functions, it quickly became clear that they also play an important role within and for the brain.

Difficulties in Interpreting the Results of Cytokine Research

During the 1970s it became clear that immune cells are not the only cells that release cytokines (19). For example, TNF-α can be produced by white blood cells, the endothelium, fat cells and other cells (46). Therefore, if we measure a specific cytokine like TNF-α in the serum or plasma, it is unclear from which cells or which organ it is derived and where this cytokine will exert its effect, as one cytokine can have different effects on different cell types (10).

As cytokine signaling often shows redundancy (53–55), synergy (47), antagonistic effects (56–59) or signaling cascades (60), it seems advisable to take all cytokines that work together or against each other into account and thus measure a whole range instead of a single cytokine.

Cytokines as Potential Biomarkers of Depression

As peripheral levels of IL-6, IL-10, IL-12, IL13, and TNF-α have been shown to be significantly elevated and IFN-γ plasma concentrations significantly lower in patients with depression compared to healthy controls in meta-analytic research (2), one could think that cytokines qualify as a biomarker of depression. This perspective could be supported by further meta-analytic findings that antidepressant treatment decreases peripheral levels of IL-6, IL-10, and TNF-α (2). However, one has to take into account that these are results comparing means of groups. There is huge overlap of the distributions of cytokine levels of depressed patients and healthy controls, and additionally, the meta-analytic research of Köhler et al. (2) did not provide evidence that reductions in peripheral inflammation are associated with antidepressant treatment response. Thus, the mentioned group effects may not be relevant on an individual level. Additionally, the described effects of antidepressants on certain cytokines may be a mere pharmacological effect of these medications on immune cells that is not necessarily related to the depressive syndrome, as it has been shown that antidepressants (69), antipsychotics (208), and mood stabilizers (209) influence cytokine production directly.

Challenges in Regard to the Current Diagnostic Criteria

People with depression exhibit heterogeneous sets of symptoms. Thus, it may be sensible to define subgroups of patients with depression with more homogenous psychopathology. It might well be that within such a subgroup, cytokines are of greater value as an individualized biomarker of disease severity and antidepressant response. However, an alternative perspective may also be worth considering: cytokines could be associated with depressive syndromes independent of the psychiatric diagnosis. For example, patients with schizophrenia can also suffer from depressive symptoms (212). Therefore, cytokine levels may not only be associated with a diagnostic category, but with transdiagnostic depressive symptoms. This might be a reason for limited specificity of cytokines as biomarkers for a certain psychiatric diagnosis, because the mentioned cytokine levels are not only elevated in depression, but also in other psychiatric disorders (see **Table 2**). Moreover, a number of environmental, social, psychological and medical factors (see **Table 3**) are also associated with cytokine changes. Therefore, cytokines may currently not be considered as specific biomarkers for depression.

Future Perspectives on Cytokines as Biomarkers for Depression

There are many immunologically important cytokines like IL-21 (65) and IL-22 (66) which have not been extensively researched in psychiatric samples yet, even though the first preliminary studies have revealed promising results (67, 69). Cytokine levels are often researched and interpreted in isolation from their origin in terms of the specific cell type and the particular tissue they are originating from, even though changes in specific cytokine-producing immune cells like T_{reg} cells have been reported in depression (213) and during antidepressant therapy (214). Additionally, their receptors and their target tissue have been neglected in psychiatric research, despite considerations that modulation of cytokine receptors might be a promising future antidepressant strategy (215).

At this point, we would like to mention that there are only few studies available that measured cytokine concentrations in the cerebrospinal fluid [e.g., (216)], even though one would assume that cytokine concentrations in the cerebrospinal fluid might better reflect cytokine signaling in the brain than cytokine levels in the serum or plasma.

Taken together, including novel cytokines, cells and tissues of their origin, their receptors and target tissues in future scientific and clinical projects might help to fill the gaps in our knowledge in immunological biomarker research.

Future Perspectives on Cytokines as a Therapeutic Target for Antidepressant Treatment

Animal experiments have shown that cytokine blockers like TNF-α blockers can be effective in the treatment of depression-like behavior (217). However, attempts to try cytokine blockers in people with depression did not show striking success (218). Furthermore, designer monoclonal antibodies to bind directly to the cytokine and soluble cytokine receptors are currently being developed which will hopefully have less severe side effects than those currently available.

AUTHOR CONTRIBUTIONS

HH, OP, NL, and BD conducted the literature search. HH and MI drafted the manuscript. All authors were involved in drafting, critiquing and approving of the manuscript, and accept responsibility for the accuracy, and integrity of this work. The authors were the only individuals who contributed to this publication.

FUNDING

BD is supported by a studentship awarded by the Department of Psychological Medicine, King's College London (KCL) and the Institute of Psychiatry, Psychology and Neuroscience (IoPPN), KCL. OP is funded by OBELIX (Obesity, Lifestyle and Learning from Extreme Phenotypes), a research framework within the Biomedical Research Centre (BRC) of the South London and Maudsley NHS Foundation Trust (SLaM) and the IoPPN.

REFERENCES

1. Köhler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, et al. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand.* (2017) 135:373–87. doi: 10.1111/acps.12698
2. Köhler CA, Freitas TH, Stubbs B, Maes M, Solmi M, Veronese N, et al. Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: systematic review and meta-analysis. *Mol Neurobiol.* (2017) 55:4195–206. doi: 10.1007/s12035-017-0632-1
3. Burgey C, Kern WV, Römer W, Sakinc T, Rieg S. The innate defense antimicrobial peptides hBD3 and RNase7 are induced in human umbilical vein endothelial cells by classical inflammatory cytokines but not Th17 cytokines. *Microbes Infect.* (2015) 17:353–9. doi: 10.1016/j.micinf.2015.01.005
4. Enzerink A, Vaheri A. Fibroblast activation in vascular inflammation. *J Thromb Haemost.* (2011) 9:619–26. doi: 10.1111/j.1538-7836.2011.04209.x
5. Tsuruda T, Imamura T, Hatakeyama K, Asada Y, Kitamura K. Stromal cell biology. *Circ J.* (2010) 74:1042–50. doi: 10.1253/circj.cj-10-0024
6. Barbierato M, Facci L, Argentini C, Marinelli C, Skaper S, Giusti P. Astrocyte-microglia cooperation in the expression of a pro-inflammatory phenotype. *CNS Neurol Disord Drug Targets* (2013) 12:608–18. doi: 10.2174/18715273113129990064
7. Lackie J, O'Callaghan CA. A Dictionary of Biomedicine. (2010) OUP Oxford
8. Stedman TL. *Stedman's Medical Dictionary* (2006). Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins
9. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. *Molecular Cell Biology* 4th ed. New York, NY: W. H. Freeman (2000).
10. Tracey KJ, Cerami A. Tumor necrosis factor and regulation of metabolism in infection: role of systemic versus tissue levels. *Exp Biol Med.* (1992) 200:233–9. doi: 10.3181/00379727-200-43426
11. Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev.* (1990) 70:963–85. doi: 10.1152/physrev.1990.70.4.963
12. Isaacs A, Lindenmann J. Virus interference. I. the interferon. *Proc Royal Soc B Biol Sci.* (1957) 147:258–67. doi: 10.1098/rspb.1957.0048
13. Wheelock EF. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. *Science* (1965) 149:310–1. doi: 10.1126/science.149.3681.310
14. Bloom BR, Bennett B. mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science* (1966) 153:80–2. doi: 10.1126/science.153.3731.80
15. David JR. Delayed hypersensitivity *in vitro*: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc Natl Acad Sci USA.* (1966) 56:72–7. doi: 10.1073/pnas.56.1.72
16. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA.* (1975) 72:3666–70. doi: 10.1073/pnas.72.9.3666
17. Hitchcock G, Isaacs A. Protection of mice against the lethal action of an encephalitis virus. *BMJ* (1960) 2:1268–70. doi: 10.1136/bmj.2.5208.1268
18. Hitchcock G, Porterfield JS. The production of interferon in brains of mice infected with an arthropod-borne virus. *Virology* (1961) 13:363–5. doi: 10.1016/0042-6822(61)90155-6
19. Cohen S, Bigazzi PE, Yoshida T. Similarities of T cell function in cell-mediated immunity and antibody production. *Cell Immunol.* (1974) 12:150–9. doi: 10.1016/0008-8749(74)90066-5
20. Blalock JE, Smith EM. Human leukocyte interferon (HuIFN-α): Potent endorphin-like opioid activity. *Biochem Biophys Res Commun.* (1981) 101:472–478. doi: 10.1016/0006-291x(81)91284-5
21. Smith EM, Dion LD, Blalock JE. Opiate receptor mediated effects of IFN-alpha and lymphocyte derived endorphin-like peptides. *Progr Clin Biol Res.* (1985) 192:259–264. doi
22. Ankel H, Chany C, Galliot B, Chevalier MJ, Robert M. Antiviral effect of interferon covalently bound to sepharose. *Proc Natl Acad Sci USA.* (1973) 70:2360–3. doi: 10.1073/pnas.70.8.2360
23. Himmerich H. Plasma levels of tumor necrosis factor α and soluble tumor necrosis factor receptors in patients with narcolepsy. *Arch Internal Med.* (2006) 166:1739. doi: 10.1001/archinte.166.16.1739
24. Himmerich H. Activity of the TNF-α System in patients with brain disorders and during psychopharmacological treatment. *Curr Pharmaceut Anal.* (2007) 3:1–5. doi: 10.2174/157341207779802412
25. Yalow RS, Berson SA. Assay of Plasma insulin in human subjects by immunological methods. *Nature* (1959) 184:1648–9. doi: 10.1038/1841648b0
26. Van Weemen BK, Schuurs AHWM. Immunoassay using antigen-enzyme conjugates. *FEBS Lett.* (1971) 15:232–6. doi: 10.1016/0014-5793(71)80319-8
27. Engvall E, Perlmann P. Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. *Immunochemistry* (1971) 8:871–4. doi: 10.1016/0019-2791(71)90454-x
28. Keustermans GCE, Hoeks SBE, Meerding, Jenny M., Prakken BJ, de Jager W. Cytokine assays: an assessment of the preparation and treatment of blood and tissue samples. *Methods* (2013) 61:10–17. doi: 10.1016/j.ymeth.2013.04.005
29. Cohen SN, Chang ACY, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids *in vitro*. *Proc Natl Acad Sci USA.* (1973) 70:3240–4. doi: 10.1073/pnas.70.11.3240
30. Otto B. Recombinant human interferons. *Arzneimittelforschung* (1985) 35:1750–2.
31. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther.* (2011) 130:226–38. doi: 10.1016/j.pharmthera.2011.01.014
32. Himmerich H, Fulda S, Linseisen J, Seiler H, Wolfram G, Himmerich S, et al. Depression, comorbidities and the TNF-α system. *Eur Psychiatry* (2008) 23:421–9. doi: 10.1016/j.eurpsy.2008.03.013
33. Schmidt FM, Lichtblau N, Minkwitz J, Chittka T, Thormann J, Kirkby KC, et al. Cytokine levels in depressed and non-depressed subjects, and masking effects of obesity. *J Psychiatr Res.* (2014) 55:29–34. doi: 10.1016/j.jpsychires.2014.04.021
34. Hussein S, Himmerich H, Willmund GD, Ibrahim MAA, Dalton B. A systematic review of tumor necoris factor-α in post-traumatic stress disorder: evidence from human and animal studies. *Psychiatr Danub.* (2017) 29:407–20. doi: 10.24869/psyd.2017.407
35. Waheed A, Dalton B, Wesemann U, Ibrahim MAA, Himmerich H. A systematic review of interleukin-1β in post-traumatic stress disorder: evidence from human and animal studies. *J Interfer Cytokine Res.* (2018) 38:1–11. doi: 10.1089/jir.2017.0088
36. Passos IC, Vasconcelos-Moreno MP, Costa LG, Kunz M, Brietzke E, Quevedo J, et al. Inflammatory markers in post-traumatic stress disorder: a systematic review, meta-analysis, and meta-regression. *Lancet Psychiatry* (2015) 2:1002–12. doi: 10.1016/S2215-0366(15)00309-0
37. Dalton B, Bartholdy S, Robinson L, Solmi M, Ibrahim MAA, Breen G, et al. A meta-analysis of cytokine concentrations in eating disorders. *J Psychiatr Res.* (2018) 103:252–64. doi: 10.1016/j.jpsychires.2018.06.002
38. Goldsmith DR, Rapaport MH, Miller BJ. A meta-analysis of blood cytokine network alterations in psychiatric patients: comparisons between

schizophrenia, bipolar disorder and depression. *Mol Psychiatry* (2016) 21:1696–709. doi: 10.1038/mp.2016.3
39. Modabbernia A, Taslimi S, Brietzke E, Ashrafi M. Cytokine alterations in bipolar disorder: a meta-analysis of 30 studies. *Biol Psychiatry* (2013) 74:15–25. doi: 10.1016/j.biopsych.2013.01.007
40. Gray SM, Bloch MH. Systematic review of proinflammatory cytokines in obsessive-compulsive disorder. *Curr Psychiatry Rep.* (2012) 14:220–8. doi: 10.1007/s11920-012-0272-0
41. Leonard WJ, Lin JX. Cytokine receptor signaling pathways. *J Allergy Clin Immunol.* (2000) 105:877–88. doi: 10.1067/mai.2000.106899
42. Koch K-C, Ye K, Clark BD, Dinarello CA. Interleukin 4 (IL) 4 up-regulates gene and surface IL 1 receptor type I in murine T helper type 2 cells. *Eur J Immunol.* (1992) 22:153–7. doi: 10.1002/eji.1830220123
43. Kasakura S. A role for T-helper type 1 and type 2 cytokines in the pathogenesis of various human diseases. *Rinsho Byori* (1998) 46:915–21.
44. Cejková S, Králová Lesná I, Froněk J, Králová A, Poledne R. Pro-inflammatory gene expression in adipose tissue in patients with atherosclerosis. *Atherosclerosis* (2016) 252:e174. doi: 10.1016/j.atherosclerosis.2016.07.820
45. Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. *Comp Med.* (2009) 59:517–26.
46. Perskidskii I, Barshtein I. Biological manifestations of the tumor necrosis factor effect and its role in the pathogenesis of various diseases. *Arkhiv Patologii* (1992) 52:5–10.
47. Del Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. *Allergy* (1992) 47:450–5.
48. Tracey MDKJ, Cerami PDA. Tumor necrosis factor: a pleiotropic cytokine and therapuetic target. *Ann Rev Med.* (1994) 45:491–503. doi: 10.1146/annurev.med.45.1.491
49. Hurdayal R, Ndlovu HH, Revaz-Breton M, Parihar SP, Nono JK, Govender M, et al. IL-4–producing B cells regulate T helper cell dichotomy in type 1- and type 2-controlled diseases. *Proc Natl Acad Sci USA.* (2017) 114:E8430–9. doi: 10.1073/pnas.1708125114
50. Noma T. Function, molecular structure and gene expression of IL-4. *Nihon Rinsho*. (1992) 50:1787–94.
51. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol.* (2003) 3:745–56. doi: 10.1038/nri1184
52. Kicielinska J, Pajtasz-Piasecka E. The role of IL-10 in the modulation of the immune response in normal conditions and the tumor environment. *Postepy Higieny Med Doświadczal.* (2014) 68:879–92. doi: 10.5604/17322693.1111123
53. Bruserud Ø, Hamann W, Patel S, Ehninger G, Pawelec G. IL2- and IL4-dependent proliferation of T-cell clones derived early after allogeneic bone marrow transplantation: Studies of patients with chronic myelogenous leukaemia. *Eur J Haematol.* (1992) 48:221–7. doi: 10.1111/j.1600-0609.1992.tb01589.x
54. Boraschi D, Censini S, Bartalini M, Ghiara P, Tagliabue A. Arachidonic acid metabolism in macrophages: regulation by interferons and interleukin 1. *Int J Immunopharmacol.* (1985) 7:359. doi: 10.1016/0192-0561(85)90339-x
55. Munk ME, Emoto M. Functions of T-cell subsets and cytokines in mycobacterial infections. *Eur Respir J Suppl*. (1995) 20:668s—75s.
56. Georas SN. T-helper cell type-2 regulation in allergic disease. *Eur Respir J.* (2005) 26:1119–37. doi: 10.1183/09031936.05.00006005
57. Ito T, Wang Y-H, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med.* (2005) 202:1213–23. doi: 10.1084/jem.20051135
58. Kane B, Fowler SJ, Niven R. Refractory asthma - beyond step 5, the role of new and emerging adjuvant therapies. *Chronic Respir Dis.* (2015) 12:69–77. doi: 10.1177/1479972314562210
59. Krumm B, Xiang Y, Deng J. Structural biology of the IL-1 superfamily: key cytokines in the regulation of immune and inflammatory responses. *Protein Sci.* (2014) 23:526–38. doi: 10.1002/pro.2441
60. Lorentz A, Bischoff SC. Regulation of human intestinal mast cells by stem cell factor and IL-4. *Immunol Rev.* (2001) 179:57–60. doi: 10.1034/j.1600-065x.2001.790106.x
61. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic Med.* (2009) 71:171–86. doi: 10.1097/psy.0b013e3181907c1b
62. Haase H, Kruse A, Rink L, editors. Die Regulation des Immunsystems und immunprivilegierte Organe. In: *Immunologie für Einsteiger*. Berlin; Heidelberg: Springer (2015). p. 101–20.
63. Breitbart W, Rosenfeld B, Tobias K, Pessin H, Ku GY, Yuan J, et al. Depression, cytokines, and pancreatic cancer. *Psycho Oncol.* (2013) 23:339–45. doi: 10.1002/pon.3422
64. Caraci F, Spampinato SF, Morgese MG, Tascedda F, Salluzzo MG, Giambirtone MC, et al. Neurobiological links between depression and AD: The role of TGF-beta1 signaling as a new pharmacological target. *Pharmacol Res.* (2018) 130:374–84. doi: 10.1016/j.phrs.2018.02.007
65. Leonard WJ, Wan CK. IL-21 Signaling in immunity. *F1000Res*. (2016) 5:224. doi: 10.12688/f1000research.7634.1
66. Alabbas SY, Begun J, Florin TH, Oancea I. The role of IL-22 in the resolution of sterile and nonsterile inflammation. *Clin Transl Immunol.* (2018) 7:e1017. doi: 10.1002/cti2.1017
67. Juncal-Ruiz M, Riesco-Dávila L, Ortiz-García de la Foz V, Martínez-Garcia O, Ramírez-Bonilla M, Ocejo-Viñals JG, et al. Comparison of the anti-inflammatory effect of aripiprazole and risperidone in 75 drug-naïve first episode psychosis individuals: A 3 months randomized study. *Schizophr Res.* (2018) 202:226–33. doi: 10.1016/j.schres.2018.06.039
68. Lenze EJ, Mulsant BH, Blumberger DM, Karp JF, Newcomer JW, Anderson SJ, et al. Efficacy, safety, and tolerability of augmentation pharmacotherapy with aripiprazole for treatment-resistant depression in late life: a randomised, double-blind, placebo-controlled trial. *Lancet* (2015) 386:2404–12. doi: 10.1016/s0140-6736(15)00308-6
69. Munzer A, Sack U, Mergl R, Schönherr J, Petersein C, Bartsch S, et al. Impact of antidepressants on cytokine production of depressed patients *in vitro*. *Toxins* (2013) 5:2227–40. doi: 10.3390/toxins5112227
70. Himmerich H, Schneider F. Psychoimmunologie. In: Schneider F, editors. *Facharztwissen Psychiatrie, Psychosomatik und Psychotherapie*. Berlin: Springer Berlin Heidelberg (2016). p. 535–40.
71. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Ann Rev Immunol.* (2009) 27:485–517. doi: 10.1146/annurev.immunol.021908.132710
72. Eyerich K, Dimartino V, Cavani A. IL-17 and IL-22 in immunity: driving protection and pathology. *Eur J Immunol.* (2017) 47:607–14. doi: 10.1002/eji.201646723
73. Dons EM, Raimondi G, Cooper DKC, Thomson AW. Induced regulatory T cells: mechanisms of conversion and suppressive potential. *Hum Immunol.* (2012) 73:328–34. doi: 10.1016/j.humimm.2011.12.011
74. Cheon Y-H, Lee S-G, Kim M, Kim H-O, Sun Suh Y, Park K-S, et al. The association of disease activity, pro-inflammatory cytokines, and neurotrophic factors with depression in patients with rheumatoid arthritis. *Brain Behav Immun.* (2018) 73:274–81. doi: 10.1016/j.bbi.2018.05.012
75. Song C, Luchtman D, Kang Z, Tam EM, Yatham LN, Su K-P, et al. Enhanced inflammatory and T-helper-1 type responses but suppressed lymphocyte proliferation in patients with seasonal affective disorder and treated by light therapy. *J Affect Disord.* (2015) 185:90–6. doi: 10.1016/j.jad.2015.06.003
76. Dahl J, Ormstad H, Aass HCD, Malt UF, Bendz LT, Sandvik L, et al. The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* (2014) 45:77–86. doi: 10.1016/j.psyneuen.2014.03.019
77. Mao R, Zhang C, Chen J, Zhao G, Zhou R, Wang F, et al. Different levels of pro- and anti-inflammatory cytokines in patients with unipolar and bipolar depression. *J Affect Disord.* (2018) 237:65–72. doi: 10.1016/j.jad.2018.04.115
78. de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, Culpepper J, et al. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. *J Immunol.* (1993) 151:6370–81.
79. Tilg H, Peschel C. Interferon-alpha and its effects on the cytokine cascade: a pro- and anti-inflammatory cytokine. *Leukem Lymphoma* (1996) 23:55–60. doi: 10.3109/10428199609054802
80. D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. *J Neurosci.* (2009). 29:7. doi: 10.1523/JNEUROSCI.3567-08.2009
81. Lichtblau N, Schmidt FM, Schumann R, Kirkby KC, Himmerich H. Cytokines as biomarkers in depressive disorder: current

standing and prospects. *Int Rev Psychiatry* (2013) 25:592–603. doi: 10.3109/09540261.2013.813442
82. Dunn AJ, Wang J. Cytokine effects on CNS biogenic amines. *Neuroimmunomodulation* (1995) 2:319–28. doi: 10.1159/000097211
83. Dunn AJ, Wang J, Ando T. Effects of cytokines on cerebral neurotransmission. Comparison with the effects of stress. *Adv Exp Med Biol.* (1999) 461:117–27. doi: 10.1007/978-0-585-37970-8_8
84. Anisman H, Matheson K. Stress, depression, and anhedonia: caveats concerning animal models. *Neurosci Behav Rev.* (2005) 29:525–46. doi: 10.1016/j.neubiorev.2005.03.007
85. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med.* (2001) 344:961–6. doi: 10.1056/NEJM200103293441303
86. Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreib R, Staab JP, et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. *Aliment Pharmacol Ther.* (2007) 25:1163–74. doi: 10.1111/j.1365-2036.2007.03316.x
87. Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis c receiving interferon-α-based immunotherapy are related to interferon-α-induced changes in the serotonergic system. *J Clin Psychopharmacol.* (2002) 22:86–90. doi: 10.1097/00004714-200202000-00014
88. Capuron L, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, Miller AH. Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. *Biol Psychiatry* (2003) 54:906–14. doi: 10.1016/S0006-3223(03)00173-2
89. Zhu C-B, Blakely RD, Hewlett WA. The Proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology* (2006) 31:2121–31. doi: 10.1038/sj.npp.1301029
90. Zhu C-B, Carneiro AM, Dostmann WR, Hewlett WA, Blakely RD. p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J Biol Chem.* (2005) 280:15649–58. doi: 10.1074/jbc.m410858200
91. Zhu C-B, Lindler KM, Owens AW, Daws LC, Blakely RD, Hewlett WA. Interleukin-1 receptor activation by systemic lipopolysaccharide induces behavioral despair linked to MAPK regulation of CNS serotonin transporters. *Neuropsychopharmacology* (2010) 35:2510–20. doi: 10.1038/npp.2010.116
92. Morón JA, Zakharova I, Ferrer JV, Merrill GA, Hope B, Lafer EM, et al. Mitogen-activated protein kinase regulates dopamine transporter surface expression and dopamine transport capacity. *J Neurosci.* (2003) 23:8480–8. doi: 10.1523/jneurosci.23-24-08480.2003
93. Capuron L, Pagnoni G, Drake DF, Woolwine BJ, Spivey JR, Crowe RJ, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch General Psychiatry* (2012) 69:1044. doi: 10.1001/archgenpsychiatry.2011.2094
94. Ida T, Hara M, Nakamura Y, Kozaki S, Tsunoda S, Ihara H. Cytokine-induced enhancement of calcium-dependent glutamate release from astrocytes mediated by nitric oxide. *Neurosci Lett.* (2008) 432:232–6. doi: 10.1016/j.neulet.2007.12.047
95. Matute C, Domercq M, Sánchez-Gómez, MV. Glutamate-mediated glial injury: Mechanisms and clinical importance. *Glia* (2006) 53:675. doi: 10.1002/glia.20323
96. Lee M, Schwab C, McGeer PL. Astrocytes are GABAergic cells that modulate microglial activity. *Glia* (2010) 59:152–65. doi: 10.1002/glia.21087
97. Dale RC, Merheb V, Pillai S, Wang D, Cantrill L, Murphy TK, et al. Antibodies to surface dopamine-2 receptor in autoimmune movement and psychiatric disorders. *Brain* (2012) 135:3453–68. doi: 10.1093/brain/aws256
98. Tanaka S, Kuratsune H, Hidaka Y, Hakariya Y, Tatsumi K-I, Takano T, et al. Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome. *Int J Mol Med.* (2003) 12:225–30. doi: 10.3892/ijmm.12.2.225
99. Besedovsky HO, Del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocr Rev.* (1996) 17:64–102. doi: 10.1210/edrv-17-1-64
100. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry* (2001) 49:391–404. doi: 10.1016/s0006-3223(00)01088-x
101. Pace TWW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: Relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav Immun.* (2007) 21:9–19. doi: 10.1016/j.bbi.2006.08.009
102. Sephton SE. Diurnal cortisol rhythm as a predictor of breast cancer survival. *J Natl Cancer Inst.* (2000) 92:994–1000. doi: 10.1093/jnci/92.12.994
103. Matthews K, Schwartz J, Cohen S, Seeman T. Diurnal cortisol decline is related to coronary calcification: CARDIA study. *Psychosomatic Med.* (2006) 68:657–61. doi: 10.1097/01.psy.0000244071.42939.0e
104. Raison CL, Borisov AS, Woolwine BJ, Massung B, Vogt G, Miller AH. Interferon-alpha effects on diurnal hypothalamic-pituitary-adrenal axis activity: relationship with proinflammatory cytokines and behavior. *Mol Psychiatry* (2010) 15:535–47. doi: 10.1038/mp.2008.58
105. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci.* (2002) 5:405–14. doi: 10.1038/nn835
106. Berthold-Losleben M, Heitmann S, Himmerich H. Anti-inflammatory drugs in psychiatry. *Inflamm Allergy Drug Targets* (2009) 8:266–76. doi: 10.2174/187152809789352221
107. Alexander GE, Crutcher MD, DeLong MR. Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Progress Brain Res.* (1991) 85:119–46.
108. Eisenberger NI, Lieberman MD. Why rejection hurts: a common neural alarm system for physical and social pain. *Trends Cogn Sci.* (2004) 8:294–300. doi: 10.1016/j.tics.2004.05.010
109. Juengling FD, Ebert D, Gut O, Engelbrecht MA, Rasenack J, Nitzsche EU, et al. Prefrontal cortical hypometabolism during low-dose interferon alpha treatment. *Psychopharmacology* (2000) 152:383–9. doi: 10.1007/s002130000549
110. Capuron L, Pagnoni G, Demetrashvili MF, Lawson DH, Fornwalt FB, Woolwine B, et al. Basal ganglia hypermetabolism and symptoms of fatigue during interferon-α therapy. *Neuropsychopharmacology* (2007) 32:2384–92. doi: 10.1038/sj.npp.1301362
111. Brydon L, Harrison NA, Walker C, Steptoe A, Critchley HD. Peripheral inflammation is associated with altered substantia nigra activity and psychomotor slowing in humans. *Biol Psychiatry* (2008) 63:1022–9. doi: 10.1016/j.biopsych.2007.12.007
112. Majer M, Welberg LAM, Capuron L, Pagnoni G, Raison CL, Miller AH. IFN-alpha-induced motor slowing is associated with increased depression and fatigue in patients with chronic hepatitis C. *Brain Behav Immun.* (2008) 22:870–80. doi: 10.1016/j.bbi.2007.12.009
113. Harrison NA, Brydon L, Walker C, Gray MA, Steptoe A, Critchley HD. Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity. *Biol Psychiatry* (2009) 66:407–14. doi: 10.1016/j.biopsych.2009.03.015
114. Capuron L, Pagnoni G, Demetrashvili M, Woolwine B, Nemeroff C, Berns G, et al. Anterior cingulate activation and error processing during interferon-alpha treatment. *Biol Psychiatry* (2005) 58:190–6. doi: 10.1016/j.biopsych.2005.03.033
115. Himmerich H, Fischer J, Bauer K, Kirkby KC, Sack U, Krugel U. Stress-induced cytokine changes in rats. *Eur Cytokine Network* (2013) 24:97–103. doi: 10.1684/ecn.2013.0338
116. Munkholm K, Brauner J, Kessing LV, Vinberg M. Cytokines in bipolar disorder vs. healthy control subjects: a systematic review and meta-analysis. *J Psychiatr Res.* (2013) 47:1119–33. doi: 10.1016/j.jpsychires.2013.05.018
117. Himmerich H, Minkwitz J, Kirkby K. Weight gain and metabolic changes during treatment with antipsychotics and antidepressants. *Endocr Metabol Immune Disorder Drug Targets* (2015) 15:252–60. doi: 10.2174/1871530315666150623092031
118. Cross-Disorder Group of the Psychiatric Genomics C. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* (2013) 381:1371–9. doi: 10.1016/S0140-6736(12)62129-1
119. Piliero LM. T-cell homeostasis in humans with thymic hypoplasia due to chromosome 22q11.2 deletion syndrome. *Blood* (2003) 103:1020–5. doi: 10.1182/blood-2003-08-2824
120. Gimeno D, Kivimäki M, Brunner EJ, Elovainio M, De Vogli R, Steptoe A, et al. Associations of C-reactive protein and interleukin-6 with cognitive

symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological Med.* (2008) 39:413. doi: 10.1017/s0033291708003723
121. Ramirez G, Toro R, Dobeli H, von Bernhardi R. Protection of rat primary hippocampal cultures from A beta cytotoxicity by pro-inflammatory molecules is mediated by astrocytes. *Neurobiol Dis.* (2005) 19:243–54. doi: 10.1016/j.nbd.2005.01.007
122. Mufaddel A, Omer AA, Salem MO. Psychiatric aspects of infectious diseases. *Open J Psychiatry* (2014) 04:202–17. doi: 10.4236/ojpsych.2014.43027
123. Steinberg H, Himmerich H. Emil Kraepelin's habilitation and his thesis: a pioneer work for modern systematic reviews, psychoimmunological research and categories of psychiatric diseases. *World J Biol Psychiatry* (2013) 14:248–57. doi: 10.3109/15622975.2011.623717
124. Himmerich H, Kohls E, Hegerl U, Rummel-Kluge C. Prädiktive Faktoren der Depression und ihrer Therapie. *Der Nervenarzt* (2014) 85:1249–54. doi: 10.1007/s00115-014-4048-0
125. Himmerich H, Lichtblau N. Prävention psychischer Erkrankungen am Beispiel der Depression. In: Hans-Wolfgang Hoefert CKH, editors. *Krankheitsprävention in der Kontroverse.* Lengerich: Pabst Science Publishers (2014). p. 166–81.
126. Muñoz RF, Cuijpers P, Smit F, Barrera AZ, Leykin Y. Prevention of major depression. *Ann Rev Clin Psychol.* (2010) 6:181–212. doi: 10.1146/annurev-clinpsy-033109-132040
127. Mills NT, Scott JG, Wray NR, Cohen-Woods S, Baune BT. Research review: the role of cytokines in depression in adolescents: a systematic review. *J Child Psychol Psychiatry* (2013) 54:816–35. doi: 10.1111/jcpp.12080
128. Fonken LK, Xu X, Weil ZM, Chen G, Sun Q, Rajagopalan S, et al. Air pollution impairs cognition, provokes depressive-like behaviors and alters hippocampal cytokine expression and morphology. *Mol Psychiatry* (2011) 16:987–95. doi: 10.1038/mp.2011.76
129. Kristiansson M, Sörman K, Tekwe C, Calderón-Garcidueñas L. Urban air pollution, poverty, violence and health – Neurological and immunological aspects as mediating factors. *Environ Res.* (2015) 140:511–3. doi: 10.1016/j.envres.2015.05.013
130. Measelle JR, Ablow JC. Contributions of early adversity to pro-inflammatory phenotype in infancy: the buffer provided by attachment security. *Attach Hum Dev.* (2018) 20:1–23. doi: 10.1080/14616734.2017.1362657
131. Hintikka J, Lehto SM, Niskanen L, Huotari A, Herzig KH, Koivumaa-Honkanen H, et al. Unemployment and ill health: a connection through inflammation? *BMC Public Health* (2009) 9:410. doi: 10.1186/1471-2458-9-410
132. Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Biobehav Rev.* (2012) 36:764–85. doi: 10.1016/j.neubiorev.2011.12.005
133. Grudet C, Malm J, Westrin A, Brundin L. Suicidal patients are deficient in vitamin D, associated with a pro-inflammatory status in the blood. *Psychoneuroendocrinology* (2014) 50:210–9. doi: 10.1016/j.psyneuen.2014.08.016. [Epub ahead of print].
134. Amini S, Jafarirad S, Amani R. Postpartum depression and vitamin D: a systematic review. *Crit Rev Food Sci Nutr.* (2018). doi: 10.1080/10408398.2017.1423276. [Epub ahead of print].
135. Husted KS, Bouzinova EV. The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. (2016) 52:139–47. doi: 10.1016/j.medici.2016.05.003
136. Dinan TG, Cryan JF. The microbiome-gut-brain axis in health and disease. *Gastroenterol Clin North Am.* (2017) 46:77–89. doi: 10.1016/j.gtc.2016.09.007
137. Schirmer M, Kumar V, Netea MG, Xavier RJ. The causes and consequences of variation in human cytokine production in health. *Curr Opin Immunol.* (2018) 54:50–8. doi: 10.1016/j.coi.2018.05.012
138. Rohleder N. Stimulation of systemic low-grade inflammation by psychosocial stress. *Psychosomatic Med.* (2014) 76:181–9. doi: 10.1097/psy.0000000000000049
139. Menard C, Pfau ML, Hodes GE, Kana V, Wang VX, Bouchard S, et al. Social stress induces neurovascular pathology promoting depression. *Nat Neurosci.* (2017) 20:1752–60. doi: 10.1038/s41593-017-0010-3
140. Levine ME, Cole SW, Weir DR, Crimmins EM. Childhood and later life stressors and increased inflammatory gene expression at older ages. *Soc Sci Med.* (2015) 130:16–22. doi: 10.1016/j.socscimed.2015.01.030
141. Cohen M, Granger S, Fuller-Thomson E. The association between bereavement and biomarkers of inflammation. *Behav Med.* (2014) 41:49–59. doi: 10.1080/08964289.2013.866539
142. Jaremka LM, Lindgren ME, Kiecolt-Glaser JK. Synergistic relationships amongst stress, depression, and troubled relationships: Insights from psychoneuroimmunology. *Depress Anxiety* (2013) 30:288–96. doi: 10.1002/da.22078
143. O'Connor TG, Scheible K, Sefair AV, Gilchrist M, Blackmore ER, Winter MA, et al. Immune and neuroendocrine correlates of temperament in infancy. *Dev Psychopathol.* (2017) 29:1589–600. doi: 10.1017/s0954579417001250
144. Kim HN, Kim BH, Cho J, Ryu S, Shin H, Sung J, et al. Pathway analysis of genome-wide association datasets of personality traits. *Genes Brain Behav.* (2015) 14:345–56. doi: 10.1111/gbb.12212
145. Golimbet VE, Alfimova MV, Korovaitseva GI, Lezheiko TV. Analysis of the association of interleukin 4 and interleukin 10 gene variants with basic personality traits. *Mol Biol.* (2016) 50:839–44. doi: 10.1134/s0026893316060054
146. Quagliato LA, Nardi AE. Cytokine alterations in panic disorder: a systematic review. *J Affect Disord.* (2018) 228:91–6. doi: 10.1016/j.jad.2017.11.094
147. Tang Z, Ye G, Chen X, Pan M, Fu J, Fu T, et al. Peripheral proinflammatory cytokines in Chinese patients with generalised anxiety disorder. *J Affect Disord.* (2018) 225:593–8. doi: 10.1016/j.jad.2017.08.082
148. Heberlein A, Käser M, Lichtinghagen R, Rhein M, Lenz B, Kornhuber J, et al. TNF-α and IL-6 serum levels: neurobiological markers of alcohol consumption in alcohol-dependent patients? *Alcohol* (2014) 48:671–6. doi: 10.1016/j.alcohol.2014.08.003
149. Moreira FP, Medeiros JR, Lhullier AC, Souza LD, Jansen K, Portela LV, et al. Cocaine abuse and effects in the serum levels of cytokines IL-6 and IL-10. *Drug Alcohol Depend.* 158:181–5. doi: 10.1016/j.drugalcdep.2015.11.024
150. Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. *J Cerebr Blood Flow Metabol.* (2012) 32:1677–98. doi: 10.1038/jcbfm.2012.88
151. Ethemoglu O, Ay H, Koyuncu I, Gönel A. Comparison of cytokines and prooxidants/antioxidants markers among adults with refractory versus well-controlled epilepsy: a cross-sectional study. *Seizure* (2018) 60:105–9. doi: 10.1016/j.seizure.2018.06.009
152. Koh S. Role of neuroinflammation in evolution of childhood epilepsy. *J Child Neurol.* (2018) 33:64–72. doi: 10.1177/0883073817739528
153. Kaur K, Gill JS, Bansal PK, Deshmukh R. Neuroinflammation - A major cause for striatal dopaminergic degeneration in Parkinson's disease. *J Neurol Sci.* (2017) 381:308–14. doi: 10.1016/j.jns.2017.08.3251
154. Tiwari PC, Pal R. The potential role of neuroinflammation and transcription factors in Parkinson disease. *Dialog Clin Neurosci.* (2017) 19:71–80.
155. Schreiner B, Becher B. Perspectives on cytokine-directed therapies in multiple sclerosis. *Swiss Med Week.* (2015) 145:w14199. doi: 10.4414/smw.2015.14199
156. Rose-John S, Winthrop K, Calabrese L. The role of IL-6 in host defence against infections: immunobiology and clinical implications. *Nat Rev Rheumatol.* (2017) 13:399–409. doi: 10.1038/nrrheum.2017.83
157. Arango-Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol.* (2014) 5:491. doi: 10.3389/fimmu.2014.00491
158. Li S, Yang D, Peng T, Wu Y, Tian Z, Ni B. Innate lymphoid cell-derived cytokines in autoimmune diseases. *J Autoimmun.* (2017) 83:62–72. doi: 10.1016/j.jaut.2017.05.001
159. Kuwabara T, Ishikawa F, Kondo M, Kakiuchi T. The Role of IL-17 and related cytokines in inflammatory autoimmune diseases. *Mediator Inflamm.* (2017) 2017:1–11. doi: 10.1155/2017/3908061
160. Igaz P, Glaz E, Racz K. Cytokines in diseases of the endocrine system. *Cell Biol Int.* (2000) 24:663–8. doi: 10.1006/cbir.2000.0536
161. Smyth MJ, Cretney E, Kershaw MH, Hayakawa Y. Cytokines in cancer immunity and immunotherapy. *Immunol Rev.* (2004) 202:275–93. doi: 10.1111/j.0105-2896.2004.00199.x

162. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS ONE* (2015) 10:e0121971. doi: 10.1371/journal.pone.0121971
163. Thormann J, Chittka T, Minkwitz J, Kluge M, Himmerich H. Obesity and depression: an overview on the complex interactions of two diseases. *Fortschr Neurol Psychiatr*. (2013) 81:145–53. doi: 10.1055/s-0032-1330351
164. Herder C, Schmitt A, Budden F, Reimer A, Kulzer B, Roden M, et al. Association between pro- and anti-inflammatory cytokines and depressive symptoms in patients with diabetes—potential differences by diabetes type and depression scores. *Transl Psychiatry* (2018) 7:11. doi: 10.1038/s41398-017-0009-2
165. Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, et al. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. *Curr Vasc Pharmacol.* (2015) 13:26–36. doi: 10.2174/15701611113119990003
166. Sousa A, Raposo F, Fonseca S, Valente L, Duarte F, Gonçalves M, et al. Measurement of cytokines and adhesion molecules in the first 72 hours after severe trauma: association with severity and outcome. *Disease Marker.* (2015) 2015:1–8. doi: 10.1155/2015/747036
167. Winckler FC, Braz AMM, Silva VND, Golim MA, Andrade VG, Machado PEA, et al. Influence of the inflammatory response on treatment of hepatitis C with triple therapy. *Rev Soc Bras Med Trop*. (2018) 51:731–6. doi: 10.1590/0037-8682-0137-2018
168. Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nat Rev Cancer* (2008) 8:887–99. doi: 10.1038/nrc2507
169. Alazawi W, Pirmadjid N, Lahiri R, Bhattacharya S. Inflammatory and immune responses to surgery and their clinical impact. *Ann Surg.* (2016) 264:73–80. doi: 10.1097/SLA.0000000000001691
170. Raj RV, Hamadani M, Szabo A, Pasquini MC, Shah NN, Drobyski WR, et al. Peripheral blood grafts for T cell-replete haploidentical transplantation increase the incidence and severity of cytokine release syndrome. *Biol Blood Marrow Transplant.* (2018) 24:1664–70. doi: 10.1016/j.bbmt.2018.04.010.
171. Weschenfelder J, Sander C, Kluge M, Kirkby KC, Himmerich H. The influence of cytokines on wakefulness regulation: clinical relevance, mechanisms and methodological problems. *Psychiatr Danub.* (2012) 24:112–26.
172. Schmidt FM, Pschiebl A, Sander C, Kirkby KC, Thormann J, Minkwitz J, et al. Impact of serum cytokine levels on EEG-measured arousal regulation in patients with major depressive disorder and healthy controls. *Neuropsychobiology* (2016) 73:1–9. doi: 10.1159/000441190
173. Szarka A, Rigo J Jr, Lazar L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol*. (2010) 11:59. doi: 10.1186/1471-2172-11-59
174. Sel'kov SA, Pavlov OV, Selyutin AV. Cytokines and placental macrophages in regulation of birth activity. *Bull Exp Biol Med.* (2000) 129:511–5. doi: 10.1007/bf02434861
175. Camilleri G, Borg M, Brincat S, Schembri-Wismayer P, Brincat M, Calleja-Agius J. The role of cytokines in cardiovascular disease in menopause. *Climacteric* (2012) 15:524–30. doi: 10.3109/13697137.2012.700743
176. Sudheimer KD, O'Hara R, Spiegel D, Powers B, Kraemer HC, Neri E, et al. Cortisol, cytokines, and hippocampal volume interactions in the elderly. *Front Aging Neurosci.* (2014) 6:153. doi: 10.3389/fnagi.2014.00153
177. Graham AM, Rasmussen JM, Rudolph MD, Heim CM, Gilmore JH, Styner M, et al. Maternal systemic interleukin-6 during pregnancy is associated with newborn amygdala phenotypes and subsequent behavior at 2 years of age. *Biol Psychiatry* (2018) 83:109–19. doi: 10.1016/j.biopsych.2017.05.027
178. Clark SM, Pocivavsek A, Nicholson JD, Notarangelo FM, Langenberg P, McMahon RP, et al. Reduced kynurenine pathway metabolism and cytokine expression in the prefrontal cortex of depressed individuals. *J Psychiatry Neurosci.* (2016) 41:386–94. doi: 10.1503/jpn.150226
179. Shadrina M, Bondarenko EA, Slominsky PA. Genetics factors in major depression disease. *Front Psychiatry* (2018) 9:334. doi: 10.3389/fpsyt.2018.00334
180. Crawford B, Craig Z, Mansell G, White I, Smith A, Spaull S, et al. DNA methylation and inflammation marker profiles associated with a history of depression. *Hum Mol Genetics* (2018) 27:2840–50. doi: 10.1093/hmg/ddy199
181. Maes M. Major depression and activation of the inflammatory response system. *Adv Exp Med Biol.* (1999) 461:25–46. doi: 10.1007/978-0-585-37970-8_2
182. Anisman H, Merali Z, Poulter MO, Hayley S. Cytokines as a precipitant of depressive illness: animal and human studies. *Curr Pharmaceut Design* (2005) 11:963–72. doi: 10.2174/1381612053381701
183. Dugue B, Leppanen E, Grasbeck R. Preanalytical factors and the measurement of cytokines in human subjects. *Int J Clin Lab Res.* (1996) 26:99–105.
184. Fagiolo U, Cossarizza A, Santacaterina S, Ortolani C, Monti D, Paganelli R, et al. Increased cytokine production by peripheral blood mononuclear cells from healthy elderly people. *Ann N Y Acad Sci.* (1992) 663:490–3. doi: 10.1111/j.1749-6632.1992.tb38712.x
185. Fagiolo U, Cossarizza A, Scala E, Fanales-Belasio E, Ortolani C, Cozzi E, et al. Increased cytokine production in mononuclear cells of healthy elderly people. *Eur J Immunol.* (1993) 23:2375–8. doi: 10.1002/eji.1830230950
186. Bastard JP, Jardel C, Delattre J, Hainque B, Bruckert E, Oberlin F. Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects. *Circulation* (1999) 99:2221–2.
187. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest*. (2003). 112:1785–8. doi: 10.1172/jci200320514
188. Capuron L, Su S, Miller AH, Bremner JD, Goldberg J, Vogt GJ, et al. Depressive symptoms and metabolic syndrome: is inflammation the underlying link? *Biol Psychiatry* (2008) 64:896–900. doi: 10.1016/j.biopsych.2008.05.019
189. Sweat V, Starr V, Bruehl H, Arentoft A, Tirsi A, Javier E, et al. C-reactive protein is linked to lower cognitive performance in overweight and obese women. *Inflammation* (2008) 31:198–207. doi: 10.1007/s10753-008-9065-3
190. Yaffe K. The metabolic syndrome, inflammation, and risk of cognitive decline. *JAMA* (2004) 292:2237. doi: 10.1001/jama.292.18.2237
191. Godbout J, Johnson R. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. *Immunol Allergy Clin N Am.* (2009) 29:321–37. doi: 10.1016/j.iac.2009.02.007
192. Layé S. Polyunsaturated fatty acids, neuroinflammation and well being. *Prostaglandins Leukot Essent Fatty Acids* (2010) 82:295–303. doi: 10.1016/j.plefa.2010.02.006
193. Johannsen A, Susin C, Gustafsson A. Smoking and inflammation: evidence for a synergistic role in chronic disease. *Periodontol 2000* (2013) 64:111–26. doi: 10.1111/j.1600-0757.2012.00456.x
194. Kluge M, Schuld A, Schacht A, Himmerich H, Dalal MA, Wehmeier PM, et al. Effects of clozapine and olanzapine on cytokine systems are closely linked to weight gain and drug-induced fever. *Psychoneuroendocrinology* (2009) 34:118–28. doi: 10.1016/j.psyneuen.2008.08.016
195. Berthold-Losleben M, Himmerich H. The TNF-alpha system: functional aspects in depression, narcolepsy and psychopharmacology. *Curr Neuropharmacol*. 6:193–202. doi: 10.2174/157015908785777238
196. Shapiro PA. Management of depression after myocardial infarction. *Curr. Cardiol Rep.* (2015) 17:10. doi: 10.1007/s11886-015-0634-4
197. Murakami Y, Ishibashi T, Tomita E, Imamura Y, Tashiro T, Watcharanurak K, et al. Depressive symptoms as a side effect of Interferon-alpha therapy induced by induction of indoleamine 2,3-dioxygenase 1. *Sci Rep.* (2016) 6:29920. doi: 10.1038/srep29920
198. Bromberg JS, Hittle L, Xiong Y, Saxena V, Smyth EM, Li L, et al. Gut microbiota-dependent modulation of innate immunity and lymph node remodeling affects cardiac allograft outcomes. *JCI Insight* (2018) 3:19. doi: 10.1172/jci.insight.121045
199. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, et al. Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry* (2001) 58:445–52. doi: 10.1001/archpsyc.58.5.445
200. Pace LA, Crowe SE. Complex Relationships Between Food, Diet, and the Microbiome. *Gastroenterol Clin North Am.* (2016) 45:253–65. doi: 10.1016/j.gtc.2016.02.004
201. Kraus T, Haack M, Schuld A, Hinze-Selch D, Koethe D, Pollmächer T. Body weight, the tumor necrosis factor system, and leptin production during

treatment with mirtazapine or venlafaxine. *Pharmacopsychiatry* (2002) 35:220–5. doi: 10.1055/s-2002-36390
202. Kast RE. Anti- and pro-inflammatory considerations in antidepressant use during medical illness: bupropion lowers and mirtazapine increases circulating tumor necrosis factor-alpha levels. *General Hosp Psychiatry* (2003) 25:495–6. doi: 10.1016/s0163-8343(03)00093-8
203. Gupta R, Gupta K, Tripathi AK, Bhatia MS, Gupta LK. Effect of mirtazapine treatment on serum levels of brain-derived neurotrophic factor and tumor necrosis factor-a in patients of major depressive disorder with severe depression. *Pharmacology* (2016) 97:184–188. doi: 10.1159/000444220
204. Wijkstra J, Lijmer J, Burger H, Geddes J, Nolen WA. Pharmacological treatment for psychotic depression. *Cochrane Database Syst Rev.* (2013) 11:Cd004044. doi: 10.1002/14651858.CD004044.pub3
205. Sumegi A. Mood stabilizers-past, present and future. *Neuropsychopharmacol Hung.* (2008) 10:31–42.
206. Himmerich H, Koethe D, Schuld A, Yassouridis A, Pollmacher T. Plasma levels of leptin and endogenous immune modulators during treatment with carbamazepine or lithium. *Psychopharmacology* (2005) 179:447–51. doi: 10.1007/s00213-004-2038-9
207. Baumeister D, Ciufolini S, Mondelli V. Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? *Psychopharmacology* (2016) 233:1575–89. doi: 10.1007/s00213-015-4044-5
208. Himmerich H, Schönherr J, Fulda S, Sheldrick AJ, Bauer K, Sack U. Impact of antipsychotics on cytokine production *in-vitro*. *J Psychiatr Res.* (2011) 45:1358–65. doi: 10.1016/j.jpsychires.2011.04.009
209. Himmerich H, Bartsch S, Hamer H, Mergl R, Schönherr J, Petersein C, et al. Impact of mood stabilizers and antiepileptic drugs on cytokine production *in-vitro*. *J Psychiatr Res.* (2013) 47:1751–9. doi: 10.1016/j.jpsychires.2013.07.026
210. Jha MK, Minhajuddin A, Gadad BS, Greer TL, Mayes TL, Trivedi MH. Interleukin 17 selectively predicts better outcomes with bupropion-SSRI combination: Novel T cell biomarker for antidepressant medication selection. *Brain Behav Immun.* (2017) 66:103–10. doi: 10.1016/j.bbi.2017.07.005
211. Del Grande da Silva G, Wiener CD, Barbosa LP, Gonçalves Araujo JM, Molina ML, San Martin P, et al. Pro-inflammatory cytokines and psychotherapy in depression: Results from a randomized clinical trial. *J Psychiatric Res.* (2016) 75:57–64. doi: 10.1016/j.jpsychires.2016.01.008
212. Lyngstad SH, Gardsjord ES, Simonsen C, Engen MJ, Romm KL, Melle I, et al. Consequences of persistent depression and apathy in first-episode psychosis — A one-year follow-up study. *Comprehen Psychiatry* (2018) 86:60–6. doi: 10.1016/j.comppsych.2018.07.015
213. Li Y, Xiao B, Qiu W, Yang L, Hu B, Tian X, et al. Altered expression of CD4+CD25+ regulatory T cells and its 5-HT1a receptor in patients with major depression disorder. *J Affect Disord.* (2010) 124:68–75. doi: 10.1016/j.jad.2009.10.018
214. Himmerich H, Milenović S, Fulda S, Plümäkers B, Sheldrick AJ, Michel TM, et al. Regulatory T cells increased while IL-1β decreased during antidepressant therapy. *J Psychiatr Res.* (2010) 44:1052–7. doi: 10.1016/j.jpsychires.2010.03.005
215. Maes M, Anderson G, Kubera M, Berk M. Targeting classical IL-6 signalling or IL-6trans-signalling in depression? *Expert Opin Therapeut Targets* (2014) 18:495–512. doi: 10.1517/14728222.2014.888417
216. Kern S, Skoog I, Börjesson-Hanson A, Blennow K, Zetterberg H, Östling S, et al. Higher CSF interleukin-6 and CSF interleukin-8 in current depression in older women. Results from a population-based sample. *Brain Behav Immun.* (2014) 41:55–8. doi: 10.1016/j.bbi.2014.05.006
217. Krügel U, Fischer J, Radicke S, Sack U, Himmerich H. Antidepressant effects of TNF-α blockade in an animal model of depression. *J Psychiatr Res.* (2013) 47:611–6. doi: 10.1016/j.jpsychires.2013.01.007
218. Schmidt FM, Kirkby KC, Himmerich H. The TNF-alpha inhibitor etanercept as monotherapy in treatment-resistant depression - report of two cases. *Psychiatr Danub*. (2014) 26:288–90.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 21 February 2019
doi: 10.3389/fpsyt.2019.00074

Sex Specific Changes in Tryptophan Breakdown Over a 6 Week Treatment Period

Eva Z. Reininghaus[1], *Nina Dalkner*[1]*, *Karin Riedrich*[1,2], *Dietmar Fuchs*[3,4], *Johanna M. Gostner*[3,4] *and Bernd Reininghaus*[1,2]

[1] Department of Psychiatry and Psychotherapeutic Medicine, Medical University of Graz, Graz, Austria, [2] TZ-Justus Park Bad Hall, Bad Hall, Austria, [3] Division of Medical Biochemistry, Biocenter, Medical University of Innsbruck, Innsbruck, Austria, [4] Division of Biological Chemistry, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

Introduction: Despite the knowledge of sex differences concerning neurobiological parameters as well as clinical course of illness in individuals with mood disorders, the literature concerning tryptophan (Trp) breakdown, specific for women and men, is sparse to date. The current study aimed to evaluate sex differences in Trp, kynurenine (Kyn) and Kyn/Trp concentrations in general, as well as differences in changes of those concentrations over the course of a 6-week rehabilitation program in individuals with life-time unipolar affective disorder. For this purpose changes in Trp and Kyn as well as the Kyn/Trp concentrations between the time of admission (t1) and discharge (t2) were analyzed in dependence of sex. Furthermore, correlations between Trp and Kyn levels and clinical parameters were performed separately for male and female participants.

Material and Methods:

Results: For the current analysis 426 individuals with lifetime affective disorder completing a 6-week rehabilitation program were included. In both sexes, psychiatric symptoms decreased significantly over time. There was a significant difference between women ($n = 242$) and men ($n = 184$) regarding the changes in Trp, Kyn, and Kyn/Trp over time even if controlled for relevant covariates [multivariate: $F_{(3,380)} = 2.663$, $\eta^2 = 0.021$, $p = 0.048$]. Kyn as well as Kyn/Trp concentrations increased significantly in men over time (Kyn $F = 4.809$, $\eta^2 = 0.012$, $p = 0.029$; Kyn/Trp $F = 7.923$, $\eta^2 = 0.020$, $p = 0.005$). Results remained the same when controlled for psychiatric symptoms.

Discussion: The main finding of the present study is the significant difference between women and men regarding the change in Trp, Kyn, and Kyn/Trp over a 6-week psychiatric treatment period, while the depression severity scores as well as general psychiatric symptoms decreased. Sex specific changes in Trp-Kyn pathways have only been explored to a very small extent to date in the literature but are of high clinical relevance in the context of personalized medicine.

Keywords: tryptophan, kynurenine, sex, sex differences, body mass index

OPEN ACCESS

Edited by:
Frank M. Schmidt, Universitätsklinikum Leipzig, Germany

Reviewed by:
Sudhakar Selvaraj, University of Texas Health Science Center at Houston, United States
Hubertus Himmerich, King's College London, United Kingdom

***Correspondence:**
Nina Dalkner
nina.dalkner@medunigraz.at

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *28 September 2018*
Accepted: *01 February 2019*
Published: *21 February 2019*

Citation:
Reininghaus EZ, Dalkner N, Riedrich K, Fuchs D, Gostner JM and Reininghaus B (2019) Sex Specific Changes in Tryptophan Breakdown Over a 6 Week Treatment Period. Front. Psychiatry 10:74. doi: 10.3389/fpsyt.2019.00074

INTRODUCTION

In recent decades, the association between the tryptophan (Trp)-kynurenine (Kyn) axis, low-grade inflammation and neuropsychiatric disorders has been shown in various studies. The Kyn/Trp ratio reflects levels of Trp breakdown and can be used as a proxy measure of indoleamine 2,3-dioxygenase-1 (IDO) activity. An increase in IDO activity is usually driven by chronic low-inflammatory processes which breaks Trp down the Kyn pathway and reduces serotonin and melatonin synthesis (1). A number of studies have proven the role of IDO in cytokine-induced depression by demonstrating correlations between cytokine-induced depression and lower Trp and raised Kyn concentrations as well as an increase in the Kyn/Trp-ratio (2, 3). Originally, the role of such processes has been shown in depressive symptoms accompanying interferon-treated hepatitis C patients (3–6).

Sex Differences

Sex differences in concentrations of clinical and neurobiological biomarkers have been reported in a multitude of studies concerning affective disorders (7–12); however, data on sex specific differences concerning the Trp-Kyn axis and psychiatric disorder are sparse to date. We could recently show a shift toward the (neurotoxic) hydroxykynurenine arm of the kynurenine pathway in adult euthymic males with bipolar disorder and an association with poorer performance in short- and long-term verbal memory (9). Those associations were not present in female individuals with bipolar disorder and point toward the involvement of the Trp-Kyn axis in sex-specific cognitive dysfunction of psychiatric diseases.

Despite the high number of studies investigating the Trp-Kyn pathway in individuals with neuropsychiatric symptoms undergoing cytokine treatment, studies in psychiatric cohorts are comparatively rare and results contradictory. As an example, a recent publication (13), as well as some older studies (14–16) found increased Kyn/Trp in depressive individuals compared to controls. In contrast, other studies found decreased Kyn and a decreased Kyn/Trp in depressed individuals compared to healthy controls (17, 18). Nevertheless, in a cohort study, no correlations between depressive symptoms and Kyn/Trp could be found (17). Inflammatory parameters correlated in most of the studies with Kyn/Trp, but inflammation did not mediate the association between Kyn/Trp and depressive symptoms (17).

Measuring clinical and neurobiological changes over time during a therapeutic process requires a very structured treatment with a predefined treatment plan that applies the same therapy levels to all patients. Psychiatric rehabilitation settings provide an intensive, multidisciplinary 6-week program for individuals with serious mental illness, most typically suffering from affective disorders and due to the structured program, treatment outcome can be easily analyzed. In this setting, the current symptoms of patients are not serious enough to require acute psychiatric care; most patients receive rehabilitative treatment following acute therapy in an psychiatric hospital. Current acute and severe psychopathology is not necessarily prevalent in all patients. The rehabilitation program is a structured and targeted setting which includes medical, psychiatric, psychological and psychotherapeutic treatments, as well as occupational therapy, physiotherapy and diet counseling. The principal goals of the rehabilitation setting include long-term management of psychiatric symptom, strengthening of social skills, active participation in everyday life, improving of cognitive functioning, and decreasing the rates of re-hospitalization and retirement due to disability (19). We recently showed that there is an association between the therapeutic response to this multimodal treatment and changes in Kyn concentrations, the Kyn/Trp ratio as well as high sensitive C-reactive protein (hsCRP) in severely depressed patients not receiving cytokine treatment (20). Importantly, Kyn increased significantly in the patient group not responding to treatment, while the Kyn/Trp ratio decreased significantly in the group of responders over time. In addition, changes in Kyn as well as hsCRP concentrations correlated significantly with changes in the body mass index (BMI) over time in this study. Nevertheless, sex specific aspects could not be considered due to small subgroup sample sizes.

Despite the knowledge of sex differences concerning neurobiological biomarkers as well as clinical course of illness in individuals with mood disorders, the literature concerning Trp breakdown, specific for women and men, is sparse to date. The current study aimed to evaluate sex differences in Trp, Kyn, and Kyn/Trp concentrations in general, as well as differences in changes of those concentrations over the course of a 6-week rehabilitation program in individuals with life-time unipolar affective disorder in an exploratory study design. As a unidirectional differential hypothesis we formulated that there is a significant difference in the change of Trp, Kyn, and Kyn/Trp concentrations over a 6-week rehabilitation treatment setting between women and men. For this purpose, changes in Trp and Kyn concentrations as well as the Kyn/Trp ratio between the time of admission (t1) and discharge (t2) were analyzed in dependence of sex. Furthermore, correlations between Trp and Kyn concentrations and clinical parameters were performed separately for male and female participants.

MATERIALS AND METHODS

Participants

The study was conducted at a psychiatric rehabilitation center with treatment focus on affective and stress-related disorders. Data of 600 individuals treated between April 2015 and April 2017 were available, of whom 426 patients had a life-time diagnosis of unipolar affective disorder (F32 and F33 according to ICD-10 diagnosis) and were included in the current analysis. The treating psychiatrist performed the respective diagnoses according to the ICD-10 diagnosis criteria [International classification of mental disorders (21)] using record reviews. None of the patients had a substance use disorder as a main diagnosis, as this was an exclusion criteria for taking part in the rehabilitation setting in general. All participants completed the 6-week rehabilitation program, consisting of weekly medical consultations, psychotherapy (single and group setting), occupational therapy, physiotherapy, physical training

TABLE 1 | *Descriptive statistics* of women and men at the time of admission.

T1	Females *M* (*SD*)	Males *M* (*SD*)	Differences between women and men
Age [years]	53.6 (7.8)	51.7 (8.0)	$z = -3.0$, $p = 0.003$**
BMI [kg/m²]	26.0 (5.0)	27.2 (4.1)	$z = -3.0$, $p = 0.002$**
Smoking severity	0.81 (1.8)	1.1 (2.1)	n.s.
Cardiovascular disease (%)	38.2%	52.5%	$t = -2.9$, $p = 0.004$**
hsCRP	2.1 (2.1)	2.0 (2.0)	n.s.
IL-6	2.1 (2.1)	2.8 (3.0)	$z = -2.5$, $p = 0.012$*
BDI-II	21.8 (9.8)	20.0 (10.8)	n.s.
HAM-D	11.3 (6.0)	12.4 (7.4)	n.s.
GSI	1.1 (0.6)	1.0 (0.6)	$z = -2.0$ $p = 0.043$*
Trp t1 [μmol]	62.4 (7.6)	68.0 (8.0)	$F_{(3,407)} = 15.154$, $p = 0.000$** Trp $F = 43.0$, $p = 0.000$**
Kyn t1 [μmol]	1.8 (0.47)	1.9 (0.42)	Kyn $F = 1.4$, $p = 0.241$
Kyn/Trp t1 [μmol]	0.0298 (0.0074)	0.0281 (0.0057)	Kyn/Trp $F = 6.5$, $p = 0.011$*

**significant for $p < 0.05$, ** significant at $p < 0.01$; BMI, body mass index; hsCRP, high sensitive reactive protein; IL-6, interleukin 6; BDI-II, Becks Depression Inventory; HAM-D, Hamilton Depression Score; GSI, Global severity index (SCL); Trp, Tryptophan; Kyn, Kynurenine.*

as well as diet counseling. Every patient had to attend 18–20 h of therapy per week, this was performed either in a single or a group setting. In general all patients attending psychiatric rehabilitation received a very similar structured and targeted program. The patients undergoing the psychiatric rehabilitation program differed in their stage of recovery. In general, the psychiatric rehabilitation setting follows acute psychiatric inpatient treatment protocols; however waiting times for therapy program initiation are different and dependent on insurance approval. The whole study enrolled current psychiatric symptoms, complete lifetime psychiatric history, anthropometric measure, fasting blood, psychological testing and various lifestyle questionnaires, data were collected by trained clinic staff.

The study has been approved by the local ethics committee of the Medical University of Linz, Austria, in accordance with *The Code of Ethics* of the World Medical Association (Declaration of Helsinki 1964; General Assembly of the World Medical Association, 2014), ICH guideline for Good Clinical Practice and current regulations (EK-number: E-24-14). Written consent was obtained from all participants at the time of admission and all received the same study procedure.

Psychological Inventories

Depressive symptoms were assessed with the Hamilton Depression Scale (HAM-D) (22) and the Beck Depression Inventory (BDI-II) (23). The Symptom-Checklist Revised (SCL-90-R; (24), a 90-item self-report inventory, was used to assess a broad range of psychological symptoms and psychological distress in the last 7 days. Cognitive, physical, and emotional symptoms of distress, and overall distress were rated on a five-point Likert scale with 53 items and the Global Severity Index (GSI) was used for the current analysis to capture fundamental psychological distress.

Biological Assays

For the measurement of serum inflammatory markers and amino acids, fasting blood samples were collected between 8.00 and 10 a.m., samples were either processed immediately for further analyses (hsCRP, IL-6) or stored at −80°C until thawed for the biological assays. Free Trp and Kyn serum concentrations were determined by high-performance liquid chromatography, as described earlier (25). Kyn/Trp was calculated as index of Trp breakdown providing a proxy of IDO activity (if it correlates with markers of immune activation). Levels of hsCRP were analyzed by Fa Abbott with Architect ci8200 and IL-6 was analyzed by Fa Roche with Cobas e411.

Statistics

Description of the Cohort at the Time of Admission

Due to distribution of data, nonparametric tests were used. For comparing means at t1, Mann-Whitney-U-Tests were used. Additionally, Spearmann-correlations between Trp, Kyn, and Kyn/ Trp and clinical variables at t1 were calculated. A multivariate analysis of co-variance (MANCOVA) with sex as the between subject factor and the concentration of Trp, Kyn, and Kyn/Trp at t1 as dependent variables was performed. Age, BMI, interleukin-6 (IL-6), GSI and cardiovascular disease were inserted as covariates as they differed between women and men at the time of admission. Moderation analysis was used to calculate if sex significantly moderates the correlations between depressive symptoms measured with BDI-II as well as HAM-D and metabolites at the time of admission (moderator variable = sex, independent variable = BDI-II or HAM-D, dependent variable = respective metabolite).

Concerning medical comorbidities, cardiovascular disease (including hypertension) was significantly more often found in men ($p = 0.004$, 53% vs. 38%), while there was no difference in the prevalence of diabetes mellitus or lipid associated diseases between men and women in our cohort.

Changes Over Time

Changes of the respective variables between t1 and t2 were calculated as a mean difference (respective variable_Diff). For statistical analyses we conducted a multivariate analysis of co-variance (MANCOVA) with sex as the between subjects factor and the changes of Trp, Kyn and Kyn/Trp as dependent variables. Age, BMI, IL-6 and cardiovascular disease were inserted as covariates as they differed between women and men at the time of admission (see **Table 1**). In a further step, psychiatric symptoms, namely HAM-D_Diff as well as GSI and BDI, were introduced as covariates. Within the sex groups, non-parametric Wilcoxon Rank Sum Test was used to analyze changes in Trp, Kyn, and Kyn/ Trp over time. Non-parametric correlations (Spearmann-Rho) between the mean change of Trp, Kyn, and Kyn/Trp between t1 and t2 and the changes in clinical variables between t1 and t2 were analyzed. Moderation analysis was used to test whether sex can significantly moderate a possible correlation between depressive symptoms measured with BDI

TABLE 2 | Associations of serum tryptophan and kynurenine concentrations with clinical parameters at the time of admission.

	Women			Men		
	Trp [μmol/L]	**Kyn [μmol/L]**	**Kyn/Trp [μmol]**	**Trp [μmol/L]**	**Kyn [μmol/L]**	**Kyn/Trp [μmol]**
Age [years]	$r = -0.025$ $p = 0.697$	***r* = 0.298**** ***p* = 0.000**	***r* = 0.308**** ***p* = 0.000**	$r = -0.131$ $p = 0.077$	***r* = 0.241**** ***p* = 0.001**	***r* = 0.331***** ***p* = 0.000**
BMI [kg/m²]	$r = 0.151^*$ $p = 0.020$	***r* = 0.238**** ***p* = 0.000**	$r = 0.161^*$ $p = 0.013$	$r = 0.047$ $p = 0.526$	***r* = 0.300***** ***p* = 0.000**	***r* = 0.269***** ***p* = 0.000**
Smoking severity	$r = 0.078$ $p = 0.228$	$r = -0.79$ $p = 0.222$	$r = -0.128$ $p = 0.048^*$	$r = -0.009$ $p = 0.909$	$r = -0.176$ $p = 0.019^*$	$r = -0.195$ $p = 0.009^*$
BDI-II	$r = -0.121$ $p = 0.064$	$r = -0.123$ $p = 0.059$	$r = -0.80$ $p = 0.221$	$r = -0.188^*$ $p = 0.012$	***r* = -0.243**** ***p* = 0.001**	$r = -0.165^*$ $p = 0.028$
HAM-D	$r = -0.143^*$ $p = 0.035$	$r = -0.081$ $p = -231$	$r = 0.007$ $p = 0.920$	$r = -0.145$ $p = 0.058$	$r = -0.181^*$ $p = 0.018$	$r = -0.113$ $p = 0.140$
GSI	$r = -0.159^*$ $p = 0.014$	$r = -0.124$ $p = 0.056$	$r = -0.68$ $p = 0.298$	$r = -0.116$ $p = 0.119$	$r = -0.115$ $p = 0.123$	$r = -0.043$ $p = 0.567$
hsCRP	$r = 0.093$ $p = 0.148$	***r* = 0.216**** ***p* = 0.001**	***r* = 0.189**** ***p* = 0.003**	$r = -0.112$ $p = 0.128$	$r = 0.186^*$ $p = 0.012$	***r* = 0.229** ***p* = 0.002****
IL-6	$r = -0.024$ $p = 0.707$	$r = 0.163^*$ $p = 0.011$	$r = 0.183$ $p = 0.004^*$	$r = -0.150$ $p = 0.042$	$r = 0.073$ $p = 0.322$	$r = 0.163^*$ $p = 0.027$

**significant at $p < 0.005$; **significant if corrected for Bonferroni at $p < 0.004$; BMI, body mass index; hsCRP, high sensitive reactive protein; IL-6, interleukin 6; BDI-II, Becks Depression Inventory; HAM-D, Hamilton Depression Score; GSI, Global severity index (SCL); Trp, Tryptophan; Kyn, Kynurenine.*

as well as HAM-D and differences in metabolites over time. Error probabilities below 0.05 were accepted to denote statistical significance for *t*-tests and multivariate analyses. Additionally, due to the multitude of clinical variables, variables at $p < 0.004$ are given for correlation analyses to mark Bonferroni corrected values.

RESULTS

Time of Admission

Participants had a mean age of 52.8 (SD 8.0) and a mean BMI of 26.5 (SD 4.7), 56.8% ($n = 242$) were female. Of all participants, 29.3% had university or polytechnic degree, 19.4% were divorced and 13.4% were single without a partner. 33.3% did exercise at least once a week, 43.4% had a therapy with Serotonin reuptake inhibitors, 23.2% with serotonin and norepinephrine reuptake inhibitors and 12.2% with atypical antipsychotics. Men had significantly lower age and higher levels in the GSI, higher BMI, IL-6 and more cardiovascular disease as well as higher Trp and lower Kyn/Trp compared to females. There was no difference in smoking severity (Fagerstroem Test for nicotine dependence), depressive symptoms or hsCRP levels at the time of admission. Further descriptive data for both groups at the time of admission are displayed in **Table 1**.

In both sexes, there was a significant correlation between Kyn and age as well as BMI. Furthermore, there was a significant correlation between Kyn/Trp and age as well as hsCRP. In women only, we found a significant correlation between Kyn and hsCRP as well as between Kyn/Trp and IL-6. In men only, we found a significant correlation between Kyn/Trp and BMI as well as a negative correlation between Kyn and BDI-II. Moderation analyses showed that sex did not significantly moderate the correlation between depressive symptoms and Trp, Kyn as well as Kyn/Trp at the time of admission.

Table 2 gives the associations between Trp, Kyn, and Kyn/Trp concentrations and clinical parameters at t1. **Table 3** gives an overview about the associations at t2.

TABLE 3 | Descriptive statistics of women and men at the time of discharge.

T2	Women	Men	
BMI t2 [kg/m²]	25.7, $SD = 4.7$	26.8, $SD = 3.8$	$t = 2.8$, $p = 0.005^{**}$
hsCRP	1.8 (1.9)	1.7 (1.7)	n.s.
IL-6	2.3 (1.8)	2.7 (2.2)	$t = 2.0$, $p = 0.042^*$
BDI-II t2	10.9 (10.4)	11.1 (10.1)	n.s.
HAM-D t2	7.4 (5.7)	7.2 (5.7)	n.s.
GSI	0.6 (0.5)	0.6 (0.5)	n.s.
TRP t2 [μmol]	62.7, $SD = 8.5$	68.3, $SD = 8.5$	$F_{(3,395)} = 12.6$, $p = 0.000^{**}$ (corrected for age, BMI, IL-6)
KYN t2 [μmol]	1.8 (0.46)	2.0 (0.42)	Trp $F = 37.6$, $p = 0.000^{**}$ Kyn $F = 10.9$, $p = 0.001^{**}$
KYN/TRP t2 [μmol]	0.0292 (0.0068)	0.0290 (0.0056)	Kyn/Trp $F = 0.094$, $p = 0.760$

**significant for $p < 0.05$, **significant at $p < 0.01$; BMI, body mass index; hsCRP, high sensitive reactive protein; IL-6, interleukin 6; BDI-II, Becks Depression Inventory; HAM-D, Hamilton Depression Score; GSI, Global severity index (SCL); Trp, Tryptophan; Kyn, Kynurenine.*

Changes Over Time

In both sexes, psychiatric symptoms decreased significantly over time (HAM-D $M = -4.641$, $SD = 0.510$, BDI $M = -10.519$, $SD = 9.538$, GSI $M = -1.141$, $SD = 0.510$). Reductions of HAM-D scores were significantly higher in men ($M = -5.262$, $SD = 5.076$) compared to women over time ($M = -4.133$, $SD = 4.584$).

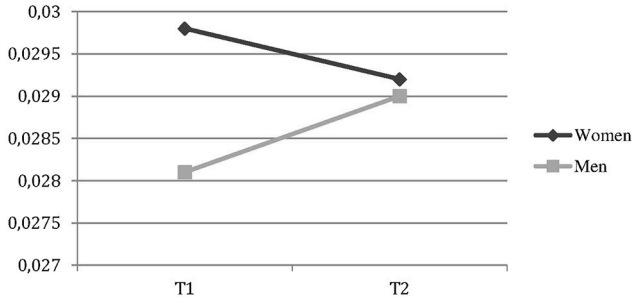

FIGURE 1 | Significantly different changes in kynurenine/tryptophan ratio in women vs. men over time. t1, time of admission; t2, time of discharge.

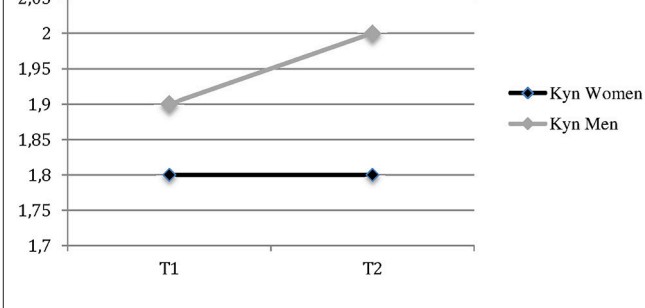

FIGURE 2 | Significantly different changes in kynurenine in women vs. men over time Kyn, Kynurenine; t1, time of admission; t2, time of discharge.

BMI, BDI-II, GSI, and hsCRP levels decreased significantly over time in both sexes, but there were no significant differences in the changes over time between women and men. The levels of IL-6 remained stable over time in both sexes.

There was a significant difference between women and men regarding the change in Trp, Kyn, and Kyn/Trp over time even if controlled for age, BMI, IL-6 and cardiovascular disease [multivariate: $F_{(3,380)} = 2.663$ $p = 0.048$, $\eta^2 = 0.021$]. Kyn as well as Kyn/Trp increased in men compared to women (Kyn_Diff $F = 4.809$, $p = 0.029$, $\eta^2 = 0.012$; Kyn/Trp_Diff $F = 7.923$, $p = 0.005$, $\eta^2 = 0.020$). Wilcoxon Rank Sum Test showed that Kyn and Kyn/Trp increased significantly in men (Kyn_Diff $z = -2.077$, $p = 0.038$; Kyn/Trp_Diff $z = -2.307$, $p = 0.21$).When psychiatric symptoms were included in the multivariate analysis (GSI, BDI, HAM_Diff) the results remained the same [multivariat: $F_{(3,346)} = 2.796$, $p = 0.040$, $\eta^2 = 0.024$] with similar significant changes in Kyn_Diff as well as Kyn/Trp_Diff. Moderation analysis showed that the interaction of the change in objective depressive symptoms (HAM_Diff) and sex significantly moderated the change in Kyn/Trp over time [$F_{(3,382)} = 5.571$, $p = 0.001$; interaction effect: $\beta = 0.107$, $T = 2.099$, $p = 0.036$].

The median Kyn and Kyn/Trp changes in men and women are shown in **Figures 1**, **2**.

There were significant positive correlations between the changes in BMI over time and changes in Trp as well as Kyn concentrations in women and between BMI changes and changes in Kyn as well as Kyn/Trp in men (Bonferroni corrected) if only Bonferroni corrected values were included. Further results with lower significance can be obtained from **Table 4**.

DISCUSSION

The aim of the current analysis was to evaluate sex dependent changes in serum concentrations of Trp, Kyn and the Kyn/Trp in individuals with life-time unipolar affective disorder over a 6-week rehabilitation treatment course. At the time of admission, Trp was significantly lower and Kyn/Trp higher in women compared to men. There was a significant difference between women and men regarding the change in Trp, Kyn and Kyn/Trp over time, with increases in Kyn as well as in Kyn/Trp in men (even if controlled for relevant covariates). In both sexes, psychiatric symptoms decreased significantly over the course of treatment, reductions of HAM-D scores were significantly higher in men compared to women but did not correlate with changes in Trp breakdown. Importantly, the interaction of sex and the change in depressive symptomatology over time measured with the HAM-D significantly moderated the change in Kyn/Trp over time.

Furthermore, inflammatory marker hsCRP correlated with Kyn in both sexes, as well as with Kyn/Trp in women at the time of admission. Importantly, BMI correlated significantly with Kyn in both sexes, while it correlated with Kyn/Trp only in men. The correlations concerning the BMI were similar with the changes over time.

Sex differences in affective disorders are most consistently displayed in the increased lifetime prevalence of major depression in women compared to men. Sex differences on a neurobiological level can converge in (1) producing the same clinical outcome, (2) different outcomes or (3) result in unclear physiologic consequence [for a review see (11)]. Female and male sex hormone interactions with Kyn pathway activation have, in a small content, been described in the literature. Trp and Kyn concentrations were found approximately 15% higher in men compared to women (25). Schröcksnadel et al. (26) found Kyn/Trp rise in pregnancy, implicating that Kyn pathway activation is associated with changes in hormone levels. In addition, estrogen and progesterone have been shown to induce and androgens to inhibit Kyn pathway activity (27–30). In concordance with our results, a recent study found that women with current as well as lifetime depression had lower levels of Trp in both serum and cerebrospinal fluid compared to men (31), in the study by Elovainio et al. (32) the Kyn/Trp was found to predict depressive symptoms in women only. Furthermore, the IDO enzyme was involved in immune regulation of early atherosclerosis (33), particularly among females (34).

Only a few studies have analyzed the general effects of psychiatric treatment on the Trp-Kyn pathway, some of them evaluated the effect of antidepressant therapy with inconsistent results. Importantly, treatment response was not evaluated in

TABLE 4 | Associations between changes of serum tryptophan and kynurenine concentrations over time.

	Women			Men		
	Trp _Diff	**Kyn _Diff**	**Kyn/ Trp _Diff**	**Trp _Diff**	**Kyn _Diff**	**Kyn/ Trp _Diff**
BMI_Diff	***r* = 0.286** *p* = 0.000**	***r* = 0.288** *p* = 0.000**	*r* = 0.097 *p* = 0.147	*r* = 0.185* *p* = 0.014	***r* = 0.357** *p* = 0.000**	***r* = 0.244** *p* = 0.001**
Smoking severity	*r* = 0.007 *p* = 0.917	*r* = 0.072 *p* = 0.281	*r* = 0.099 *p* = 0.140	*r* = 0.195* *p* = 0.010	*r* = 0.163* *p* = 0.032	*r* = −0.019 *p* = 0.800
BDI_Diff	*r* = 0.077 *p* = .0291	*r* = 0.053 *p* = 0.472	*r* = 0.034 *p* = 0.642	*r* = −0.024 *p* = 0.772	*r* = 0.077 *p* = 0.355	*r* = 0.092 *p* = 0.267
HAM-D_Diff	*r* = −0.097 *p* = 0.160	*r* = −0.178* *p* = 0.009	*r* = −0.090 *p* = 0.189	*r* = −0.076 *p* = 0.323	*r* = –0.024 *p* = 0.752	*r* = 0.077 *p* = 0.320
GSI_Diff	*r* = −0.023 *p* = 0.750	*r* = −0.047 *p* = 0.506	*r* = −0.004 *p* = 0.959	*r* = −0.027 *p* = 0.740	*r* = 0.079 *p* = 0.324	*r* = 0.131 *p* = 0.099
hsCRP_Diff	*r* = 0.030 *p* = 0.655.	*r* = 0.171* *p* = 0.010	*r* = 0.191* *p* = 0.004	*r* = −0.056 *p* = 0.485	*r* = 0.171* *p* = 0.022	*r* = 0.217* *p* = 0.004
IL6_Diff	*r* = 0.119 *p* = 0.075	*r* = 0.164* *p* = 0.013	*r* = 0.124 *p* = 0.063	*r* = −0.089 *p* = 0.239	*r* = 0.060 *p* = 0.427	*r* = 0.163* *p* = 0.030

**significant at $p < 0.005$, **significant after Bonferroni correction at $p < 0.004$; Diff, Difference; BMI, body mass index; hsCRP, high sensitive reactive protein; IL-6, interleukin 6; BDI-II, Becks Depression Inventory; HAM-D, Hamilton Depression Score; GSI, Global severity index; Trp, Tryptophan; Kyn, Kynurenine.*

all these studies as well as no stratification for sex has been performed. Reductions in Kyn pathway neurotoxic metabolites and Kyn/Trp, as well as increased Kyn/Trp after antidepressant treatment have been observed by Halaris et al. (35) and Myint et al. (15). In another study Dahl et al. (36), no changes in Kyn and metabolites in medication free depressive individuals were found after 12 weeks of treatment. Interestingly, Kyn/Trp increased during the treatment with electroconvulsive therapy (37). Sample size was small in this hitherto literature, including between 19 and 58 patients only.

Another important result of the current study was the significant positive correlation between changes in BMI and Kyn in both sexes, as well as with Kyn/Trp only in men. We could previously show a general correlation between changes in Kyn and BMI over time in a depressive extreme-group subsample of this cohort (20), this correlation could be confirmed in the current general sample. Overweight and obesity have been shown to be associated with increased immune activation and Trp breakdown in mentally healthy individuals (38–41), even more, IDO expression in adipose and hepatic tissue was higher in obese compared to lean women (42). In bipolar disorder, Kyn/Trp ratio was increased in overweight/obese compared to normal-weight patients (43). It has been assumed that overweight/obesity and metabolic syndrome, as well as associated somatic comorbidities, may be linked to the etiology, course and treatment of psychiatric disorders. The activation of the Kyn pathways in overweight/obese individuals may be an important factor of how BMI interacts with mood dysregulation.

Furthermore, also the brain-gut-axis is associated with Trp-Kyn metabolism (44). Changes in the composition of gut microbes can modulate plasma concentrations of Trp and his metabolites (45). In line with this, the microbiota is known to play a role in the regulation of serotonin synthesis, which is potentially mediated by IDO expression and stress response axis (46). Dietary and stress factors may mediate some of their effects on the gut microbiome by influencing gut permeability as well as low-level immune-inflammatory responses. Leblhuber et al. (47). Poor diet in obesity can negatively affect gut permeability, thereby contributing to an increase in gut-linked immune-inflammatory processes, with increased pro-inflammatory cytokines feeding back to further increase gut permeability.

TRP via serotonin and melatonin, is involved in the regulation of satiety and caloric intake. Intake of food rich in TRP can increase Trp availability in the body and induce the enzymatic machinery. However, this does not necessarily lead to an increase of serotonin availability in the brain because tryptophan has to pass the blood brain barrier in competition with the so-called large neutral amino acids. Nutrients rich in Trp usually also contain other amino acids in high concentrations and Trp is therefore not effectively transported into the brain. Since the release of insulin after ingestion of non-fructose carbohydrate can shift this ratio toward TRP, an individual with decreased levels of serotonin would crave carbohydrate-rich food as compensatory to serotonin depletion. In contrast, food rich in antioxidants is assumed to have two positive effects on serotonin production rates: they support serotonin biosynthesis and they slow down production of inflammatory products and associated Trp breakdown (48). After dietary intake, systemic Trp levels are regulated by hepatic trytpophan 2,3 dioxygenase (TDO). Dietary antioxidants can therefore increase brain serotonin availability but Trp does not necessarily increase in the blood. Serotonin, by regulating carbohydrate and fat intake, can lower caloric intake. Nevertheless, it is not just the amount of dietary Trp, which determines tryptophan availability, the immune system status can have a drastic influence to lower Trp levels in case of continuous activation (48). In this context it is also interesting that Kyn and the Kyn/Trp correlated positively with

high levels of carbohydrate craving in a former study (49). The correlation of increased Kyn with food craving, especially carbohydrate craving, probably indicates a regulatory deficit in the maintenance of chronic inflammatory processes in obesity and BD. Sex dependent changes in stress management as well as nutritional behavior during psychiatric rehabilitation may have influenced changes in Kyn as well as Kyn/Trp in our sample.

LIMITATIONS

As it is a naturalistic clinical study, patients were not free from medication and somatic comorbidities were present to some content. We did not analyze possible influences of psychotropic medication has due to the large inhomogeneity of drugs and doses taken. As we know from clinical studies that especially antidepressant medication might have influence on Trp and Kyn pathways, changes in Kyn as well as Kyn/Trp over time might have been associated with the respective medication. Due to the naturalistic nature and moderate sample size of our study it was not feasible to factor in all parameters that could potentially alter the association between Kyn downstream catabolits and cognitive performance. Accounting, for instance, for each and every medication would have yielded a myriad of permutations of psychopharmacological treatment combinations not fit for statistical analysis. In line with this, we also want to state that patients received a mixture of different treatments and therefore changes in the Kyn pathway cannot be linked to a specific therapeutic mechanism.

Amino acids concentrations were only measured in the serum of patients and may not accurately reflect central concentrations. However, cytokine induced increases in plasma Kyn in individuals treated with IFN- alpha correlated with increased Kyn in the cerebrospinal fluid (CSF) as well as with activated inflammatory responses and behavioral changes (50, 51). A more recent study also found significant correlations between Trp, Kyn and Kyn/Trp between serum and CSF in individuals with current as well as life-time depression (31).

The patients undergoing the psychiatric rehabilitation program differed in their stage of recovery; not all were depressive at the time of admission. However, the current study was not designed to investigate the association between a specific treatment and Kyn pathway activity, and we therefore cannot make any claims related to specific antidepressants or psychological therapies, nor on their respective effects on study outcomes.

CONCLUSION

Knowledge about general changes in the Trp-Kyn pathway in individuals with affective disorders as well as about specific dysregulations in subsets of patients or differences between women and men will be of particular relevance when selecting subjects for future treatment trials targeting the Trp-Kyn pathway. The main finding of the present study was the significant difference between women and men regarding the change in Trp, Kyn and Kyn/Trp over a 6-week psychiatric treatment period, when the depression severity scores as well as general psychiatric symptoms decreased. Also, associations with BMI support previous findings of the importance of metabolic processes in affective disorders.

AUTHOR CONTRIBUTIONS

ER and ND: analysis of data and drafting the work; ER, KR, ND, and BR: coordination of study; DF and JG: measurement of laboratory parameters concerning Trp breakdown; All authors: revising the paper critically for important intellectual content, substantial contributions to the conception or design of the work, contributed to manuscript revision, read and approved the submitted version, interpretation of data, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Fuchs D, Moller AA, Reibnegger G, Stockle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *J Acquir Immune Defic Syndr.* (1990) 3:873–6.
2. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry* (2002) 7:468–73. doi: 10.1038/sj.mp.4000995
3. Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, et al. Interferon-alpha-induced changes in tryptophan metabolism. Relationship to depression and paroxetine treatment. *Biol Psychiatry* (2003) 54:906–14. doi: 10.1016/S0006-3223(03)00173-2
4. Bonaccorso S, Marino V, Biondi M, Grimaldi F, Ippoliti F, Maes M. Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus. *J Affect Disord.* (2002) 72:237–41. doi: 10.1016/S0165-0327(02)00264-1
5. Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. *J Clin Psychopharmacol.* (2002) 22:86–90. doi: 10.1097/00004714-200202000-00014
6. Capuron L, Dantzer R. Cytokines and depression: the need for a new paradigm. *Brain Behav Immun.* (2003) 17 (Suppl. 1):S119–24. doi: 10.1016/S0889-1591(02)00078-8
7. Queissner R, Pilz R, Dalkner N, Birner A, Bengesser SA, Platzer M, et al. The relationship between inflammatory state and quantity of affective episodes in bipolar disorder. *Psychoneuroendocrinology* (2018) 90:61–67. doi: 10.1016/j.psyneuen.2018.01.024
8. Fellendorf FT, Kainzbauer N, Platzer M, Dalkner N, Bengesser SA, Birner A, et al. Gender differences in the association between physical activity and cognitive function in individuals with bipolar disorder. *J Affect Disord.* (2017) 221:232–7. doi: 10.1016/j.jad.2017.06.048
9. Platzer M, Dalkner N, Fellendorf FT, Birner A, Bengesser SA, Queissner R, et al. Tryptophan breakdown and cognition in bipolar disorder. *Psychoneuroendocrinology* (2017) 81:144–50. doi: 10.1016/j.psyneuen.2017.04.015
10. Birner A, Seiler S, Lackner N, Bengesser SA, Queissner R, Fellendorf FT, et al. Cerebral white matter lesions and affective episodes correlate in

male individuals with bipolar disorder. *PLoS ONE* (2015) 10:e0135313. doi: 10.1371/journal.pone.0135313
11. Rubinow DR, Schmidt PJ. Sex differences and the neurobiology of affective disorders. *Neuropsychopharmacology* (2018) 44:111–28. doi: 10.1038/s41386-018-0148-z.
12. Bengesser SA, Lackner N, Birner A, Fellendorf FT, Platzer M, Mitteregger A, et al. Peripheral markers of oxidative stress and antioxidative defense in euthymia of bipolar disorder–Gender and obesity effects. *J Affect Disord.* (2015) 172:367–74. doi: 10.1016/j.jad.2014.10.014
13. Messaoud A, Mensi R, Douki W, Neffati F, Najjar MF, Gobbi G, et al. Reduced peripheral availability of tryptophan and increased activation of the kynurenine pathway and cortisol correlate with major depression and suicide. *World J Biol Psychiatry* (2018) 23:1–9. doi: 10.1080/15622975.2018.1468031
14. Gabbay V, Klein RG, Katz Y, Mendoza S, Guttman LE, Alonso CM, et al. The possible role of the kynurenine pathway in adolescent depression with melancholic features. *J Child Psychol Psychiatry* (2010) 51:935–43. doi: 10.1111/j.1469-7610.2010.02245.x
15. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J Affect Disord.* (2007) 98:143–51. doi: 10.1016/j.jad.2006.07.013
16. Sublette ME, Galfalvy HC, Fuchs D, Lapidus M, Grunebaum MF, Oquendo MA, et al. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. *Brain Behav Immun.* (2011) 25:1272–8. doi: 10.1016/j.bbi.2011.05.002
17. Quak J, Doornbos B, Roest AM, Duivis HE, Vogelzangs N, Nolen WA, et al. Does tryptophan degradation along the kynurenine pathway mediate the association between pro-inflammatory immune activity and depressive symptoms? *Psychoneuroendocrinology* (2014) 45:202–10. doi: 10.1016/j.psyneuen.2014.03.013
18. Pompili M, Lionetto L, Curto M, Forte A, Erbuto D, Montebovi F, et al. Tryptophan and kynurenine metabolites: are they related to depression? *Neuropsychobiology* (2018) 77:23–8. doi: 10.1159/000491604
19. Hinterhuber H, Meise U. The importance of medical-psychiatric rehabilitation. *Neuropsychiatr* (2007) 21:S1–4.
20. Reininghaus B, Riedrich K, Dalkner N, Bengesser SA, Birner A, Platzer M, et al. Changes in the tryptophan-kynurenine axis in association to therapeutic response in clinically depressed patients undergoing psychiatric rehabilitation. *Psychoneuroendocrinology* (2018) 94:25–30. doi: 10.1016/j.psyneuen.2018.04.029
21. Dilling H, Freyberger HJ. *Taschenführer zur ICD-10-Klassifikation psychischer Störungen*. Vol. 5, Bern: Verlag Hans Huber (2010). p. 1–532.
22. Hamilton, M. Hamilton depression scale. In: Guy W editor. *ECDEU Assessment Manual For Psychopharmacology, rev. edn.* Rockville, MD: National Institute of Mental Health (1978). p. 179–92.
23. Hautzinger M, Keller F, Kühner C. *Beck Depressions-Inventar (BDI-II).* Frankfurt: Harcourt Test Services (2006).
24. Derogatis LR, Unger R. *Symptom Checklist-90-Revised*. Hoboken, NJ: John Wiley & Sons (2010).
25. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin Chem.* (1997) 43:2424–6.
26. Schrocksnadel K, Widner B, Bergant A, Neurauter G, Schrocksnadel H, Fuchs D. Tryptophan degradation during and after gestation. *Adv Exp Med Biol.* (2003) 527:77–83. doi: 10.1007/978-1-4615-0135-0_8
27. Braidman IP, Rose DP. Effects of sex hormones on three glucocorticoid-inducible enzymes concerned with amino acid metabolism in rat liver. *Endocrinology* (1971) 89:1250–5. doi: 10.1210/endo-89-5-1250
28. Rose DP, Braidman IP. Excretion of tryptophan metabolites as affected by pregnancy, contraceptive steroids, and steroid hormones. *Am J Clin Nutr.* (1971) 24:673–83. doi: 10.1093/ajcn/24.6.673
29. Rose DP, Adams PW. Oral contraceptives and tryptophan metabolism: effects of oestrogen in low dose combined with a progestagen and of a low-dose progestagen (megestrol acetate) given alone. *J Clin Pathol.* (1972) 25:252–8. doi: 10.1136/jcp.25.3.252
30. Moursi GE, Abdel-Daim MH, Kelada NL, Abdel-Tawab GA, Girgis LH. The influence of sex, age, synthetic oestrogens, progestogens and oral contraceptives on the excretion of urinary tryptophan metabolites. *Bull World Health Organ* (1970) 43:651–61.
31. Hestad KA, Engedal K, Whist JE, Farup PG. The relationships among tryptophan, kynurenine, indoleamine 2,3-dioxygenase, depression, and neuropsychological performance. *Front Psychol.* (2017) 8:1561. doi: 10.3389/fpsyg.2017.01561
32. Elovainio M, Hurme M, Jokela M, Pulkki-Raback L, Kivimaki M, Hintsanen M, et al. Indoleamine 2,3-dioxygenase activation and depressive symptoms: results from the Young Finns study. *Psychosom Med.* (2012) 74:675–81. doi: 10.1097/PSY.0b013e318266d0f5
33. Murr C, Grammer TB, Kleber ME, Meinitzer A, Marz W, Fuchs D. Low serum tryptophan predicts higher mortality in cardiovascular disease. *Eur J Clin Invest.* (2015) 45:247–54. doi: 10.1111/eci.12402
34. Pertovaara M, Raitala A, Juonala M, Lehtimaki T, Huhtala H, Oja SS, et al. Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the cardiovascular risk in Young Finns study. *Clin Exp Immunol.* (2007) 148:106–11. doi: 10.1111/j.1365-2249.2007.03325.x
35. Halaris A, Myint AM, Savant V, Meresh E, Lim E, Guillemin G, et al. Does escitalopram reduce neurotoxicity in major depression? *J Psychiatr Res.* (2015) 66–67:118–26. doi: 10.1016/j.jpsychires.2015.04.026
36. Dahl J, Andreassen OA, Verkerk R, Malt UF, Sandvik L, Brundin L, et al. Ongoing episode of major depressive disorder is not associated with elevated plasma levels of kynurenine pathway markers. *Psychoneuroendocrinology* (2015) 56:12–22. doi: 10.1016/j.psyneuen.2015.02.011
37. Guloksuz S, Arts B, Walter S, Drukker M, Rodriguez L, Myint AM, et al. The impact of electroconvulsive therapy on the tryptophan-kynurenine metabolic pathway. *Brain Behav Immun.* (2015) 48:48–52. doi: 10.1016/j.bbi.2015.02.029
38. Brandacher G, Winkler C, Aigner F, Schwelberger H, Schroecksnadel K, Margreiter R, et al. Bariatric surgery cannot prevent tryptophan depletion due to chronic immune activation in morbidly obese patients. *Obes Surg.* (2006) 16:541–8. doi: 10.1381/096089206776945066
39. Brandacher G, Hoeller E, Fuchs D, Weiss HG. Chronic immune activation underlies morbid obesity: is IDO a key player? *Curr Drug Metab.* (2007) 8:289–95. doi: 10.2174/138920007780362590
40. Favennec M, Hennart B, Caiazzo R, Leloire A, Yengo L, Verbanck M, et al. The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. *Obesity* (2015) 23:2066–74. doi: 10.1002/oby.21199
41. Mangge H, Summers KL, Meinitzer A, Zelzer S, Almer G, Prassl R, et al. Obesity-related dysregulation of the tryptophan-kynurenine metabolism: role of age and parameters of the metabolic syndrome. *Obesity* (2013) 22:195–201. doi: 10.1002/oby.20491
42. Wolowczuk I, Hennart B, Leloire A, Bessede A, Soichot M, Taront S, et al. Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: an attempt to maintain immune homeostasis and vascular tone. *Am J Physiol Regul Integr Comp Physiol.* (2012) 303:R135–43. doi: 10.1152/ajpregu.00373.2011
43. Reininghaus EZ, McIntyre RS, Reininghaus B, Geisler S, Bengesser SA, Lackner N, et al. Tryptophan breakdown is increased in euthymic overweight individuals with bipolar disorder: a preliminary report. *Bipolar Disord.* (2014) 16:432–40. doi: 10.1111/bdi.12166
44. O'Mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav Brain Res.* (2015) 277:32–48. doi: 10.1016/j.bbr.2014.07.027
45. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: gut microbiota: the neglected endocrine organ. *Mol Endocrinol.* (2014) 28:1221–38. doi: 10.1210/me.2014-1108
46. Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. *Brain Behav Immun.* (2010) 24:9–16. doi: 10.1016/j.bbi.2009.05.058
47. Leblhuber F, Steiner K, Schuetz B, Fuchs D, Gostner JM. Probiotic supplementation in patients with Alzheimer's dementia - an explorative intervention study. *Curr Alzheimer Res.* (2018) 15:1106–13. doi: 10.2174/1389200219666180813144834
48. Strasser B, Gostner JM, Fuchs D. Mood, food, and cognition: role of tryptophan and serotonin. *Curr Opin Clin Nutr Metab Care* (2016)19:55–61. doi: 10.1097/MCO.0000000000000237
49. Dalkner N, Platzer M, Bengesser SA, Birner A, Fellendorf FT, Queissner R, et al. The role of tryptophan metabolism and food craving in the

relationship between obesity and bipolar disorder. *Clin Nutr.* (2018) 37:1744–51. doi: 10.1016/j.clnu.2017.06.024

50. Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol Psychiatry* (2010) 15:393–403. doi: 10.1038/mp.2009.116
51. Raison CL, Borisov AS, Woolwine BJ, Massung B, Vogt G, Miller AH. Interferon-alpha effects on diurnal hypothalamic-pituitary-adrenal axis activity: relationship with proinflammatory cytokines and behavior. *Mol Psychiatry* (2010) 15:535–47. doi: 10.1038/mp.2008.58

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 28 February 2019
doi: 10.3389/fpsyt.2019.00101

Is the HPA Axis as Target for Depression Outdated, or Is There a New Hope?

*Andreas Menke**

Center of Mental Health, Department of Psychiatry, Psychosomatics and Psychotherapy, University Hospital of Wuerzburg, Wuerzburg, Germany

Major depressive disorder (MDD) is a very common stress-related mental disorder that carries a huge burden for affected patients and the society. It is associated with a high mortality that derives from suicidality and the development of serious medical conditions such as heart diseases, diabetes, and stroke. Although a range of effective antidepressants are available, more than 50% of the patients do not respond to the first treatment they are prescribed and around 30% fail to respond even after several treatment attempts. The heterogeneous condition of MDD, the lack of biomarkers matching patients with the right treatments and the situation that almost all available drugs are only targeting the serotonin, norepinephrine, or dopamine signaling, without regulating other potentially dysregulated systems may explain the insufficient treatment status. The hypothalamic-pituitary-adrenal (HPA) axis is one of these other systems, there is numerous and robust evidence that it is implicated in MDD and other stress-related conditions, but up to date there is no specific drug targeting HPA axis components that is approved and no test that is routinely used in the clinical setting identifying patients for such a specific treatment. Is there still hope after these many years for a breakthrough of agents targeting the HPA axis? This review will cover tests detecting altered HPA axis function and the specific treatment options such as glucocorticoid receptor (GR) antagonists, corticotropin-releasing hormone 1 (CRH_1) receptor antagonists, tryptophan 2,3-dioxygenase (TDO) inhibitors and FK506 binding protein 5 (FKBP5) receptor antagonists.

Keywords: precision medicine, personalized medicine, biomarker, depression, HPA axis, glucocorticoid receptor, CRH1, FKBP5

OPEN ACCESS

Edited by:
Christian Sander,
Leipzig University, Germany

Reviewed by:
Casimiro Cabrera Abreu,
Queens University, Canada
Andrea Norcini Pala,
Columbia University, United States

***Correspondence:**
Andreas Menke
Menke_A@ukw.de

Specialty section:
This article was submitted to Mood and Anxiety Disorders, a section of the journal Frontiers in Psychiatry

Received: *15 October 2018*
Accepted: *11 February 2019*
Published: *28 February 2019*

Citation:
Menke A (2019) Is the HPA Axis as Target for Depression Outdated, or Is There a New Hope? Front. Psychiatry 10:101. doi: 10.3389/fpsyt.2019.00101

INTRODUCTION

With a life-time prevalence around 20% major depressive disorder (MDD) is a very common disorder. In Europe it is one of the three most disabling conditions, next to dementias and alcohol abuse (1) and the burden of disease is projected to climb (2). MDD is associated with a substantially increased mortality due to suicide and an increased risk for serious medical conditions such as heart diseases, diabetes, and stroke (3). Although a range of effective antidepressants are available, more than 50% of patients do not respond to the first antidepressant treatment they are prescribed (4) and around 30% fail to respond even after several treatment approaches (5). Of note, even remitted patients suffer from a functional impairment (6). These non-sufficient treatment options that are

currently available are reflected by the high personal and societal burden with increased rates of sick leave and early retirement (1). The commonly used treatment options do not only struggle with high rates of partial or no response, but also with a delayed onset of treatment effects and uncomfortable or even threatening adverse side effects (3). Various factors may explain the current situation: MDD is a heterogeneous condition with poorly defined endophenotypes or subgroups, the currently available drugs have very similar treatment mechanisms and target almost only components of the serotonin, norepinephrine, or dopamine signaling, and there are no biomarkers to predict the response or side effects to specific interventions (7). Moreover, the diagnostic process and treatment choice are solely based on clinical experience and intuition. Fortunately, initiatives are under way to provide individualized treatment options for each patient: personalized medicine and precision medicine are employed to match individual patients with the most effective treatment options (7). Personalized and precision medicine are often used interchangeable, however, they describe two different concepts. Personalized treatment has been administered for the last decades, physicians considered sex, age, weight, co-medication together with renal, and liver functioning, comorbidities, core-symptoms (disturbances of sleep and appetite, psychotic vs. non-psychotic, agitated vs. non-agitated . . .) and patients preferences in the selection process of a suitable antidepressant. However, this personalization resembles a trial and error process and is highly dependent on the experience and the knowledge of the physician (8). The objective of precision medicine is to improve the selection of effective antidepressants with best possible response and minimal side effects using genetic markers or biomarkers derived from peripheral blood, imaging, neuropsychological tests, or behavioral measures (9–11). Given the high prevalence of MDD, another task of precision medicine will be the identification of individuals at risk and then to deliver specific interventions to avoid the full development of MDD (12).

GENETIC AND ENVIRONMENTAL FACTORS CONTRIBUTING TO MDD

A meta-analysis with more than 21,000 individuals observed a heritability of MDD around 40%, common environmental factors had very small effects, but individual environmental factors showed a substantial contribution of around 60% (13). In fact, the development of MDD is crucially dependent on gene x environment interactions (14–17). Aversive environmental events such as sexual, physical, or emotional childhood trauma have been robustly associated with MDD (18). But still it is not understood how early aversive events interact with genetic and epigenetic factors to confer vulnerability to MDD and how to treat patients who have experienced early life adversity. Meanwhile there is growing evidence showing that childhood trauma substantially shapes biological systems that are responsible for a fight-or-flight response, such as the hypothalamic-pituitary-adrenal (HPA) axis. In fact, childhood trauma may lead to an increased sensitivity of the HPA axis and to heightened responses to subsequent stressors (19, 20). Thus, the HPA axis may be a suitable target for specific interventions.

HPA AXIS

Environmental stress activates the release of the monoamines serotonin, norepinephrine and dopamine from the amygdala, hippocampus, and other brain regions. Subsequently, the paraventricular nucleus (PVN) of the hypothalamus synthesizes corticotrophin-releasing hormone (CRH), that binds to corticotropin-releasing hormone 1 (CRH_1) and CRH_2 receptors in the anterior pituitary. Then ACTH is secreted in the circulation (see **Figure 1**). ACTH activates the production and release of glucocorticoids (GC) in the adrenal glands. To reinstate homeostasis negative feedback mechanisms are initiated: GCs bind to glucocorticoid receptors (GR) of the hippocampus, the PNV and the anterior pituitary gland and thus inhibit the further release of CRH (22). In MDD the sensitivity of the GR is impaired leading to a reduced negative feedback mechanism and subsequently to a central hypersecretion of CRH and an increased production of GCs (23, 24). The sensitivity of the GR is substantially regulated by *FKBP5*, encoding the FK 506 binding protein 51 or FKBP51, a co-chaperone of heat-shock protein 90 (hsp90) (25). When FKBP51 is bound to the GR complex, the affinity for glucocorticoid-binding is reduced and the GR is translocated into the nucleus less efficiently. *FKBP5* mRNA and protein expression are induced by GR activation and provide an ultra-short negative feedback loop for GR sensitivity (25). Polymorphisms within *FKBP5* have been shown to be associated with differential regulation of *FKBP5* mRNA expression after activation of GR and differences in GR sensitivity (26, 27). *FKBP5* has been implicated in several mental disorders and stress-related conditions such as major depression (26), bipolar disorder (28), childhood trauma and posttraumatic stress disorder (29), aggressive and suicidal behavior (30, 31). Above the cellular level these genetic variants in combination with epigenetic alterations were associated with structural and functional changes in several brain regions (32–34) and with impaired working memory and cardiac stress reactivity (35). Recently *FKBP5* has been associated with metabolic function, diabetes and obesity (36–38) and pain (39, 40).

Stress-induced cortisol excess was also observed to impact the kynurenine pathway by enhancing the hepatic activity of tryptophan 2,3-dioxygenase (TDO) (41–43). Aside indoleamine 2,3-dioxygenase (IDO) TDO is the first and rate-limiting enzyme that catalyzes the conversion of tryptophan into N-formyl-kynurenine (NFK) (44). Downstream several kynurenine pathways metabolites have been associated with the development of major depression as they exert neurotoxic effects, e.g., by activating N-methyl-D-aspartate (NMDA) receptors or enhancing free radical production (45–48).

TESTS DETECTING THE FUNCTION OF THE HPA AXIS

Different tests have been developed to measure the function of the HPA axis (21). The dexamethasone suppression test [DST, (49)] identifies an impaired suppression of dexamethasone on cortisol, as observed in depressed patients (50–52). However, the DST has not reached clinical relevance as a diagnostic

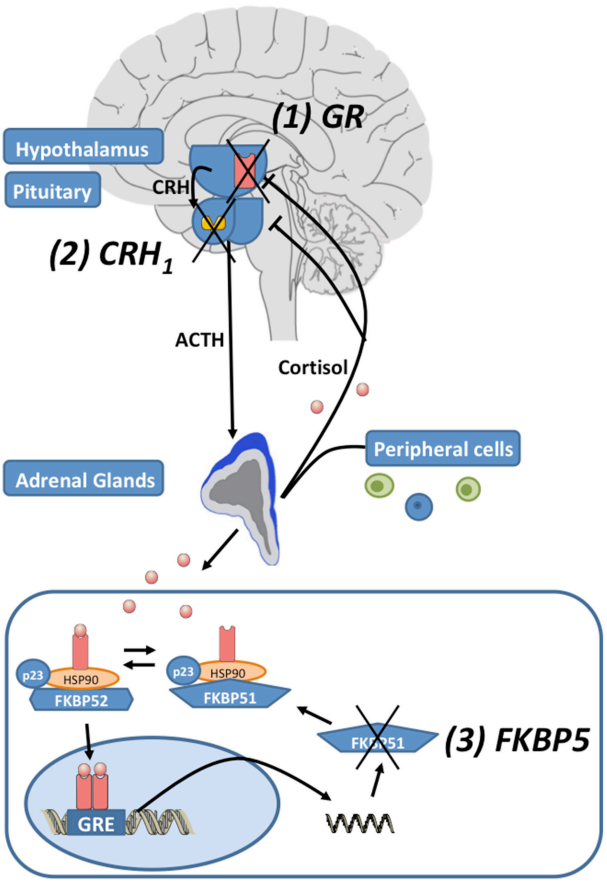

FIGURE 1 | The hypothalamic-pituitary-adrenal (HPA) axis: Corticotropin-releasing hormone (CRH) is released by neurons in the paraventricular nucleus of the hypothalamus. Subsequently CRH_1 receptors are activated and the secretion of adrenocorticotropic hormone (ACTH) from the pituitary is induced. ACTH induces the release of glucocorticoids (cortisol) by the adrenal glands. After the activation of the HPA axis, negative feedback loops are activated to reinstate homeostasis by cortisol activating glucocorticoid receptors (GR). The unliganded GR complex consists of the co-chaperones FKBP51 or FKBP52 (encoded by their respective genes *FKBP5* and *FKBP4*), p23 (a co-chaperone molecule) and hsp90 dimer. When FKBP51 binds to the GR-complex via hsp90, the GR affinity for cortisol is reduced. When glucocorticoids bind to the GR, FKBP51 is exchanged against FKBP52 and the nuclear translocation of the ligand-bound GR is enabled. The GR directly binds to the DNA via glucocorticoid response elements (GREs) and induces FKBP5 mRNA expression and subsequently FKBP51 production, inducing an ultra-short negative feedback loop on GR sensitivity. Drugs regulating the function of the HPA axis target: (1) the GR, (2) the CRH_1 receptors, and (3) *FKBP5*/FKBP51 (modified after Leistner and Menke (21); Copyright (2018), with permission from Elsevier).

tool because of its low sensitivity, which ranges between 20 and 50% (52–54). To increase the sensitivity and the specificity the DST was combined with the CRH stimulation test, the dexamethasone-corticotropin-releasing hormone [dex-CRH, (55, 56)] test, which actually led to an improved sensitivity in detecting alterations of the HPA axis with a successful classification of up to 80% of depressed patients (55, 56). These findings could be replicated in several studies (57–59), but others observed negative results when analyzing case-control differences (60–62). Interestingly, in addition to its ability to identify depressed patients, several studies observed that the dex-CRH test may allow a stratification of depressed patients and predict treatment outcome and disease course (21). Studies reported an increased cortisol response to the dex-CRH test in patients after remission at risk of relapse (59, 63, 64), in subjects with violent suicide attempts and suicide completion (65) and in melancholic patients compared to non-melancholic depressed patients (66). Contrary, a reduced cortisol response in the dex-CRH test was found in depressed patients with suicidal behavior (67) and women with chronic social stressors (68). An early normalization of the dex-CRH test results has been associated with response to antidepressant medication (59). However, we observed previously, that the readouts of the dex-CRH test are substantially dependent on the plasma concentrations of dexamethasone, thus several factors that influence the plasma concentration do also impact the readout of the test (69). Recently we reported on the potential use of the dexamethasone-induced gene expression changes as an additional indicator for alterations of the HPA axis and as a potential biomarker in depression (70) and other stress-related mental disorders such as job-related exhaustion (71). For this test, before and 3 h after a GR activation by dexamethasone cortisol, ACTH, blood count, and gene expression signatures are measured to detect GR sensitivity alterations (21). Of note, this test was not dependent on dexamethasone plasma concentrations (69). Applying this test we observed an increased GR sensitivity in patients with anxious depression compared with non-anxious depression, an enhanced leukocyte reactivity in patients with childhood trauma (72) and an increased GR sensitivity in healthy women compared to healthy men (73). In a broader, stimulated expression quantitative trait locus (eQTL) approach we combined these gene expression signatures after GR-activation with genome-wide single nucleotide polymorphism (SNP) data and found that common genetic variants that modulate the transcriptional response to GR-activation mediate the risk for MDD as well as other mental disorders (74).

SPECIFIC TARGETS OF THE HPA AXIS

GR Antagonists

Based on numerous findings of a HPA axis hyperactivity in patients with psychotic depression open-label and double-blind trials with the GR antagonist (and also progesterone antagonist) mifepristone were conducted (75) (**Figure 1**). The studies using dosages between 300 and 1,200 mg /d showed mixed results, with both positive studies and failed trials (75). A combined analysis of similarly designed double-blind phase 2 or 3 studies assessing the efficacy and safety of 7 day mifepristone treatment revealed a meaningful efficacy ($p < 0.004$) for mifepristone in reducing psychotic symptoms, adverse events were similar in mifepristone and placebo treated patients (76). Interestingly, high mifepristone plasma concentrations were associated with the strongest response, followed by changes in cortisol and ACTH (76). There is also accumulating evidence that mifepristone ameliorates cognitive deficits in major depression and bipolar

disorder (77). Thus, for depressed patients with psychotic features a GR antagonist such as mifepristone may be an individualized treatment option.

CRH_1 Receptor Antagonists

In preclinical models central administration of CRH produces behavioral effects that closely resemble the symptoms of depression in humans (78, 79). These effects are attenuated by central administration of a specific CRH receptor antagonist (79, 80). Moreover, also clinical studies provided evidence of a CRH hyperactivity in depression and anxiety (79). A clinical trial using the CRH_1 receptor antagonist R121919 in the treatment of major depression revealed significant reductions in the Hamilton Depression Rating Scale (HAMD) over the 30 day treatment period (81). The stress-elicited secretion of cortisol was reduced, however, it did not impair the CRH-induced release of ACTH and cortisol and thus the stress hormone system responsivity to CRH remained unchanged (81). However, the study did not include design components such as blinding, randomization or a placebo control and R121919 was withdrawn due to liver enzyme elevations. A further trial using another CRH_1 receptor antagonist, CP-316,311 did not observe a significant difference between patients treated with CP-316,311 and placebo (82). Other trials using CRH_1 receptor antagonists in patients with major depression, social and generalized anxiety disorder and suicidal ideation could also not reveal beneficial effects (83). In a trial with anxious, alcohol-dependent women the CRH_1 receptor antagonist Verucerfont (also GSK561679) produced a dampening of the HPA axis response to social stressors and attenuated amygdala response to negative affective stimuli, while alcohol craving was unaffected (84). Recently a double-blind, randomized and placebo-controlled trial investigated the efficacy of the same CRH_1 receptor antagonist in women suffering from Posttraumatic stress disorder (85, 86). The trial did not observe a significant improvement of PTSD symptoms in patients treated with GSK561679 compared to placebo overall (86). However, subjects with a moderate or severe history of childhood abuse and a certain CRH1 receptor SNP genotype did only response to GSK561679, not to placebo (86). Nevertheless, the authors concluded that CRH_1 receptor antagonists as a class are ineffective as monotherapy for stress-related mental disorders (86) and the question arose whether it is time to call it quits for the CRH_1 receptor antagonists (83, 87). CRH is a key regulator of the stress response and controls endocrine activity by direct modulation of the HPA axis. As stated above, numerous preclinical and clinical data support the involvement of CRH and CRH_1 receptors in stress-related mental disorder (88). However, some of the tested agents did only show meaningful effects in some of the preclinical stress tests, moreover, preclinical data not always translate to clinical trials without complications (83, 87). For CRH_1 receptor antagonists, the traditional clinical trial design is probably not suitable. Instead, patients with an overactivity of the CRH—CRH1 receptor signaling should be identified by reliable biological measures in terms of precision medicine, that is already well-established in other medical fields, such as oncology (87), and then included in a respective trial. Thus, CRH_1 receptor antagonists are still promising agents for stress-related mental disorders, but probably only in those patients who are subject to a significant CRH signaling dysfunction.

TDO Inhibitors

TDO inhibition by directly targeting the kynurenine production is supposed to decrease neurotoxic metabolites and thus ameliorate depressive symptoms (44). TDO inhibition is a mechanism shared by the largest number of antidepressants, e.g., citalopram effectively decreases TDO activity (44, 89). Interestingly, agents inhibiting glucocorticoids such as RU486 showed antidepressive properties by inhibiting TDO activity (90, 91). Additionally, co-treatment with allopurinol, also a TDO inhibitor, improved chronic stress induced depressive-like behavior (92). Recently, the agent NSC 36398, a flavonoid compound, was observed to be a first selective TDO inhibitor (93).

FKBP5 Antagonists

As described above *FKBP5*, respectively, FK506 binding protein 51/FKBP51 regulates the responsiveness of the GR and the HPA axis and is also implicated in important gene x environment interactions underlying stress-related mental disorders (25, 94), making it a promising drug target. In fact, several research groups have consistently observed protective effects of *FKBP5* knock-out or knock-down on stress-coping behavior and stress endocrinology in preclinical models of depression and anxiety (95). The prototypic FKBP ligand FK506 and rapamycin showed the principal druggability. In addition, FKBP51 is highly suited for X-ray cocrystallography, which facilitates the rational drug design (96). Sulfonamide analogs have been found that possess FKBP51 binding properties (97). However, drug discovery has been hampered by the inability that all known ligands cannot differentiate FKBP51 and the opposing homolog FKBP52 (98, 99). Recently with SAFit1 and SAFit2 two promising potent and highly selective inhibitors of FKBP51 were discovered, that achieved selectivity by an induced-fit mechanism and improved neuroendocrine feedback and stress-coping behavior in mice (100, 101). Of note, co-application of SAFit2 with the selective serotonin reuptake inhibitor escitalopram, a common antidepressant, lowered the efficacy of escitalopram in anxiety-related tests but improved stress coping behavior in a mouse model (102). *FKBP5* antagonists may be promising new treatment options for patients suffering from stress-related mental disorders and who have an altered functioning of *FKBP5*/GR/HPA axis signaling.

CONCLUSION

Despite the very strong preclinical and clinical data of a dysregulation of the HPA axis in stress-related mental disorders, such as major depression, no drug has been approved that targets specific components of the HPA axis. In addition, no test is routinely used in the clinical setting to identify patients with a measureable HPA axis dysfunction. In fact, there is evidence that not all depressed patients display alterations of the HPA axis, and therefore not all of them would benefit of

a very specific treatment, targeting only HPA axis components. This has become abundantly clear with the failing CRH_1 receptor antagonists for major depression and posttraumatic stress disorder. However, even in the failed trials, there were initial hints that subgroups of patients carrying certain genetic risk variants or having a history of childhood trauma would indeed benefit from these very specific treatment options (86). Therefore, precision medicine has to be employed to match the specific antidepressant agent to the specific underlying biological alteration and the individual patient. Biological markers derived from tests detecting the HPA axis function, GR sensitivity, or *FKBP5* dysregulation are necessary to identify suitable patients for these specific agents. In addition, clinical variables such as psychotic symptoms or history of childhood trauma combined with certain genetic risk variants may further improve the accuracy of such a test. Still in its infancy, the dexamethasone-induced gene expression test may become a promising tool to assess the GR sensitivity and FKBP5 function (21), because it combines neuroendocrine results with molecular genetic patterns of a GR challenged gene expression integrating genetic risk polymorphisms and additional clinical data (21). Thus, the future algorithms defining the treatment of major depression or other stress-related mental disorders will incorporate tests that stratify patients groups and match individual patients with highly specific agents, for example that target HPA axis components such as the GR, CRH_1 receptors or *FKBP5*. GR antagonists, especially mifepristone, have provided very promising results for the treatment of psychotic depression so far and therefore could gain more relevance (76). CRH_1 receptor antagonists have experienced a setback, but after employing suitable tests to find susceptible patients this development could be reversed. FKBP5, representing a molecular hub modulating many cellular pathways, is a novel and very promising candidate to target component of the stress hormone system and to ameliorate stress-related mental disorders and other sequelae of stress.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This publication was funded by the German Research Foundation (DFG) and the University of Wuerzburg in the funding programme Open Access Publishing.

REFERENCES

1. Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B, et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol.* (2011) 21:655–79. doi: 10.1016/j.euroneuro.2011.07.018
2. Kessler RC, Bromet EJ. The epidemiology of depression across cultures. *Annu Rev Public Health.* (2013) 34:119–38. doi: 10.1146/annurev-publhealth-031912-114409
3. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. *Nat Rev Dis Primers.* (2016) 2:16065. doi: 10.1038/nrdp.2016.65
4. Ruhe HG, Huyser J, Swinkels JA, Schene AH. Switching antidepressants after a first selective serotonin reuptake inhibitor in major depressive disorder: a systematic review. *J Clin Psychiatry.* (2006) 67:1836–55. doi: 10.4088/JCP.v67n1203
5. Rush AJ, Trivedi MH, Wisniewski SR, Stewart JW, Nierenberg AA, Thase ME, et al. Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *N Engl J Med.* (2006) 354:1231–42. doi: 10.1056/NEJMoa052963
6. Ormel J, Oldehinkel AJ, Nolen WA, Vollebergh W. Psychosocial disability before, during, and after a major depressive episode: a 3-wave population-based study of state, scar, and trait effects. *Arch Gen Psychiatry.* (2004) 61:387–92. doi: 10.1001/archpsyc.61.4.387
7. Menke A. Precision pharmacotherapy: psychiatry's future direction in preventing, diagnosing, and treating mental disorders. *Pharmacogenom Pers Med.* (2018) 11:211–22. doi: 10.2147/PGPM.S146110
8. Perlis RH. Abandoning personalization to get to precision in the pharmacotherapy of depression. *World Psychiatry.* (2016) 15:228–35. doi: 10.1002/wps.20345
9. Collins FS, Varmus H. A new initiative on precision medicine. *N Engl J Med.* (2015) 372:793–5. doi: 10.1056/NEJMp1500523
10. Jameson JL, Longo DL. Precision medicine–personalized, problematic, and promising. *N Engl J Med.* (2015) 372:2229–34. doi: 10.1056/NEJMsb1503104
11. Holsboer F. How can we realize the promise of personalized antidepressant medicines? *Nat Rev Neurosci.* (2008) 9:638–46. doi: 10.1038/nrn2453
12. van Zoonen K, Buntrock C, Ebert DD, Smit F, Reynolds CF III, Beekman AT, et al. Preventing the onset of major depressive disorder: a meta-analytic review of psychological interventions. *Int J Epidemiol.* (2014) 43:318–29. doi: 10.1093/ije/dyt175
13. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry.* (2000) 157:1552–62. doi: 10.1176/appi.ajp.157.10.1552
14. Klengel T, Binder EB. Epigenetics of stress-related psychiatric disorders and gene x environment interactions. *Neuron.* (2015) 86:1343–57. doi: 10.1016/j.neuron.2015.05.036
15. Binder EB. Dissecting the molecular mechanisms of gene x environment interactions: implications for diagnosis and treatment of stress-related psychiatric disorders. *Eur J Psychotraumatol.* (2017) 8(Suppl. 5):1412745. doi: 10.1080/20008198.2017.1412745
16. Kendler KS, Davis CG, Kessler RC. The familial aggregation of common psychiatric and substance use disorders in the National Comorbidity Survey: a family history study. *Br J Psychiatry.* (1997) 170:541–8. doi: 10.1192/bjp.170.6.541
17. Kessler RC, Davis CG, Kendler KS. Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. *Psychol Med.* (1997) 27:1101–19. doi: 10.1017/S0033291797005588
18. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry.* (2001) 49:1023–39. doi: 10.1016/S0006-3223(01)01157-X
19. Danese A, Lewis SJ. Psychoneuroimmunology of early-life stress: the hidden wounds of childhood trauma? *Neuropsychopharmacology.* (2017) 42:99–114. doi: 10.1038/npp.2016.198
20. Kuhlman KR, Chiang JJ, Horn S, Bower JE. Developmental psychoneuroendocrine and psychoneuroimmune pathways from childhood adversity to disease. *Neurosci Biobehav Rev.* (2017) 80:166–84. doi: 10.1016/j.neubiorev.2017.05.020
21. Leistner C, Menke A. How to measure glucocorticoid receptor's sensitivity in patients with stress-related psychiatric disorders. *Psychoneuroendocrinology.* (2018) 91:235–60. doi: 10.1016/j.psyneuen.2018.01.023
22. de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci.* (2005) 6:463–75. doi: 10.1038/nrn1683

23. Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology.* (2000) 23:477–501. doi: 10.1016/S0893-133X(00)00159-7
24. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biol Psychiatry.* (2001) 49:391–404. doi: 10.1016/S0006-3223(00)01088-X
25. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology.* (2009) 34(Suppl. 1):S186–95. doi: 10.1016/j.psyneuen.2009.05.021
26. Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Putz B, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet.* (2004) 36:1319–25. doi: 10.1038/ng1479
27. Menke A, Klengel T, Rubel J, Bruckl T, Pfister H, Lucae S, et al. Genetic variation in FKBP5 associated with the extent of stress hormone dysregulation in major depression. *Genes Brain Behav.* (2013) 12:289–96. doi: 10.1111/gbb.12026
28. Willour VL, Chen H, Toolan J, Belmonte P, Cutler DJ, Goes FS, et al. Family-based association of FKBP5 in bipolar disorder. *Mol Psychiatry.* (2008) 14:261–268. doi: 10.1038/sj.mp.4002141
29. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, et al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA.* (2008) 299:1291–305. doi: 10.1001/jama.299.11.1291
30. Roy A, Gorodetsky E, Yuan Q, Goldman D, Enoch MA. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology.* (2010) 35:1674–83. doi: 10.1038/npp.2009.236
31. Bevilacqua L, Carli V, Sarchiapone M, George DK, Goldman D, Roy A, et al. Interaction between FKBP5 and childhood trauma and risk of aggressive behavior. *Arch Gen Psychiatry.* (2012) 69:62–70. doi: 10.1001/archgenpsychiatry.2011.152
32. Grabe HJ, Wittfeld K, Van der Auwera S, Janowitz D, Hegenscheid K, Habes M, et al. Effect of the interaction between childhood abuse and rs1360780 of the FKBP5 gene on gray matter volume in a general population sample. *Hum Brain Mapp.* (2016) 37:1602–13. doi: 10.1002/hbm.23123
33. Han KM, Won E, Sim Y, Kang J, Han C, Kim YK, et al. Influence of FKBP5 polymorphism and DNA methylation on structural changes of the brain in major depressive disorder. *Sci Rep.* (2017) 7:42621. doi: 10.1038/srep42621
34. Tozzi L, Farrell C, Booij L, Doolin K, Nemoda Z, Szyf M, et al. Epigenetic changes of FKBP5 as a link connecting genetic and environmental risk factors with structural and functional brain changes in major depression. *Neuropsychopharmacology.* (2018) 43:1138–45. doi: 10.1038/npp.2017.290
35. Lovallo WR, Enoch MA, Acheson A, Cohoon AJ, Sorocco KH, Hodgkinson CA, et al. Early-life adversity interacts with FKBP5 genotypes: altered working memory and cardiac stress reactivity in the oklahoma family health patterns project. *Neuropsychopharmacology.* (2016) 41:1724–32. doi: 10.1038/npp.2015.347
36. Balsevich G, Hausl AS, Meyer CW, Karamihalev S, Feng X, Pohlmann ML, et al. Stress-responsive FKBP51 regulates AKT2-AS160 signaling and metabolic function. *Nat Commun.* (2017) 8:1725. doi: 10.1038/s41467-017-01783-y
37. Ortiz R, Joseph JJ, Lee R, Wand GS, Golden SH. Type 2 diabetes and cardiometabolic risk may be associated with increase in DNA methylation of FKBP5. *Clin Epigenetics.* (2018) 10:82. doi: 10.1186/s13148-018-0513-0
38. Lee RS, Mahon PB, Zandi PP, McCaul ME, Yang X, Bali U, et al. DNA methylation and sex-specific expression of FKBP5 as correlates of one-month bedtime cortisol levels in healthy individuals. *Psychoneuroendocrinology.* (2018) 97:164–73. doi: 10.1016/j.psyneuen.2018.07.003
39. Bortsov AV, Smith JE, Diatchenko L, Soward AC, Ulirsch JC, Rossi C, et al. Polymorphisms in the glucocorticoid receptor co-chaperone FKBP5 predict persistent musculoskeletal pain after traumatic stress exposure. *Pain.* (2013) 154:1419–26. doi: 10.1016/j.pain.2013.04.037
40. Linnstaedt SD, Riker KD, Rueckeis CA, Kutchko KM, Lackey L, McCarthy KR, et al. A functional riboSNitch in the 3' untranslated region of FKBP5 alters MicroRNA-320a binding efficiency and mediates vulnerability to chronic post-traumatic pain. *J Neurosci.* (2018) 38:8407–20. doi: 10.1523/JNEUROSCI.3458-17.2018
41. Badawy AA. Tryptophan: the key to boosting brain serotonin synthesis in depressive illness. *J Psychopharmacol.* (2013) 27:878–93. doi: 10.1177/0269881113499209
42. Knox WE. Two mechanisms which increase *in vivo* the liver tryptophan peroxidase activity: specific enzyme adaptation and stimulation of the pituitary adrenal system. *Br J Exp Pathol.* (1951) 32:462–9.
43. Salter M, Pogson CI. The role of tryptophan 2,3-dioxygenase in the hormonal control of tryptophan metabolism in isolated rat liver cells. Effects of glucocorticoids and experimental diabetes. *Biochem J.* (1985) 229:499–504. doi: 10.1042/bj2290499
44. Qin Y, Wang N, Zhang X, Han X, Zhai X, Lu Y. IDO and TDO as a potential therapeutic target in different types of depression. *Metab Brain Dis.* (2018) 33:1787–800. doi: 10.1007/s11011-018-0290-7
45. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J.* (2012) 279:1356–65. doi: 10.1111/j.1742-4658.2012.08485.x
46. Maes M, Galecki P, Verkerk R, Rief W. Somatization, but not depression, is characterized by disorders in the tryptophan catabolite (TRYCAT) pathway, indicating increased indoleamine 2,3-dioxygenase and lowered kynurenine aminotransferase activity. *Neuro Endocrinol Lett.* (2011) 32:264–73.
47. Savitz J, Drevets WC, Wurfel BE, Ford BN, Bellgowan PS, Victor TA, et al. Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder. *Brain Behav Immun.* (2015) 46:55–9. doi: 10.1016/j.bbi.2015.02.007
48. Stone TW, Stoy N, Darlington LG. An expanding range of targets for kynurenine metabolites of tryptophan. *Trends Pharmacol Sci.* (2013) 34:136–43. doi: 10.1016/j.tips.2012.09.006
49. Carroll BJ, Martin FI, Davies B. Resistance to suppression by dexamethasone of plasma 11-O.H.C.S. levels in severe depressive illness. *Br Med J.* (1968) 3:285–7. doi: 10.1136/bmj.3.5613.285
50. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, et al. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. *Arch Gen Psychiatry.* (1981) 38:15–22. doi: 10.1001/archpsyc.1981.01780260017001
51. Nelson JC, Davis JM. DST studies in psychotic depression: a meta-analysis. *Am J Psychiatry.* (1997) 154:1497–503. doi: 10.1176/ajp.154.11.1497
52. Nierenberg AA, Feinstein AR. How to evaluate a diagnostic marker test. Lessons from the rise and fall of dexamethasone suppression test. *JAMA.* (1988) 259:1699–702. doi: 10.1001/jama.1988.03720110061036
53. Holsboer F, Bender W, Benkert O, Klein HE, Schmauss M. Diagnostic value of dexamethasone suppression test in depression. *Lancet.* (1980) 2:706. doi: 10.1016/S0140-6736(80)92755-5
54. Arana GW, Baldessarini RJ, Ornsteen M. The dexamethasone suppression test for diagnosis and prognosis in psychiatry. Commentary and review. *Arch Gen Psychiatry.* (1985) 42:1193–204. doi: 10.1001/archpsyc.1985.01790350067012
55. Bardeleben U, Holsboer F. Cortisol response to a combined dexamethasone-human corticotrophin-releasing hormone challenge in patients with depression. *J Neuroendocrinol.* (1989) 1:485–8. doi: 10.1111/j.1365-2826.1989.tb00150.x
56. Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res.* (1994) 28:341–56. doi: 10.1016/0022-3956(94)90017-5
57. Modell S, Yassouridis A, Huber J, Holsboer F. Corticosteroid receptor function is decreased in depressed patients. *Neuroendocrinology.* (1997) 65:216–22. doi: 10.1159/000127275
58. Kunugi H, Ida I, Owashi T, Kimura M, Inoue Y, Nakagawa S, et al. Assessment of the Dexamethasone/CRH test as a state-dependent marker for Hypothalamic-Pituitary-Adrenal (HPA) axis abnormalities in major depressive episode: a multicenter study. *Neuropsychopharmacology.* (2006) 31:212–20. doi: 10.1038/sj.npp.1300868
59. Ising M, Horstmann S, Kloiber S, Lucae S, Binder EB, Kern N, et al. Combined dexamethasone/corticotropin releasing hormone test predicts treatment response in major depression - a potential biomarker? *Biol Psychiatry.* (2007) 62:47–54. doi: 10.1016/j.biopsych.2006.07.039
60. Oshima A, Yamashita S, Owashi T, Murata T, Tadokoro C, Miyaoka H, et al. The differential ACTH responses to combined dexamethasone/CRH

administration in major depressive and dysthymic disorders. *J Psychiatr Res.* (2000) 34:325–8. doi: 10.1016/S0022-3956(00)00021-2
61. Gervasoni N, Bertschy G, Osiek C, Perret G, Denis R, Golaz J, et al. Cortisol responses to combined dexamethasone/CRH test in outpatients with a major depressive episode. *J Psychiatr Res.* (2004) 38:553–7. doi: 10.1016/j.jpsychires.2004.04.008
62. Carpenter LL, Ross NS, Tyrka AR, Anderson GM, Kelly M, Price LH. Dex/CRH test cortisol response in outpatients with major depression and matched healthy controls. *Psychoneuroendocrinology.* (2009) 34:1208–13. doi: 10.1016/j.psyneuen.2009.03.009
63. Zobel AW, Nickel T, Sonntag A, Uhr M, Holsboer F, Ising M. Cortisol response in the combined dexamethasone/CRH test as predictor of relapse in patients with remitted depression. a prospective study. *J Psychiatr Res.* (2001) 35:83–94. doi: 10.1016/S0022-3956(01)00013-9
64. Appelhof BC, Huyser J, Verweij M, Brouwer JP, van Dyck R, Fliers E, et al. Glucocorticoids and relapse of major depression (dexamethasone/corticotropin-releasing hormone test in relation to relapse of major depression). *Biol Psychiatry.* (2006) 59:696–701. doi: 10.1016/j.biopsych.2005.09.008
65. Coryell W, Schlesser M. The dexamethasone suppression test and suicide prediction. *Am J Psychiatry.* (2001) 158:748–53. doi: 10.1176/appi.ajp.158.5.748
66. Kaestner F, Hettich M, Peters M, Sibrowski W, Hetzel G, Ponath G, et al. Different activation patterns of proinflammatory cytokines in melancholic and non-melancholic major depression are associated with HPA axis activity. *J Affect Disord.* (2005) 87:305–11. doi: 10.1016/j.jad.2005.03.012
67. Pfennig A, Kunzel HE, Kern N, Ising M, Majer M, Fuchs B, et al. Hypothalamus-pituitary-adrenal system regulation and suicidal behavior in depression. *Biol Psychiatry.* (2005) 57:336–42. doi: 10.1016/j.biopsych.2004.11.017
68. Rydmark I, Wahlberg K, Ghatan PH, Modell S, Nygren A, Ingvar M, et al. Neuroendocrine, cognitive and structural imaging characteristics of women on longterm sickleave with job stress-induced depression. *Biol Psychiatry.* (2006) 60:867–73. doi: 10.1016/j.biopsych.2006.04.029
69. Menke A, Arloth J, Best J, Namendorf C, Gerlach T, Czamara D, et al. Time-dependent effects of dexamethasone plasma concentrations on glucocorticoid receptor challenge tests. *Psychoneuroendocrinology.* (2016) 69:161–71. doi: 10.1016/j.psyneuen.2016.04.003
70. Menke A, Arloth J, Putz B, Weber P, Klengel T, Mehta D, et al. Dexamethasone stimulated gene expression in peripheral blood is a sensitive marker for glucocorticoid receptor resistance in depressed patients. *Neuropsychopharmacology.* (2012) 37:1455–64. doi: 10.1038/npp.2011.331
71. Menke A, Arloth J, Gerber M, Rex-Haffner M, Uhr M, Holsboer F, et al. Dexamethasone stimulated gene expression in peripheral blood indicates glucocorticoid-receptor hypersensitivity in job-related exhaustion. *Psychoneuroendocrinology.* (2014) 44:35–46. doi: 10.1016/j.psyneuen.2014.02.013
72. Menke A, Lehrieder D, Fietz J, Leistner C, Wurst C, Stonawski S, et al. Childhood trauma dependent anxious depression sensitizes HPA axis function. *Psychoneuroendocrinology.* (2018) 98:22–9. doi: 10.1016/j.psyneuen.2018.07.025
73. Rampp C, Eichelkraut A, Best J, Czamara D, Rex-Haffner M, Uhr M, et al. Sex-related differential response to dexamethasone in endocrine and immune measures in depressed in-patients and healthy controls. *J Psychiatr Res.* (2018) 98:107–15. doi: 10.1016/j.jpsychires.2017.12.020
74. Arloth J, Bogdan R, Weber P, Frishman G, Menke A, Wagner KV, et al. Genetic differences in the immediate transcriptome response to stress predict risk-related brain function and psychiatric disorders. *Neuron.* (2015) 86:1189–202. doi: 10.1016/j.neuron.2015.05.034
75. Schatzberg AF. Anna-Monika Award Lecture, DGPPN Kongress, 2013: the role of the hypothalamic-pituitary-adrenal (HPA) axis in the pathogenesis of psychotic major depression. *World J Biol Psychiatry.* (2015) 16:2–11. doi: 10.3109/15622975.2014.916414
76. Block TS, Kushner H, Kalin N, Nelson C, Belanoff J, Schatzberg A. Combined analysis of mifepristone for psychotic depression: plasma levels associated with clinical response. *Biol Psychiatry.* (2018) 84:46–54. doi: 10.1016/j.biopsych.2018.01.008
77. Soria V, Gonzalez-Rodriguez A, Huerta-Ramos E, Usall J, Cobo J, Bioque M, et al. Targeting hypothalamic-pituitary-adrenal axis hormones and sex steroids for improving cognition in major mood disorders and schizophrenia: a systematic review and narrative synthesis. *Psychoneuroendocrinology.* (2018) 93:8–19. doi: 10.1016/j.psyneuen.2018.04.012
78. Owens MJ, Nemeroff CB. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol Rev.* (1991) 43:425–73.
79. Holsboer F. The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiatr Res.* (1999) 33:181–214. doi: 10.1016/S0022-3956(98)90056-5
80. Berridge CW, Dunn AJ. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. *Horm Behav.* (1987) 21:393–401. doi: 10.1016/0018-506X(87)90023-7
81. Zobel AW, Nickel T, Kunzel HE, Ackl N, Sonntag A, Ising M, et al. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J Psychiatr Res.* (2000) 34:171–81. doi: 10.1016/S0022-3956(00)00016-9
82. Binneman B, Feltner D, Kolluri S, Shi Y, Qiu R, Stiger T. A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH1 antagonist) in the treatment of major depression. *Am J Psychiatry.* (2008) 165:617–20. doi: 10.1176/appi.ajp.2008.07071199
83. Spierling SR, Zorrilla EP. Don't stress about CRF: assessing the translational failures of CRF1antagonists. *Psychopharmacology.* (2017) 234:1467–81. doi: 10.1007/s00213-017-4556-2
84. Schwandt ML, Cortes CR, Kwako LE, George DT, Momenan R, Sinha R, et al. The CRF1 antagonist verucerfont in anxious alcohol-dependent women: translation of neuroendocrine, but not of anti-craving effects. *Neuropsychopharmacology.* (2016) 41:2818–29. doi: 10.1038/npp.2016.61
85. Dunlop BW, Rothbaum BO, Binder EB, Duncan E, Harvey PD, Jovanovic T, et al. Evaluation of a corticotropin releasing hormone type 1 receptor antagonist in women with posttraumatic stress disorder: study protocol for a randomized controlled trial. *Trials.* (2014) 15:240. doi: 10.1186/1745-6215-15-240
86. Dunlop BW, Binder EB, Iosifescu D, Mathew SJ, Neylan TC, Pape JC, et al. Corticotropin-releasing factor receptor 1 antagonism is ineffective for women with posttraumatic stress disorder. *Biol Psychiatry.* (2017) 82:866–74. doi: 10.1016/j.biopsych.2017.06.024
87. Murrough JW, Charney DS. Corticotropin-releasing factor type 1 receptor antagonists for stress-related disorders: time to call it quits? *Biol Psychiatry.* (2017) 82:858–60. doi: 10.1016/j.biopsych.2017.10.012
88. Lloyd RB, Nemeroff CB. The role of corticotropin-releasing hormone in the pathophysiology of depression: therapeutic implications. *Curr Top Med Chem.* (2011) 11:609–17. doi: 10.2174/1568026611109060609
89. Ara I, Bano S. Citalopram decreases tryptophan 2,3-dioxygenase activity and brain 5-HT turnover in swim stressed rats. *Pharmacol Rep.* (2012) 64:558–66. doi: 10.1016/S1734-1140(12)70851-4
90. Gjerde H, Morland J, Olsen H. The antiglucocorticoid RU486 inhibits the ethanol-induced increase of tryptophan oxygenase. *J Steroid Biochem.* (1985) 23:1091–2. doi: 10.1016/0022-4731(85)90074-3
91. Wolkowitz OM, Reus VI. Treatment of depression with antiglucocorticoid drugs. *Psychosom Med.* (1999) 61:698–711. doi: 10.1097/00006842-199909000-00011
92. Gibney SM, Fagan EM, Waldron AM, O'Byrne J, Connor TJ, Harkin A. Inhibition of stress-induced hepatic tryptophan 2,3-dioxygenase exhibits antidepressant activity in an animal model of depressive behaviour. *Int J Neuropsychopharmacol.* (2014) 17:917–28. doi: 10.1017/S1461145713001673
93. Pantouris G, Mowat CG. Antitumour agents as inhibitors of tryptophan 2,3-dioxygenase. *Biochem Biophys Res Commun.* (2014) 443:28–31. doi: 10.1016/j.bbrc.2013.11.037
94. Matosin N, Halldorsdottir T, Binder EB. Understanding the molecular mechanisms underpinning gene by environment interactions in psychiatric disorders: the FKBP5 model. *Biol Psychiatry.* (2018) 83:821–30. doi: 10.1016/j.biopsych.2018.01.021
95. Schmidt MV, Paez-Pereda M, Holsboer F, Hausch F. The prospect of FKBP51 as a drug target. *ChemMedChem.* (2012) 7:1351–9. doi: 10.1002/cmdc.201200137

96. Gopalakrishnan R, Kozany C, Gaali S, Kress C, Hoogeland B, Bracher A, et al. Evaluation of synthetic FK506 analogues as ligands for the FK506-binding proteins 51 and 52. *J Med Chem.* (2012) 55:4114–22. doi: 10.1021/jm201746x
97. Gopalakrishnan R, Kozany C, Wang Y, Schneider S, Hoogeland B, Bracher A, et al. Exploration of pipecolate sulfonamides as binders of the FK506-binding proteins 51 and 52. *J Med Chem.* (2012) 55:4123–31. doi: 10.1021/jm201747c
98. Gaali S, Gopalakrishnan R, Wang Y, Kozany C, Hausch F. The chemical biology of immunophilin ligands. *Curr Med Chem.* (2011) 18:5355–79. doi: 10.2174/092986711798194342
99. Blackburn EA, Walkinshaw MD. Targeting FKBP isoforms with small-molecule ligands. *Curr Opin Pharmacol.* (2011) 11:365–71. doi: 10.1016/j.coph.2011.04.007
100. Gaali S, Kirschner A, Cuboni S, Hartmann J, Kozany C, Balsevich G, et al. Selective inhibitors of the FK506-binding protein 51 by induced fit. *Nat Chem Biol.* (2015) 11:33–7. doi: 10.1038/nchembio.1699
101. Kolos JM, Voll AM, Bauder M, Hausch F. FKBP Ligands—where we are and where to go? *Front Pharmacol.* (2018) 9:1425. doi: 10.3389/fphar.2018.01425
102. Pohlmann ML, Hausl AS, Harbich D, Balsevich G, Engelhardt C, Feng X, et al. Pharmacological modulation of the psychiatric risk factor FKBP51 alters efficiency of common antidepressant drugs. *Front Behav Neurosci.* (2018) 12:262. doi: 10.3389/fnbeh.2018.00262

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 01 May 2019
doi: 10.3389/fpsyt.2019.00266

Detection Study of Bipolar Depression Through the Application of a Model-Based Algorithm in Terms of Clinical Feature and Peripheral Biomarkers

*Yanqun Zheng[1], Shen He[1], Tianhong Zhang[1,2], Zhiguang Lin[3], Shenxun Shi[4], Yiru Fang[1], Kaida Jiang[1] and Xiaohua Liu[1,2]**

[1] Department of Psychiatry, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2] Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [3] Biochemistry Laboratory, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [4] Department of Psychiatry, Huashan Hospital affiliated to Fudan University, Shanghai, China

OPEN ACCESS

Edited by:
Christian Sander, Leipzig University, Germany

Reviewed by:
Saori C. Tanaka, Advanced Telecommunications Research Institute International (ATR), Japan
Ives Cavalcante Passos, Federal University of Rio Grande do Sul, Brazil

***Correspondence:**
Xiaohua Liu
drliuxiaohua@gmail.com

Specialty section:
This article was submitted to Computational Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *06 July 2018*
Accepted: *08 April 2019*
Published: *01 May 2019*

Citation:
Zheng Y, He S, Zhang T, Lin Z, Shi S, Fang Y, Jiang K and Liu X (2019) Detection Study of Bipolar Depression Through the Application of a Model-Based Algorithm in Terms of Clinical Feature and Peripheral Biomarkers. Front. Psychiatry 10:266. doi: 10.3389/fpsyt.2019.00266

Objectives: The nature of the diagnostic classification of mood disorder is a typical dichotomous data problem and the method of combining different dimensions of evidences to make judgments might be more statistically reliable. In this paper, we aimed to explore whether peripheral neurotrophic factors could be helpful for early detection of bipolar depression.

Methods: A screening method combining peripheral biomarkers and clinical characteristics was applied in 30 patients with major depressive disorder (MDD) and 23 patients with depressive episode of bipolar disorder. By a model-based algorithm, some information was extracted from the dataset and used as a "model" to approach penalized regression model for stably differential diagnosis for bipolar depression.

Results: A simple and efficient model of approaching the diagnosis of individuals with depressive symptoms was established with a fitting degree (90.58%) and an acceptable cross-validation error rate. Neurotrophic factors of our interest were successfully screened out from the feature selection and optimized model performance as reliable predictive variables.

Conclusion: It seems to be feasible to combine different types of clinical characteristics with biomarkers in order to detect bipolarity of all depressive episodes. Neurotrophic factors of our interest presented its stable discriminant potentiality in unipolar and bipolar depression, deserving validation analysis in larger samples.

Keywords: bipolar depression, model-based algorithm, neurotrophic factor, clinical feature, biomarker

INTRODUCTION

The *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (*DSM-5*) separates the mood disorders into two sections: depressive and related disorders, and bipolar and related disorders. The new version of the diagnostic manual of bipolar disorders emphasizes more specific manifestations related to hypomanic and mixed manic states, which are considered to be a separate class of mood

disorder "in terms of symptomatology, family history and genetics" (1–3). From a statistical point of view, the nature of the diagnostic classification of depressive disorder can be clearly taken as a typical dichotomous problem with only two possible outcomes: one is bipolar depressive disorder (BPD) and the other is major depressive disorder (MDD).

In spite of its clear-cut division in diagnostic manual, a definite diagnosis for bipolar disorder remains an elusive goal. In a 10-year follow-up study of 290 unipolar depressed patients, Holmskov et al. have reported that the overall risk of conversion from initial diagnosis of unipolar depression to later bipolar disorder reaches up to 20.7% (4). The major challenge for clinical decision is that the identification of psychopathology still relies on the clinician's subjective judgment. Generally speaking, the classification of mood disorder or the diagnosis of bipolar disorder can be made without difficulty with a manic episode. But in the absence of specific symptoms, the clinical decision is hard to be made, especially for those patients with bipolar depression who initially come for medical help as depressive or other nonspecific symptoms being fairly laid open to doctor.

The search for objective or subjective assessment of whether a depressive episode is potentially subordinate to bipolar disorder or not is of clinical relevance, since patients at high risk may be missing the optimized opportunity of therapy. There have been several clinical studies that focused on relevant risk factors of bipolar disorder in terms of clinical symptoms for early detection. In the study mentioned above, Holmskov et al. have performed analysis for the risk factor for conversion at baseline: a rising number of previous depression recurrences [hazard ratio (HR) 1.18, 95% confidence interval (CI) (1.10–1.26)] and no strong relationship between gender, age at onset, subtype of depression, and any of the investigated Hamilton Depression Scale (HAM-D) subscales with the conversion, among others (4). It was said that it would take an average of 10 years for misdiagnosed patients to get the right diagnosis and treatment of BD (5). As for the children and youth cohort, a systematic review of cross-sectional studies reported that pediatric patients with bipolar depression had higher levels of depression severity, psychiatric comorbidity, and family history (6).

Using clinical characteristics alone is not a precise and stable solution for identification of bipolar disorder. It is prone to a certain probability of misjudgment when clinicians use individual or several indicators for clinical classification. In view of the overlap of clinical manifestations of unipolar and bipolar disorder as well as the limitations of clinician's subjective experience, methods integrating different dimensions of evidences to make judgments might be more statistically reliable and sufficient than independent variables. The scientific research has made some progress in searching for biomarkers being objectively indicative of mood disorders. Chang et al. have found that C-reactive protein could be a differential biomarker making out bipolar II depression versus MDD (7), although it was challenged by another study as confounding factors in a case–control study (8). Morphometric analyses using voxel-based morphometry by Redlich et al. have demonstrated that structural abnormalities in neural regions supporting emotion processing, such as gray matter volumes in the hippocampus and amygdala and white matter volumes within the cerebellum and hippocampus, could be good markers (9). The pattern classification approach was announced, reaching up to 79% accuracy, but the model did not survive the Alpha-Sim correction in validation data (false-positive rate is too high when applied in test data).

In our previous work, we have found that levels and trends of serum neurotrophic factors differed between patients with unipolar and bipolar depression, which may give us some inspiration. Factors such as fibroblast growth factor (FGF)-2, vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and insulin-like growth factor (IGF)-1 might be potential candidate biomarkers for bipolar disorder. Drug-naïve patients with bipolar disorder with manic episode showed increased serum levels of FGF-2, NGF, and IGF-1, while patients with MDD showed decreased serum FGF-2 levels that are probably associated with their compensatory roles of neuroprotection and angiogenesis, which are involved in their specific pathophysiology in these two disorders and thus be able to differentiate from each other (10, 11). To our knowledge, their clinical application for diagnostic assistance of mood disorders remains uncertain so far, although these neurotrophic factors potentially could be robust and biologically interpretable biomarkers.

In this study, we present a correlation-based feature selection and a reliability-based optimization strategy to extract enough information from unipolar depression and bipolar depression samples. Here, not only would we aim to investigate whether and to what extent neurotrophic factors and their individual components can be related to either unipolar or bipolar depression, we would also try to establish simple artificial intelligence system for stably differential diagnoses for bipolar depression by combining biological biomarkers and clinical characteristics. To our knowledge, there are few similar studies so far. We hypothesized that peripheral blood biomarkers can be successfully screened out from the feature selection and optimize model performance as reliable predictive variables.

MATERIALS AND METHODS

Subjects, Blood Sample Collection, and Laboratory Test

Patients in a depressive episode, including 30 patients with MDD and 23 patients with BPD, were recruited in Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine in 2014. The inclusion criteria were as follows: 1) age 18–60 years; 2) met the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, *4th edition* (*DSM-IV*, 12) for major depressive episode and depressive episode of bipolar disorder; and 3) patients not taking any psychiatric medications at least 2 weeks before treatment. Patients with severe physical illness and other mental illness associated with depressive state were excluded from this study. Subjects who are currently pregnant or lactating were also excluded.

The demographic information was collected during enrollment. The 24-item Hamilton Depression Scale (HAMD-24), the Montgomery–Åsberg Depression Rating Scale (MADRS), and the Hamilton Anxiety Scale (HAMA) were measured to assess the clinical symptoms of patients. The neurotrophic factors (FGF-2, NGF, IGF-1, and VEGF) in peripheral blood

of all patients with MDD and BPD were measured by enzyme-linked immunosorbent assay (ELISA) technique. All patients received 8 weeks of personalized therapy, among which 10% MDD patients and 60% of BPD patients took mood stabilizers. Both clinical symptom assessment and blood test took place at baseline and after treatment. Detailed information was published in our previous paper (10, 11).

The study protocol was approved by the Ethics Committee of Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine. All subjects provided informed consent for this study.

Statistical Analysis and Model-Based Diagnostic Algorithm

Patients' characteristics of a three-dimensional dataset containing peripheral levels of neurotrophic factors, clinical scale scores, and demographic features (as shown in **Table 1**) were used for feature selection, of which discriminatory power was evaluated stepwise and search strategy was approached for global optima. Then, a model-based algorithm was applied to reduce the dimension and boost model performance. Identification of robust biomarkers and model performance was supervised by significant level of analysis of covariance and size effect as well as error rates based on cross-validation.

Statistical analyses were performed using SAS9.4 software for Windows (SAS Institute Inc., Cary, NC, USA). The demographic and clinical data of the two groups of patients were listed in the attached table (see **Supplementary Materials**), as were the serum neurotrophic levels we measured.

A stepwise discriminant analysis (method Forward Stepwise) was made to select variables for use in discriminating between the two groups, as measured by Wilks' lambda, the likelihood ratio criterion (13). At each step, discriminant analysis evaluates all the variables and enters the one contributing most to the discriminatory power between groups. When none of the unselected variables meet the entry criterion, the forward selection process stops. Then, 11 variables in the dataset were found to have potential discriminatory power. Results of the selection process were summarized.

After that, the stepwise model was utilized for testing using the SAS Glmselect procedure. The "Glmselect" procedure, which is suitable for small sample research, has built-in penalties for model overfitting and internal collinearity of variables (14). Tenfold cross-validation was specified as a tuning method to choose an optimum model with minimum estimated prediction error (15). Multicollinearity was also a concern and was assessed by tolerance. Multiple logistic regression models were used to give maximum sensitivity and specificity as well as further analysis.

RESULTS

Demographic and Clinical Characteristics

Of the 53 patients, 30 were MDD and 23 were BPD. The descriptive information on demographic and clinical characteristics is listed in **Table S1**. No significant difference in age and gender were found between the two groups. In our study, 79% of our patients were recurrent with an average age of 46 years old. Comparison of two groups showed that the duration of disorder ($Z = 2.2559$, $p = 0.0241$), number of previous episode ($Z = 3.4131$, $p = 0.0006$), and presence of family history ($\chi^2 = 5.2170$, $p = 0.0308$) were significantly higher in the BPD patients. Besides, there were no significant differences in the age, gender, educational level, marital status, age at onset, duration of the present episode, and the proportion of patients with psychotic symptoms between the two groups.

Also, no statistical differences were found between groups in the baseline HAMD Scale score, as well as the MADRS and the HAMA, as shown in **Table S2**. During the 8 weeks of follow-up, all patients finished a personalized therapy and 90.57% patients got a clinical remission with a reducing rate of Hamilton scale score ≥75% without group difference at the end. Additionally, we could not find any marked differences in the overall reducing rate between groups.

The serum levels of FGF-2, IGF-1, VEGF, and NGF in the two groups were shown as mean ± standard deviation (SD) in detail. No obvious differences between these four neurotrophic factors between groups were found at baseline and after treatment. However, the concentration trend of serum FGF-2 levels was completely different between groups (effect sizes = −2.118, $p = 0.034$); while MDD patients showed a distinct decline after treatment ($d = 18.36 \pm 94.06$, $p = 0.016$), BPD patients maintained an insignificant change ($d = -4.74 \pm 92.58$, $p = 0.270$) compared to the baseline level. No similar situation occurred in the other three neurotrophic factors. Notably, no correlation was shown between the serum FGF-2 concentration and the treatment wherein patients received a mood stabilizer or not ($Z = 1.233$, $p = 0.218$).

Preliminary Screening of Predictive Variables by Discriminant Analysis

Stepwise discriminant analysis was conducted and the results are presented in **Table 2**. Since the test of homogeneity of within covariance matrices showed a significant χ^2 value, the within covariance matrices were used in the discriminant function. Eleven variables in the dataset were found to have potential discriminatory power: 1) age at onset (years); 2) IGF-1 at baseline (ng/ml); 3) VEGF at baseline (pg/ml); 4) presence of family history; 5) presence of psychotic symptoms; 6) item 5 score of MADRS Scale (Loss of appetite); 7) HAMA Factor 1 at baseline (Psychological anxiety); 8) HAMD Factor 4 at baseline (Diurnal variation); 9) delta for FGF-2 (pg/ml); 10) delta for NGF (pg/ml); and 11) delta effect of HAMD Factor 2 (Weight loss). The total classification error rate of preliminary screening by discriminant analysis method was 0.2000 by re-substitution and was 0.3551 by cross-validation at this step. By cross-validation, only one patient (0.0435%) in BPD was misclassified into MDD while 20 patients (66.6667%) in MDD were misclassified into BPD. The results showed that the variables below together could simulate the patients in BPD well but held a high false-positive rate.

Dimensionality Reduction and Model Selection

Regularization was conducted and a penalized regression model was established. Graphical summaries of the selection search

TABLE 1 | Variables that belonged to the "three-dimensional dataset" for feature selection.

Variable	Variable no./Symptom code	Additional information/Composed item
Sociodemographic and clinical characteristics		
Age (years)	age1	
Age at onset (years)	age2	
Presence of psychotic symptoms	Psycho_categor	
Presence of family history	History_categor	
HAMD-24		
Factor 1: Anxiety/Somatization		
Baseline	dfactor1b	Item 10 score of the HAMD Scale (Anxiety-Psychic)
Delta effect	ddfactor1	Item 11 score of the HAMD Scale (Anxiety-Somatic)
		Item 12 score of the HAMD Scale (Somatic symptoms-gastrointestinal)
		Item 15 score of the HAMD Scale (Hypochondriasis)
		Item 17 score of the HAMD Scale (Insight)
Factor 2: Weight loss		
Baseline	dfactor2b	Item 16 score of the HAMD Scale (Loss of weight)
Delta effect	ddfactor2	
Factor 3: Cognitive dysfunction		
Baseline	dfactor3b	Item 2 score of the HAMD Scale (Feeling of guilt)
Delta effect	ddfactor3	Item 3 score of the HAMD Scale (Suicide)
		Item 9 score of the HAMD Scale (Agitation)
Factor 4: Diurnal variation		
Baseline	dfactor4b	Item 18 score of the HAMD Scale (Diurnal variation)
Delta effect	ddfactor4	
Factor 5: Loss of motivated behavior		
Baseline	dfactor5b	Item 1 score of the HAMD Scale (Depressed mood)
Delta effect	ddfactor5	Item 7 score of the HAMD Scale (Work and interests)
		Item 8 score of the HAMD Scale (Retardation)
		Item 14 score of the HAMD Scale (Genital symptoms)
Factor 6: Sleep disturbance		
Baseline	dfactor6b	Item 4 score of the HAMD Scale (Insomnia-Initial)
Delta effect	ddfactor6	Item 5 score of the HAMD Scale (Insomnia-Middle)
		Item 6 score of the HAMD Scale (Insomnia-Delayed)
Factor 7: Despair/Sadness		
Baseline	dfactor7b	Item 22 score of the HAMD Scale (Sense of decline in ability)
Delta effect	ddfactor7	Item 23 score of the HAMD Scale (Feeling of despair)
		Item 24 score of the HAMD Scale (Feeling of inferiority)
MADRS		
Item 1 score of the MADRS Scale (Apparent sadness)		
Baseline	mads1b	
Delta effect	dmads1	
Item 2 score of the MADRS Scale (Reported sadness)		
Baseline	mads2b	
Delta effect	dmads2	
Item 3 score of the MADRS Scale (Inner tension)		
Baseline	mads3b	
Delta effect	dmads3	
Item 4 score of the MADRS Scale (Reduced sleep)		
Baseline	mads4b	
Delta effect	dmads4	
Item 5 score of the MADRS Scale (Loss of appetite)		
Baseline	mads5b	
Delta effect	dmads5	

(Continued)

TABLE 1 | Continued

Variable	Variable no./Symptom code	Additional information/Composed item
Item 6 score of the MADRS Scale (Concentration difficulties)		
Baseline Delta effect	mads6b dmads6	
Item 7 score of the MADRS Scale (Lassitude)		
Baseline Delta effect	mads7b dmads7	
Item 8 score of the MADRS Scale (Inability to feel)		
Baseline Delta effect	mads8b dmads8	
Item 9 score of the MADRS Scale (Pessimistic thoughts)		
Baseline Delta effect	mads9b dmads9	
Item 10 score of the MADRS Scale (Suicidal thoughts)		
Baseline Delta effect	mads10b dmads10	
HAMA		
Factor 1: Psychological anxiety factor		
Baseline Delta effect	afactor1b dafactor1	Item 1 score of the HAMA Scale (Anxious mood) Item 2 score of the HAMA Scale (Tension) Item 3 score of the HAMA Scale (Fears) Item 4 score of the HAMA Scale (Insomnia) Item 5 score of the HAMA Scale (Intellectual) Item 6 score of the HAMA Scale (Depressed mood) Item 14 score of the HAMA Scale (Behavior at interview)
Factor 2: Somatic anxiety factor		
Baseline Delta effect	afactor2b dafactor2	Item 7 score of the HAMA Scale (Somatic-muscular) Item 8 score of the HAMA Scale (Somatic-sensory) Item 9 score of the HAMA Scale (Cardiovascular symptoms) Item 10 score of the HAMA Scale (Respiratory symptoms) Item 11 score of the HAMA Scale (Gastrointestinal symptoms) Item 12 score of the HAMA Scale (Genitourinary symptoms) Item 13 score of the HAMA Scale (Autonomic symptoms)
FGF-2		
Baseline Delta for FGF-2	FGF-2_B dFGF-2	
IGF-1		
Baseline Delta for IGF-1	IGF_B dIGF	
VEGF		
Baseline Delta for VEGF	VEGF_B dVEGF	
NGF		
Baseline Delta for NGF	NGF_B dNGF	

HAMD-24, 24-item Hamilton Depression Scale; MADRS, Montgomery–Åsberg Depression Rating Scale; HAMA, Hamilton Anxiety Scale.

are presented in **Figure 1A** and **B**, and parameter estimations of variable entry in the final step are summarized in **Table 3**. Three predictive variables ["HAMA Factor 1 at baseline (Psychological anxiety)", "item 5 score of MADRS Scale (Loss of appetite)", and "HAMD Factor 4 at baseline (Diurnal variation)"] were dropped out as meeting the cross-validation criterion and one variable ("Presence of psychotic symptoms") was excluded by the researcher according to clinical experience.

The final multiple diagnostic model using predictors combining neurotrophic biomarkers and clinical characteristics is shown in **Figure 2**, as well as biomarker predictor model alone and clinical characteristic predictor model alone. As seen in **Figure 2**, the multivariate model based on "Age at onset (years)," "Presence of family history," "IGF at baseline (ng/ml)," "VEGF at baseline (pg/ml)," "delta for FGF-2 (pg/ml)," "delta for NGF (pg/ml)," and "delta effect of HAMD Factor 2 (Weight loss)"

TABLE 2 | Results of stepwise discriminant analysis.

Step	Variable	Partial R^2	Pr > F	Average squared canonical correlation	Wilks' Lambda
1	Presence of family history	0.0984	0.0222	0.0984	0.9016
2	Age at onset (years)	0.0652	0.0705	0.2344	0.7655
3	Presence of psychotic symptoms	0.0763	0.0521	0.2929	0.7070
4	Delta effect of HAMD Factor 2 (Weight loss)	0.0794	0.0524	0.4100	0.5900
5	Delta for FGF-2	0.0492	0.1340	0.4390	0.5610
6	IGF-1 at baseline	0.0536	0.1216	0.4691	0.5309
7	VEGF at baseline	0.0494	0.1374	0.4718	0.5282
8	Delta for NGF	0.1289	0.0154	0.5399	0.4601
9	HAMD Factor 4 at baseline (Diurnal variation)	0.1495	0.0087	0.6030	0.3970
10	The item 5 score of the MADRS Scale at baseline (Loss of appetite)	0.0689	0.0851	0.6304	0.3696
11	HAMA Factor 1 at baseline (Psychological anxiety)	0.0503	0.1480	0.6490	0.3510

presented a good performance in detecting bipolar depressive disorder [Area Under Curve (AUC) = 0.9058, $P < 0.05$].

Models' contrast estimation and testing results are also listed in **Table 4**. What can be clearly seen from the table is that the diagnostic model using biomarkers alone (AUC = 0.7725, $P < 0.05$) showed no significant difference with the diagnostic model using clinical characteristics (AUC = 0.8007, $P < 0.05$) in the level of consistency ($P > 0.05$).

In addition, "Presence of family history" and "Age at onset" in clinical data and "delta for FGF-2" in biomarker data showed a better predictive effect for the outcome.

DISCUSSION

This paper first explored the application of objective biological markers combined with clinical features in the field of psychiatric diagnosis. In this study, we found that peripheral neurotrophic factors had a stably good performance in identifying patients with bipolar disorder among depressive patients. Indeed, the distribution of neurotrophic factors had the same discriminatory power as clinical characteristics and could optimize prediction model performance as reliable indicators. This association persisted both pre-treatment and post-treatment.

The main findings in this study are consistent with those in previous clinical studies and multivariate biomarker discovery in mood disorder. Eleven variables including four for neurotrophic factors, two for demographic data, and five for symptomatic characteristics were identified as risk factors in discriminant analysis step. All 11 variables were considered as potential indicators of bipolar disorder that have been reported in other studies. In our study, these 11 variables together perfectly simulated the characteristics of bipolar disorder with a good fitting degree, while the false-positive rate reached up to 0.36%. After the penalized regression methods, clinical characteristics "Age at onset," "Presence of family history," and "delta effect of HAMD Factor 2 (Weight loss)" have frequently been identified as reliable distinguishable biomarkers for unipolar depression and bipolar depression. Similarly, the finding that higher distribution levels and trends of neurotrophic factors can effectively distinguish two types of mood disorders is consistent with those in our previous studies (10, 11).

There are also some inconsistencies between our study and other published studies. For example, variables that were considered as reliable discriminators in other studies failed to enter our final diagnostic model: "HAMD Factor 4 at baseline (Diurnal variation)," "item 5 score of MADRS Scale (Loss of appetite)," and "HAMA Factor 1 at baseline (Psychological anxiety)." In our preliminary feature selection by discriminant analysis, the three variables listed above were also identified as having a certain degree of discriminatory power. The major reason that they failed to enter the final model was that they did not meet the cross-validation criterion in the regularization step, which meant each of these variables would raise the model misclassification error rate. In the context of linear regression, cross-validation was a popular penalized regression method that enabled an assessment of the optimal complexity of a model and minimized the residual sum of squares by using a penalty on the size of the regression coefficients so as to improve the overall prediction error (16). It was often exploited to decide a best-fit model that generally included only a subset of deemed truly informative features under the given data. But at the same time, this penalty might cause coefficient estimates to be biased (in order to ensure cross-validation error), and that would remove some discriminatory variables out of the model if they lack enough effect size or potentially have a cross-correlation with other variables (17, 18). In summary, variables dropped in the regularization step not only help fit the statistical model but also lead to a higher risk of misclassification. That may also account for the inconsistency between different studies.

As mentioned above, the final model predictors included four baseline effects and three delta effects. All the predictors in the final model not only had adequate discrimination but also showed a stable and robust performance to reduce total misclassification error. Among them, "Presence of family history" and "delta for FGF-2" demonstrated both a univariate and a multivariate significant difference between the two groups and passed the regularization into the final model, showing their reliable and independent discriminant performance. Also, baseline effects "IGF-1" and "VEGF" that entered the stepwise discriminant model in the sixth and seventh steps showed a certain discernment. However, we should note that "VEGF" would slightly increase the cross-validation press of it and the tolerance was not high when entering the final regression

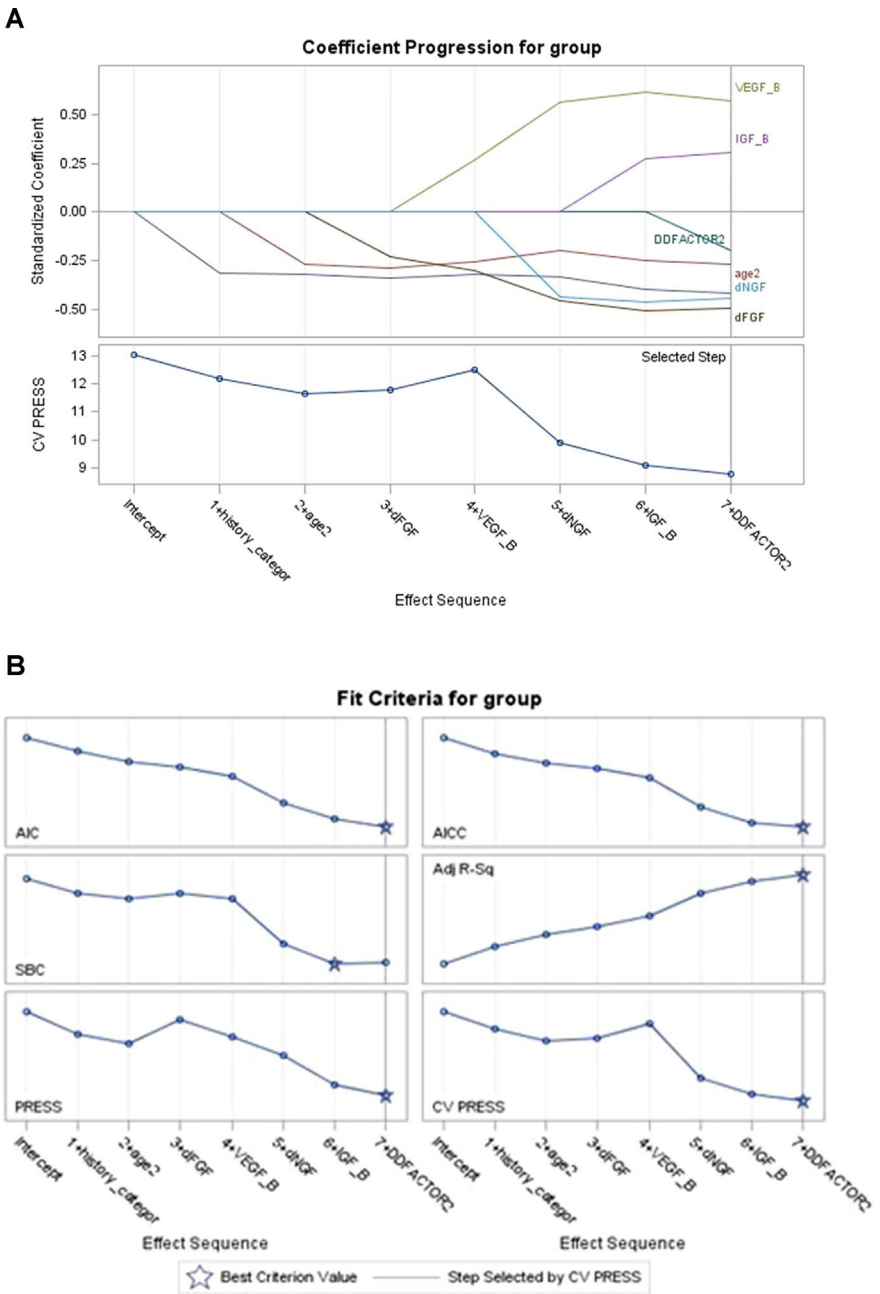

FIGURE 1 | Coefficient of model selection procession **(A** and **B)**. The variables entered the model in turn (AIC criteria) while keeping the model false-positive rate steadily decreasing; "presence of family history" and "age at onset" in clinical data and "dFGF-2" in biomarkers data showed their best predictive effect for the outcome; "VEGF" slightly increased the cross-validation press of the model. Variables not shown in the figures mean that they met the cross-validation criterion in regularization step and had been dropped out ("diurnal mood variation," "loss of appetite," "psychological anxiety at baseline"). VEGF = vascular endothelial growth factor.

model, as shown in **Table 3** and **Figure 1**. Moreover, "Age at onset" and "delta effect of HAMD Factor 2 (Weight loss)" were the other two predictions that show no statistical difference but pass the stepwise discriminant analysis as well as the penalized regression method. It was a matter of effect size versus statistical significance. As a general agreement (19, 20), effect size informed us about "the magnitude or practical importance of observed sample results" while statistical significance only evaluates the probability of obtaining the "Null hypothesis: A = B" outcome by chance. To be more specific, "Age at onset" held a good effect size but poor statistical significance. It helped to reduce the degree of overlapping between the two groups, but the differences in means were hard to be detected under the current sample size. According to **Table 3**, patients' age at onset under 41 years old

TABLE 3 | Parameter estimation of variables in the penalized regression model.

Variable entry	MLE estimate	Standard error	Pr > Chi-sq	Odds ratio	95% Wald CI		Tolerance value	*Cut-point
					Lower	Upper		
Baseline effect								
Age at onset (years)	–0.063	0.036	0.085	0.939	0.875	1.009	0.907	41.00
Presence of family history	4.559	1.525	0.003	95.50	4.812	>1000	0.919	1.000
IGF at baseline (ng/ml)	0.013	0.005	0.023	1.013	1.002	1.023	0.848	158.7
VEGF at baseline (pg/ml)	0.017	0.007	0.010	1.018	1.004	1.031	0.492	87.84
Delta effect								
Delta for FGF-2 (pg/ml)	–0.043	0.015	0.004	0.958	0.930	0.986	0.733	–8.170
Delta for NGF (pg/ml)	–0.187	0.075	0.012	0.829	0.716	0.960	0.548	1.411
Delta effect of HAMD Factor 2 (Weight loss)	–0.518	0.621	0.405	0.596	0.176	2.013	0.935	1.000

**Youden criterion.*

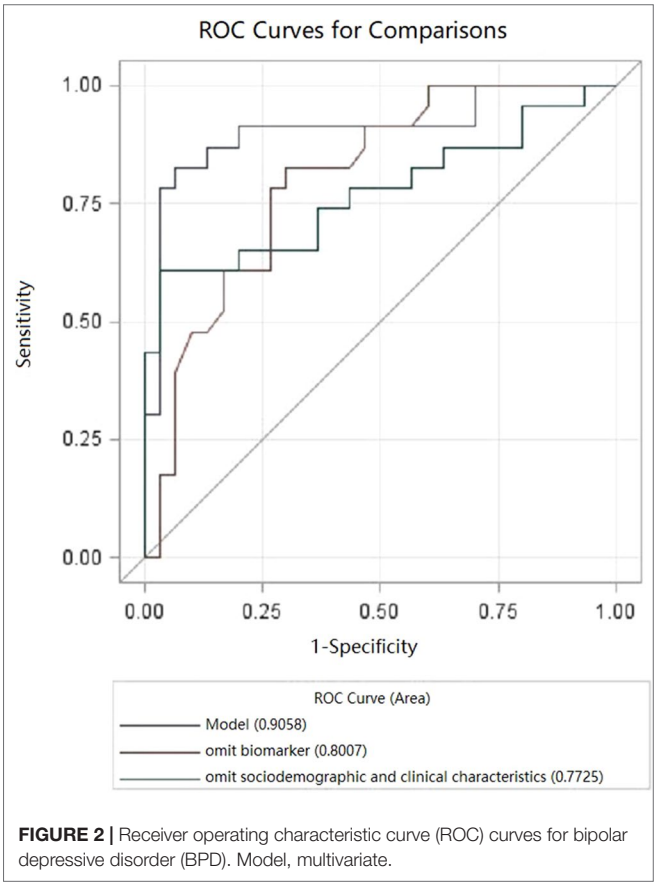

FIGURE 2 | Receiver operating characteristic curve (ROC) curves for bipolar depressive disorder (BPD). Model, multivariate.

was more likely to be a bipolar one, and it was consistent with an earlier age at onset that had been published in many clinical studies (21). Notably, its 95% confidence interval of ratio was high and that might be the reason why it has not been detected. The same situation occurred in the variable "delta effect of HAMD Factor 2 (Weight loss)". A depressed patient showing an improvement in weight loss was less likely to be a bipolar one (22). By adding this variable to the model, the Bayesian information criterion (SBC) would have a very small increase, but the Akaike information criterion (AIC) (23) would still decrease, which would still improve the model fit and cross-validation (**Figure 1**). This prompted us that, under the current sample, its convergence to the sample had reached its limit compared to other variables. In the small sample data feature selection, if only a univariate and unsupervised approach was applied, it was likely to be ignored. However, in the large sample of data, it might be easier to be identified and to obtain a good fit with better performance in different sample coherences. Larger samples were needed for further validation of the discriminant effect size of "Age at onset" and "delta effect of HAMD Factor 2 (Weight loss)".

The current study further clarified our previous findings about the neurotrophic factor classification system in mood disorders and presented details regarding a high-dimensionality biomarker discovery in the clinical study (24). The search for biomarkers of psychiatric diseases was still in its infancy. In the absence of sufficient evidence, identification of only one or two types of biological signals might be a nontrivial task. Among published studies on biomarker detection, multivariate analysis was commonly used. For patients with mood disorders, there might be minor changes in the body's multiple systems that was not individually significant. Biomarkers could only be identified by truly multivariate approaches (25, 26). However, the number of biomarkers selected should be less than seven times the number of observations. To kick out redundant information, there were common mistakes in many studies that controlled the number of variables to some notably identified ones by univariate tests (e.g., *t* test or *F* test). What's worse, the penalized method has already had been applied to the dataset before feature and model selection. It would take a great risk of losing important discriminatory information and holding a strong univariate bias. An algorithm combining non-automatic data processing has its benefit but should avoid eliminating discriminatory information at the preprocessing step. Also, noise detection based on variable correlation analysis was neither efficient nor safe as it neglects the biological interpretation of biomarkers and the possibility of related variables' cooperative discriminant power (25). In this sense, our algorithm nicely and efficiently circumvents this problem by adopting a supervised "wide in strict out strategy". Since we exhaustively

TABLE 4 | ROC association statistics and contrast estimation and testing results.

	ROC association statistics				Contrast estimation and testing**	
ROC Model	**Area**	**Standard error**	**95% Wald CI**		**Estimate**	**Pr > Chi-sq**
			Lower	**Upper**		
Model	0.9058	0.0472	0.8134	0.9982		
Model omit biomarkers	0.8007	0.0608	0.6816	0.9199	0.1333	0.0467
Model omit clinical characteristics' effect	0.7725	0.0709	0.6336	0.9114	0.1051	0.0463
Model omit biomarkers vs. Model omit clinical characteristics' effect					0.0283	0.7783*+

*Mann–Whitney; **Wald test.

+Model using biomarkers alone showed no significant difference with model using clinical characteristics alone.

filtered variables of discrimination into the model and then strictly kicked out variables by combining automatic and non-automatic "punish" to an appropriate number under the current sample size, the results had considerable reliability and stability.

What makes our study different from other studies was that we separated original feature selection and classification system building. Many research designs often directly incorporated supervised/unsupervised algorithms into variable selection, such as using SVM, or principal component analysis, in order to screen for statistically significant variables. However, this algorithm was prone to loss of important discriminatory variables when it comes to proteins and gene analysis (22, 23), resulting in low research consistency when applied into the real world. This type of study neglects the importance of the biological interpretation of biomarkers and might completely drive the statistical analysis into a completely wrong discriminatory direction (26). Including all of the discriminatory information in the preliminary feature selection and then applying a supervised algorithm to boost the model performance may be a better alternative for biomarker detection. The intrinsic relationship might be muddier at this step, but stepwise analysis was listed vividly and was good for further analysis. As science cannot claim absolute truth, what we could approach was "tentative or approximate truth," especially on psychiatry research that greatly relied on phenomenon-based diagnosis. By using supervised learning algorithms, we could be close to the biomarkers specific to bipolar disorder as much as possible. The model conducted an exhaustive search strategy and "Glmselect" in this paper may reflect some of the truth. It effectively removed irrelevant and redundant features and was computationally efficient while showing detail. Glmselect was one of the easily conducted methods with the higher prediction accuracy and computational efficiency of penalized regression. There remained a wide variability in specific biomarkers that can distinguish bipolar depression from all depression; thus, simple and efficient screening tools that could be widely used in different samples should be widely applied.

Undoubtedly, there might be statistical weakness, since it was a small sample size data analysis. To solve it, we conducted a cross-validation and stepwise discrimination in the feature selection. In case of a small sample size, the use of a 10-fold cross-validation and sequential forward selection was confirmed to be a better choice than a simple wrapper (26). Also, ranking feature sets was often based on error estimation and regularization served to reduce the overfitting problem. Therefore, the sample size was appropriate to achieve reasonable precision in the validation.

Identification of bipolar disorder was a historically difficult problem. To date, there is no single biological indicator or classification system combining biological indicators that can distinguish bipolar depression from depression and that has a stable and specific good discernment (27). Just like looking for a needle in a haystack, we need a standard and efficient way to screen variables. Algorithms with carefully built-in feature selection often provide a better alternative. Not only should we focus on the screening of biomarkers, but we also need to establish a more standardized statistics strategy for clinical data. At the same time, neurotrophic factors of interest showed a good performance in comparison with clinical scales, deserving validated analysis in other larger samples.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Ethics Committee of Shanghai Mental Health Center with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of Shanghai Mental Health Center.

AUTHOR CONTRIBUTIONS

YZ performed the statistical analyses and wrote the manuscript. SH completed all of the data entry. ZL finished all of the laboratory work. YF, KJ, and SS were responsible for the diagnosis and clinical assessment of the participants. TZ provided assistance for the statistical analyses. XL designed and wrote the study protocol, managed the literature searches and analyses, and reviewed the manuscript. All authors approved the final version of this manuscript.

FUNDING

This work was supported by projects from the Shanghai Health Bureau (2009098), the Natural Science Foundation of Shanghai (15ZR1435400), the National Natural Science Foundation of China (81000588), "Shanghai health system young talents

training plan" in Shanghai Health Bureau (XYQ2011016), and the National Key Clinical Disciplines at Shanghai Mental Health Center (OMA-MH, 2011-873).

ACKNOWLEDGMENTS

The authors would like to thank all the patients and healthy volunteers for participation and the medical staff for collecting specimens.

SUPPLEMENTARY MATERIALS

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2019.00266/full#supplementary-material

TABLE S1 | Sociodemographic and clinical characteristics of MDD patients and BPD patients.

TABLE S2 | Scale scores and serum neurotrophic levels in MDD and BPD patients before and after treatment.

REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders 4th edn (DSM–IV). Washington, DC: APA (1994). ISBN: 978-0890420614
2. American Psychiatric Association. Diagnostic and Statistical Manual of mental disorders 5th edn (DSM–V). Arlington: APA (2013). ISBN: 978-0890425541.
3. Möller HJ, Bandelow B, Bauer M, Hampel H, Herpertz SC, Soyka M, et al. DSM-5 reviewed from different angles: goal attainment, rationality, use of evidence, consequences—part 2: bipolar disorders, schizophrenia spectrum disorders, anxiety disorders, obsessive–compulsive disorders, trauma- and stressor-related disorders, personality disorders, substance-related and addictive disorders, neurocognitive disorders. *Eur Arch Psychiatry Clin Neurosc* (2015) 265(2):87–106. doi: 10.1007/s00406-014-0521-9
4. Holmskov J, Licht RW, Andersen K, Bjerregaard Stage T, Mørkeberg Nilsson F, Bjerregaard Stage K, et al. Diagnostic conversion to bipolar disorder in unipolar depressed patients participating in trials on antidepressants. *Eur Psychiatry* (2017) 40:76–81. doi: 10.1016/j.eurpsy.2016.08.006
5. Drancourt N, Etain B, Lajnef M, Henry C, Raust A, Cochet B, et al. Duration of untreated bipolar disorder: missed opportunities on the long road to optimal treatment. *Acta Psychiatr Scand* (2013) 127(2):136–44. doi: 10.1111/j.1600-0447.2012.01917.x
6. Uchida M, Serra G, Zayas L, Kenworthy T, Faraone SV, Biederman J. Can unipolar and bipolar pediatric major depression be differentiated from each other? A systematic review of cross-sectional studies examining differences in unipolar and bipolar depression. *J Affect Disord* (2015) 176:1–7. doi: 10.1016/j.jad.2015.01.037
7. Chang HH, Wang T-Y, Lee IH, Lee SY, Chen KC, Huang SY, et al. C-reactive protein: a differential biomarker for major depressive disorder and bipolar II disorder. *World J Biol Psychiatry* (2017) 18(1):63–70. doi: 10.3109/15622975.2016.1155746
8. Fond G, Brunel L, Boyer L. C-reactive protein as a differential biomarker of bipolar II depression versus major depressive disorder. *World J Biol Psychiatry* (2017) 18(1):71–2. doi: 10.1080/15622975.2016.1208842
9. Redlich R, Almeida JJ, Grotegerd D, Opel N, Kugel H, Heindel W, et al. Brain morphometric biomarkers distinguishing unipolar and bipolar depression. A voxel-based morphometry-pattern classification approach. *JAMA Psychiatry* (2014) 71(11):1222–30. doi: 10.1001/jamapsychiatry.2014.1100
10. Shen H, Zhang T, Hong B, Peng D, Su H, Lin Z, et al. Decreased serum fibroblast growth factor-2 levels in pre- and post-treatment patients with major depressive disorder. *Neurosci Lett* (2014) 579:168–72. doi: 10.1016/j.neulet.2014.07.035
11. Liu X, Zhang T, He S, Hong B, Chen Z, Peng D, et al. Elevated serum levels of FGF-2, NGF and IGF-1 in patients with manic episode of bipolar disorder. *Psychiatry Res* (2014) 218:54–60. doi: 10.1016/j.psychres.2014.03.042
12. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders 4th edn (DSM–IV). Washington, DC: APA (1994).
13. Costanza MC, Afifi AA. Comparison of stopping rules in forward stepwise discriminant analysis. *J Am Stat Assoc* (1979) 74:777–85. doi: 10.1080/01621459.1979.10481030
14. Ingersoll, KS. Which patient characteristics among cocaine users with HIV relate to drug use and adherence outcomes following a dual-focused intervention? *AIDS Behav* (2016) 20(3):633–45. doi: 10.1007/s10461-015-1119-6
15. Frank. Training set vs. test set vs. validation set—what's the deal? (2017) (Accessed April 1, 2017) Available from: www.machinelearningtutorial.net.
16. Picard RR, Cook RD. Cross-validation of regression models. *J Am Stat Assoc* (1984) 79(387):575–83. doi: 10.1080/01621459.1984.10478083
17. Hastie T, Tibshirani R, Friedman J. In: *The elements of statistical learning: data mining, inference, and prediction.* New York: Springer-Verlag (2001), ISBN: 0-387-95284-5.
18. Penalized regression methods for linear models in SAS/STAT® FundaGunes, SAS Institute Inc (2010). (Accessed February 25, 2018). Available from https://support.sas.com.
19. Fan X, Konold TR. Statistical significance versus effect size. In: Peterson P, Baker E, McGaw B, editors. *International encyclopedia of education*, 3rd ed. New York: Elsevier (2010), ISBN: 9780080448947. p. 444–50. doi: 10.1016/B978-0-08-044894-7.01368-3
20. Vacha-Haase T, Thompson B. How to estimate and interpret various effect sizes. *J Counsel Psychol* (2004) 51:473–81. doi: 10.1037/0022-0167.51.4.473
21. Egeland JA, Blumenthal RL, Nee J, Sharpe L, Endicott J. Reliability and relationship of various ages of onset criteria for major affective disorder. *J Affect Disord* (1987) 12:159–65. doi: 10.1016/0165-0327(87)90009-7
22. Dziuda DM. *Data mining for genomics and proteomics: analysis of gene and protein expression data*. New Jersey: Wiley-Interscience (2010), ISBN: 9780470163733. doi: 10.1002/9780470593417
23. Hand DJ, Mannila H, Smyth. P. *Principles of data mining. Adaptive computation and machine learning*. The United States: A Bradford Book (2001), ISBN: 9780262082907.
24. Dziuda DM. Current trends in multivariate biomarker discovery. *Issues in Toxicology* (2015) 137–61. ISSN: 17577179
25. Silver N. *The signal and the noise: why most predictions fail—but some don't*. The United States: Penguin Group (2012), ISBN: 978-1-59-420411-1.
26. Borra S, Di Ciaccio A. Measuring the prediction error. A comparison of cross-validation, bootstrap and covariance penalty methods. *Comput Stat Data Anal* (2010) 54:2976–89. doi: 10.1016/j.csda.2010.03.004
27. Efron B, Hastie T, Johnstone I, Tibshirani R. Least angle regression. *Ann Stat* (2004) 32(2):407–99. doi: 10.1214/009053604000000067

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership