



Heterogeneity in major depressive disorder: The need for biomarker-based personalized treatments

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Abstract

Major Depressive Disorder (MDD) or depression is a pathological mental condition affecting millions of people worldwide. Identification of objective biological markers of depression can provide for a better diagnostic and intervention criteria; ultimately aiding to reduce its socioeconomic health burden. This review provides a comprehensive insight into the major biomarker candidates that have been implicated in depression neurobiology. The key biomarker categories are covered across all the “omics” levels. At the epigenomic level, DNA-methylation, non-coding RNA and histone-modifications have been discussed in relation to depression. The proteomics system shows great promise with inflammatory markers as well as growth factors and neurobiological alterations within the endocannabinoid system. Characteristic lipids implicated in depression together with the endocrine system are reviewed under the metabolomics section. The chapter also examines the novel biomarkers for depression that have been proposed by studies in the microbiome. Depression affects individuals differentially and explicit biomarkers identified by robust research criteria may pave the way for better diagnosis, intervention, treatment, and prediction of treatment response.

Abbreviations

5-HT	5-hydroxytryptamine
ACTH	adrenocorticotrophic hormone
AEA	N-arachidonylethanolamide
BDNF	brain-derived neurotrophic factor
CB₁	cannabinoid receptor type 1
CNS	central nervous system
CpG	cytosine-phosphate-guanine
CREB	cAMP-response element binding protein
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DAMPs	danger associated molecular patterns
DHA	docosahexaenoic acid
DNMT	DNA methyltransferases
DSM	Diagnostic and Statistical Manual of Mental Disorders
DST	Dexamethasone Suppression Test
ECB	endocannabinoid
ECT	electroconvulsive therapy
EGR1	early growth response 1
EPA	eicosapentaenoic acid
ERK	extracellular-signal-regulated kinase
FA	fatty acids
FAAH	fatty acid amide hydrolase
FGF	fibroblast growth factor
GABA	gamma aminobutyric acid
GABA_A	gamma aminobutyric acid A (receptor)
HDAC	histone deacetylase
HDL	high-density lipoprotein

HPA	hypothalamic-pituitary-adrenal
IDO	indoleamine 2,3-dioxygenase
IFN-α	interferon- α
IL	interleukin
KA	kynurenic acid
LDL	low-density lipoprotein
LPS	lipopolysaccharide
MDD	major depressive disorder
miRNAs	microRNAs
NAc	nucleus accumbens
ncRNAs	non-coding RNAs
NF-κB	Nuclear factor kappa B
NMDA	N-methyl-D-aspartate
PEA	palmitoylethanolamide
PFC	pre-frontal cortex
PPAR-α	peroxisome proliferator-activated receptor
PRIMA1	proline rich membrane anchor 1
PTSD	post-traumatic stress disorder
PUFA	polyunsaturated fatty acids
QA	quinolinic acid
SCFAs	short-chain fatty acids
SNPs	single-nucleotide polymorphisms
SSRI	selective serotonin reuptake inhibitors
TNF	tumor necrosis factor
TrkB	tropomyosin receptor kinase B
VEGF	vascular endothelial growth factor



1. Introduction

Some of the earliest accounts of depression have been found in writings dating back to the second millennium B.C.E in Mesopotamia [1]. With extensive advances in science, today we know Major Depressive Disorder (MDD) or major depression as a pathological mental condition affecting more than 264 million people worldwide [2]. MDD can differ from person to person depending on the symptoms, which indicate mild, moderate, or severe depression. The extremes of depression often lead to suicidality, which affects nearly 800,000 people worldwide per year [2]. Furthermore, MDD is characterized by feelings of sadness, irritability, loss of interest, tiredness, troubled thinking, worthlessness and disturbances in appetite and sleep. Not only does depression affect the wellbeing of individuals and families, but also

possesses a professional risk due to presenteeism, costing the workplace [3]. MDD poses a huge economic burden of roughly \$210.5 billion per year [2]. The need for more effective intervention is therefore crucial.

MDD can be assessed by various psychometric rating scales, such as the Hamilton Depression Rating Scale, the self-rating Beck Depression Inventory, and the Montgomery-Asberg Depression Rating Scales. All of these psychometric scales use a symptomatic intensity-based approach. Diagnosis on the other hand occurs primarily through subjective symptom-based analysis by a clinician. The most common guidelines used to assess mental health conditions are the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) and the international Classification of Diseases, both of which contain a symptomatic approach to diagnosis [4,5]. Diagnostic assessment is based on patients' subjective views and clinicians' assessment. None of the psychometric approaches offer a measurable biological marker to classify the disease condition. Numerous reports have shown misdiagnosis which may prevent amelioration of the mental condition [6–8]. For example, bipolar disorder is often incorrectly assessed as MDD [9]. Hirschfeld et al. [10] reported a 69% rise in bipolar disorder misdiagnosis for unipolar depression and over a third of patients waited 10 years before accurate diagnosis, highlighting the physicians' lack of ability in understanding and diagnosis of bipolar disorder and other mood disorders. Incorrect diagnosis often leads to false treatments and an unnecessary rise in medical costs due to poor outcomes [10,11].

In addition, the DSM-V lacks distinction between the scales of depression, hence affecting accurate diagnosis of alternate subtypes of MDD [4]. Subtypes of MDD can range from severe depressive illness or melancholia to diminished mood due to changes in life circumstances [12]. Other subtypes have been proposed, including suicidal depression [13], anxious depression [14], pseudo-demented depression [15] and dysthymia [15,16]. However, depression is widely accepted as a universal condition in clinical dogma and distinguishing etiology is often neglected [17]. It is therefore important to identify specific distinctions between different forms of MDD for a more throughput diagnosis.

As well as the diagnostic limitations highlighted above, a complex array of social, psychological, genetic and biological factors influence the etiology and progression of depression [18–20]. As a result, MDD constitute a complex and heterogenous disorder, which is difficult to diagnose accurately among individuals due to different subtypes of depression that include dysthymia, psychotic depression, seasonal depression, atypical depression,

bipolar disorder, misex depression with anxiety disorder, and perinatal depression; multiple pathways can lead to these various conditions [21,22]. Many clinicians view depression as a neurobiological condition that requires unilateral pharmacological intervention. Overdiagnosis of MDD routinely occurs in patients where prescribed antidepressants may be unnecessary [23–26]. A study on clinician-identified depression (sample = 5639) revealed that only 38.4% of patients with clinician-identified depression met the DSM criteria for major depressive episodes for 12 months [25]. Another survey revealed the prescription of antidepressants with no psychiatric diagnoses increased from 59.5% to 72.7% between 1996 and 2007 [26]. This stresses the importance of identifying factors that contribute to this trend; using this information adequate national policies can be developed to tackle this problem (e.g., improvement towards providers' prescribing practices). The rise in mental-health conditions necessitates the need for improved access to better treatments as well as better screening strategies for the individuals who might have been misdiagnosed.

Antidepressants or antipsychotics have been traditionally utilized for severe MDD, such as melancholia [27]. Studies have shown a notably improved drug-placebo difference in cases of severe depression, whereas moderate depression showed little to no change [28]. This indicates antidepressants are most efficient for conditions on the extreme scale [12]. This is important as it highlights the need for alternative treatments for a disorder as heterogeneous as MDD [18–20]. Cognitive behavioral therapy, for example, can be used to alleviate depressive symptoms alone or in addition with antidepressants [29]. The American Psychiatric Association (APA) favors treatment protocols that adopt prescription of antidepressants for MDD, regardless of the spectrum of severity. These antidepressants are advocated on the basis of counteracting neurochemical imbalances underlying the neurobiology of MDD that have often been advocated but with little conclusive evidence [30].

The current strategy in modern psychiatry is the use of symptom-based approach to describe mental health conditions. This poses risks such as overdiagnosis, misdiagnosis, and ineffective treatment strategies, all of which affect individuals at risk on the personal and socioeconomic level. The introduction of objective biological markers of MDD could significantly improve the diagnostic process between clinicians and clarify different depressive subtypes among individuals, ultimately minimizing the risks by developing *ad personam* treatments.



2. Biomarkers

A biomarker is an objective measurable characteristic that can be assessed to monitor biological, physiological and pathogenic processes in order to guide therapeutic interventions [31]. It is important to distinguish between biomarkers and idiosyncrasies of a disease, i.e., the clinical outcome assessment. The clinical outcome assessment measures how a patient feels, functions, or survives and can be used as an intervention for therapeutics. Biomarkers on the other hand serve to make a connection between different clinical outcome assessment predictions [32]. Effective biomarkers can only be approved following rigorous evidence-based testing that reaches a conclusive endpoint, which connects to the disease in question [31]. Biomarkers can further be classified into subtypes depending on their applicative purposes. *Diagnostic biomarkers* are used to confirm the presence or absence of illness, as well as to identify individuals who have a disease subtype [31]. *Monitoring biomarkers* evaluate and assess the disease condition to illustrate or detect exposure to a medical agent [32]. *Treatment biomarkers* are useful in calculating an optimal treatment from a range of options, a type of approach that is useful in individualized medication. *Response biomarkers* are particularly useful in clinical and early treatment setting where they can assess changes in biomarker levels following administration of a medical product. Long-term response biomarkers can also be utilized to assess final treatment outcome. A *predictable biomarker* indicates whether a person or a group of individuals will have a favorable or unfavorable reaction to a medicinal product. This type of biomarker is very beneficial for enrichment strategies in clinical trials and can be used to assess if a specific treatment has any significant effect on an individual [33,34]. *Prognostic biomarkers* are tailored to patients with medical conditions to predict the risk of clinical occurrence, disease recurrence, and progression. They are frequently used in clinical trials for entry and exclusion criteria. Furthermore, biomarkers are important in determining the risk of an event; for example, institutes use biomarker-related data to determine the length of stay of a patient before it is safe to discharge them from medical care. A *safety biomarker* is used for evaluation before or after a medical procedure to determine the possibility, existence, or severity of toxicity of an unwanted event. Lastly, *risk biomarkers* estimate the potential to develop a medical condition in individuals who are currently disease free or healthy [32] (See Fig. 1).

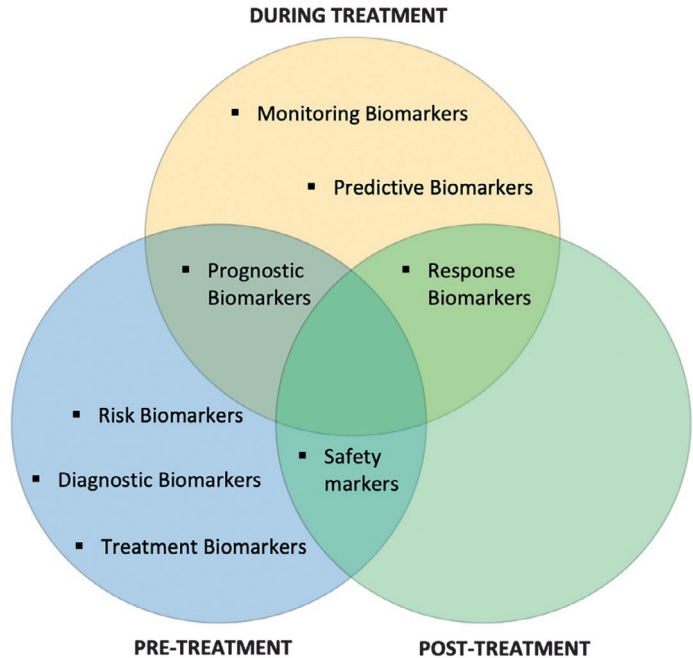


Fig. 1 Venn diagram of major biomarkers subtypes. Blue—Pre-treatment markers. Yellow—biomarkers during treatment. Green—Post-treatment markers [35].

2.1 “Omics” systems in depression

In depression and other health conditions, biomarkers can be examined across a wide variety of levels, known as “omics.” Biological systems, their molecular pathways, and their effect in modern psychiatry can be assessed by the following omics: genomics, epigenomics, transcriptomics, proteomics, metabolomics, and the microbiome. By analyzing systems at different levels, we can characterize and quantify biomarkers at that “omic” level and link their function to the effects on an individual organism [36]. The systems affected in depression include genetic, inflammatory, endocrine, neurotrophic, metabolic, neurotransmitter, and the neurosteroid and endocannabinoid systems [37,38]. It is important to denote that many of these systems can be found across different omic dimensions. With technological advances and throughput screening, a wide variety of biomarkers have been proposed for MDD [37]. This review will highlight some of the emerging evidence-based biomarkers for depression, underscoring the clinical advantages by assessing objective measures to improve diagnosis and by improving treatment response of this multifaceted disorder [38].



3. Epigenomics and depression

Epigenetics investigates the changes in gene expression without altering the genetic code itself. These modifications can be heritable or modified due to the environment and provide a useful tool to understand the etiology of disease conditions [39]. Along with this, epigenetic regulation also plays an important role in pathological processes, which include mental health diseases, such as major depression [40,41]. Traditional epigenetic mechanisms affect DNA-protein interactions, cellular differentiation, embryogenesis, genomic imprinting, and X-chromosome inactivation [39]. The primary epigenetic modifications involved in MDD consist of DNA methylation, chromatin remodeling, and non-coding RNA-mediated regulation [42].

Epigenetic changes can take place due to stress factors, which can be both physical and psychological. Depending on the severity of stressors in vulnerable individuals, the brain regions can be especially affected through changes within the chromatin structure of specific genomic loci [43] (Fig. 2A). This leads to significant alterations in genetic expression that may contribute towards depression. These types of changes can occur stochastically during development and can be used as a benchmark to determine the spectrum of an individual's vulnerability or resistance towards stress related events in the future. Epigenetic inheritance can also take place across generations, which may lead to MDD. Although research is incomplete in this field, it provides a means to analyze specific biomarkers across familial generations as a path for early treatment intervention.

Epigenetic mechanisms play an influential role in shaping our day-to-day health and well-being. Due to this, it is important to understand the epigenetics of depression, which can occur at the level of DNA methylation, non-coding RNA-mediated regulation and histone modifications [42].

3.1 DNA methylation

DNA methylation constitutes the addition of a methyl group by DNA methyltransferases (DNMTs) to the 5' position at the cytosine nucleic acid in cytosine-phosphate-guanine dinucleotides (CpG) (Fig. 2D). These modifications repress transcription of genes and are generally located at promoter regions, also known as CpG islands.

3.1.1 BDNF and methylation

In humans, gestational stress during pregnancy leads to increased risk towards depression and anxiety in adults [44]. It is therefore very important

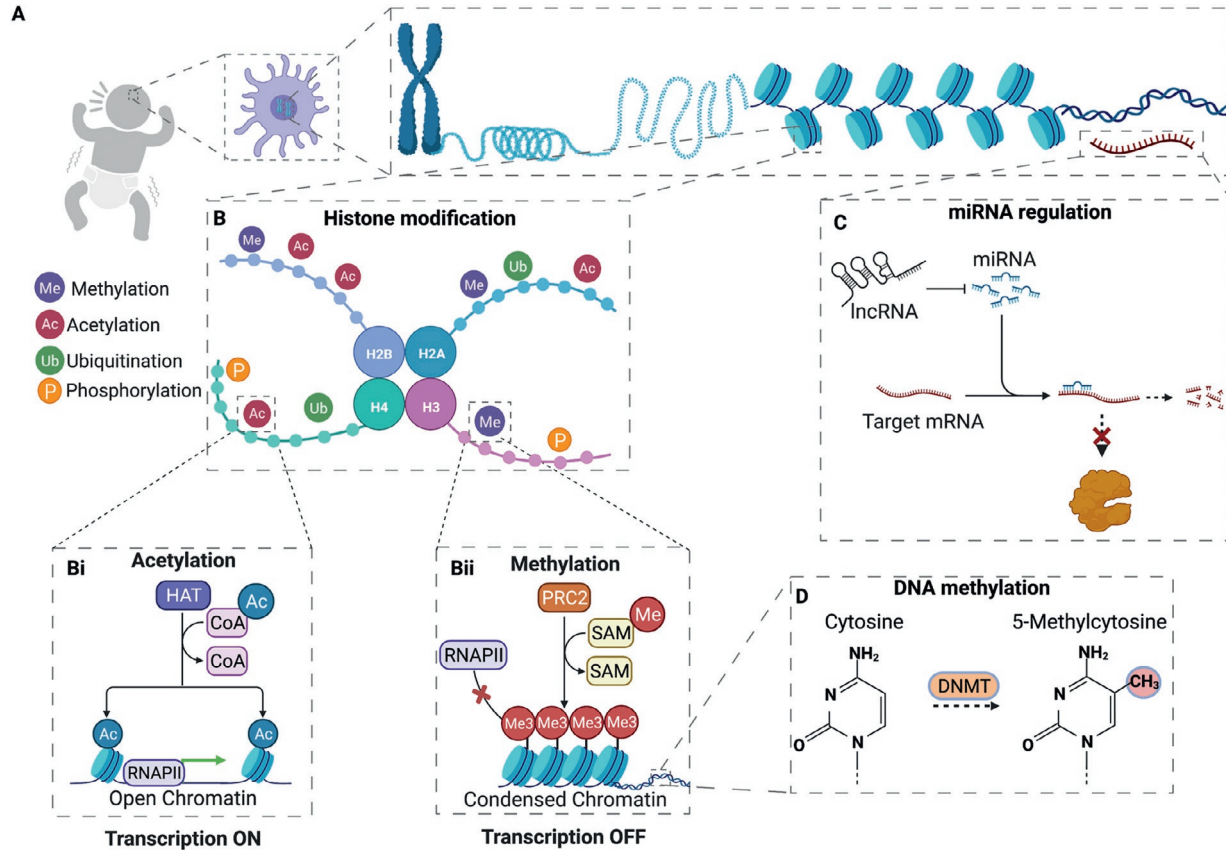


Fig. 2 See figure legend on next page.

to consider the intrauterine conditions during development that could lead to MDD due to epigenetic modifications, including DNA methylation [45]. Studies have shown an association between stress and reduced brain-derived neurotrophic factor (*BDNF*) gene as an early precursor of MDD [46–50]. Following gestational stress in mice, Zheng et al. [47] showed a decrease in *BDNF* and increased expression of DNMT1, histone deacetylase (HDAC) 1, and HDAC2 in the hippocampus of stress-induced mice when compared to non-stress offspring. As well as increased methylation at exon 1 of the *BDNF* promoter [51], experiments also revealed a reduced binding of AcH3K14 at *BDNF* CpG sites [47]. Another study by Fuchikami et al. [46] revealed an increase in methylation in peripheral blood of depressed Japanese patients compared to healthy controls at the CpG I site of the *BDNF* gene. Under normal circumstances, *BDNF* promotes neurogenesis, synaptogenesis, proliferation, differentiation, and neural plasticity [50]. The strong correlation between decreased *BDNF* and depression may indicate a disruption in neuronal plasticity and cognitive function due to the lack of *BDNF* expression [52]. The level of *BDNF* may potentially be used as a marker to assess depressive symptoms.

3.1.2 *CRH* and *5-HT*

Early life adversity and polymorphic abnormalities in the serotonin transporter, i.e., 5-hydroxytryptamine (*5-HT*) gene affects the DNA

Fig. 2 Epigenetic mechanism of gene expression regulation in the brain. (A) Stressful events during developmental stage, pre- or post-natal, or in adulthood can lead to epigenetic dysregulation that elevates the chance of acquiring depression. Epigenetic changes can take place on an organizational level in the chromosomes either at the histone level or in the DNA. (B) Chromatin structure can be broken down into histone units which contains DNA (147 bp) wrapped around histone octamers (two of each: H2A, H2B, H3, H4). N-terminal histone tails can undergo modifications (methylation, acetylation, phosphorylation, and ubiquitination), all of which control the expression of specific genes at gene loci. (B.i) Epigenetic mechanism of acetylation. Catalyzed by CoA: *N*-acetyltransferases; this modification produces an open state of chromatin and leads to gene expression by RNAPII. (B.ii) Epigenetic mechanism of methylation; polycomb-repressive complex 2 (PRC2) modulates HMT activity and the transfer of a methyl group from S-Adenosyl methionine (SAM) to lysine or arginine residues. This kind of modification can lead to activation or repression but histone trimethylation almost always leads to gene silencing. (C) miRNA modulation of gene expression. Upon miRNA formation from ncRNA, miRNA binds to target mRNA and degrades it, leading to transcriptional repression. This affects the expression of downstream proteins. (D) DNA methylation. Under stress, there is an upregulation of DNMTs, which can add a methyl group to the 5' position of cytosine at CpG sites. This modification generally leads to the repression of gene expression [35].

methylation at the corticotropin-releasing hormone (*CRH*) gene promoter in the central amygdala of adult male rats [53]. This resulted in an increased glucocorticoid release upon exposure to stress. The study also reported a strong correlation between mRNA *CRH* levels in the central amygdala and methylation of the CpG-36 site [53]. These results are indicative of stress-coping behavior in learned helplessness paradigms. A similar study by Elliot et al. [54] showed results where long-term demethylation was induced in *CRH* gene region upon social distress to adult mice. Knockdown of the *CRH* and imipramine treatment ameliorated the social avoidance upon stress [54]. The hypothalamic-pituitary-adrenal (HPA) axis mediates *CRH* release upon stress. Social distress heavily impacts *CRH* gene function in the periventricular nucleus and central amygdala regions of mice as seen by decreased methylation status of *CRH*. In female stressed-mice, however, DNA methylation was increased at the periventricular nucleus of the *CRH* gene, hinting at a sex-specific regulation of the HPA axis [55].

3.1.3 Monozygotic twin studies

Twin studies provide a valuable insight into epigenetic status due to the presence of shared genes among the twins but which often show variable phenotypic expression [56–58]. Although profiles may be similar due to shared DNA and equivalent pre and post-natal environment [59], differences in methylation status can be attributed to specific environmental stimuli [60]. A study by Córdova-Palomera et al. [61] assessed changes in DNA methylation profiles of monozygotic twins at differentially methylated probes and variably methylated probes. The majority of differentially methylated probes were discovered in genes that had previously been linked to neuropsychiatric diseases. One such example is the WD Repeat Domain 26 gene that has been related to MDD from previous data [62]. Genes in variably methylated probes showed enrichment for glucocorticoid stimulation (see *CRH*) and included insulin-like Growth Factor 2, Calcium Channel, Voltage-Dependent, L type Alpha 1C and P38 mitogen-activated protein kinase [61]. Methylation of the latter two proteins have been associated as risk-factors for depressive phenotypes [61,63,64]. These studies provide information on the epigenetics of distinct genomic-loci and the importance of differential and variable methylation in MDD.

3.1.4 Post-mortem studies – PRIMA1

Due to the difficulty of obtaining in vivo brain tissue from humans, post-mortem brain samples of MDD patients provide an interesting insight into methylation markers for depression. However, it must be noted that

neurobiological alterations observed in these samples should always be correlated and validated within peripheral alterations measured by non-invasive determinations in blood, saliva or urine in order to facilitate their future implementation in the clinical settings. Regardless, these genes may prove useful as screening tools. Sabunciyan et al. [65] performed an MDD study analyzing methylation in 3.5 million CpG sites. The author's classified 224 sites, with the bulk of the genes expressed in neuronal growth and development regions being categorized as having a methylation status of greater than 10% in MDD samples compared to controls. The Proline Rich Membrane Anchor 1 (*PRIMA1*) gene in MDD individuals showed the greatest enrichment; a 12–15% increase and correlated negatively with gene expression. Although significant for *PRIMA1*, it must be noted however the brain samples tested were relatively small ($n = 16$) and require further reproducibility before they can be defined as consistent markers. MDD is associated with reduced *PRIMA1* expression and enhanced cholinergic transmission. As *PRIMA1* methylation increased in the MDD brain, acetylcholinesterase immunoreactivity decreased. However, the evidence presented is inconclusive but highlights the role epigenetics plays in neuronal development and suggests a potential way to detect MDD [65].

3.1.5 Peripheral blood studies

Due to the inaccessibility of brain tissue samples, a wide range of non-invasive peripheral blood studies have been carried out to investigate blood diagnostic methylation biomarkers of MDD. Numata et al. [66] analyzed 363 CpG sites in medication-free MDD patients and controls. DNA methylation in MDD was found in Cell Cycle Associated Protein 1, cAMP-response element binding (*CREB*) protein, Diacylglycerol Kinase, Glycogen Synthase Kinase 3 β and Serum/Glucocorticoid Regulated Kinase 1; all of these genes have been linked to prior studies on depression [67–69].

Numerous studies on DNA methylation have shown positive correlations to MDD as indicative markers of depression [51,53,61]. DNA methylation is one of many epigenetic mechanisms that influences the activities and behavior of vulnerable individuals, and its importance provides a useful potential screening tool for psychiatric conditions.

3.2 Non-coding RNAs

Non-Coding RNAs (ncRNAs) are a group of RNA subtypes that do not code for a protein. Contrary to traditional genomic dogma which states that genetic information must be translated to a functional phenotype such as a protein, recent evidence has indicated that the majority of the genome in

eukaryotes and complex organisms is in fact transcribed into ncRNAs [70,71]. Some of these ncRNAs include microRNAs (miRNAs), small nucleolar RNAs, large intergenic non-coding RNAs, PIWI-interacting RNAs and transcribed ultraconserved regions. These ncRNAs play an important role in the maintenance of the genome through downstream signals, as they are responsible for transcription, RNA splicing, epigenetic memory and translation [72]. All of these mechanisms converge for our future understanding of neuropsychiatric diseases, such as MDD. Although the role of these ncRNA in MDD is still being established, they play a vital role in the regulation of MDD signaling pathways that mediate genes, neurogenesis, and synaptic plasticity.

3.2.1 *MicroRNAs*

Out of all the ncRNAs present, miRNAs are the most prevalent and thoroughly investigated. miRNAs are a class of endogenous, short non-coding RNA's, typically between 19 and 25 nucleotides [73]. These molecules regulate gene expression on a post-translational level. Traditionally, they have been reported to bind to mRNA, resulting in their degradation (Fig. 2C) or prevent translation due to steric hindrance of the protein synthesis vehicle, overall contributing towards translational repression or downregulation [74,75]. As well as differentiation, proliferation, apoptosis, and development, miRNAs are involved in diseases, such as MDD, where genes involved in specific disease processes were downregulated [76].

A study by Maffioletti et al. [77] identified expression levels in 1733 miRNAs from the peripheral blood of 20 depressive and 20 control subjects. The findings showed changes in 5 miRNAs (hsa-let-7a-5p, hsa-let-7d-5p, hsa-let-7f-5p, hsa-miR-24-3p and hsa-miR-425-3p), indicative of miRNA dysregulation in MDD. The study also detected similarities and differences in miRNA changes in patients with bipolar disorder, providing a means to differentiate potential biomarkers of MDD and bipolar disorder. miRNA profiles in cerebrospinal fluid (CSF) and serum of MDD patients was carried out by Wan et al. [78]. The study showed an upregulation of three miRNAs (let-7d-3p, miR-34a-5p, miR-221-3p) and downregulation of miR-451a. Additional validation tests confirmed the specificity and sensitivity of these miRNAs in relation to MDD. A genome-wide expression study by Garbett et al. [79] analyzed the transcriptomic profile in dermal fibroblasts to identify peripheral markers for MDD in patients. Quantitative PCR analysis of miRNAs showed subsequent alteration in mRNA levels for specific molecular pathways (e.g., *MET*, *PCDH10*, *PPL* and *TNXB* genes) which has-miR-122 showing greatest prominence after increased expression in the

hippocampus [79]. The findings suggest a viable difference in mRNA and miRNA expression pathways in healthy and MDD subjects.

Following antidepressant treatment, circulating miRNA levels can be used to assess the treatment outcome and to distinguish healthy miRNAs from miRNAs involved in MDD pathophysiology. Balzeaux et al. [80] examined miRNA expression in peripheral blood mononuclear cells of MDD and healthy patients, 2 and 8 weeks after antidepressant treatment. Two miRNAs were overexpressed following 8 weeks (miR-589 and miR-941). Furthermore, these miRNAs were shown to target four genes (i.e., *PPT1*, *TNF*, *IL1B* and *HIST1H1E*) which may behave as response biomarkers following treatment. Another study by Bocchio-Chiavetto et al. [81] examined changes in blood miRNA following 12 weeks of escitalopram uptake in 10 MDD patients. Quantitative real-time PCR revealed 28 miRNAs were upregulated while two miRNAs were highly downregulated. miR-132 stood out as it has been involved in synaptic plasticity and neurogenesis while miR-26a, miR-26b and miR-183 correspond towards BDNF function [81]. These miRNAs point towards positive signs of escitalopram treatment efficacy. With robust replications of these findings also including the study of several other antidepressants, these miRNAs could be adopted as treatment response biomarkers (see Fig. 1) for MDD upon antidepressant intake.

3.2.2 miRNAs, BDNF levels and glucocorticoid receptor expression downregulation

Research has shown the *BDNF* gene is implicated the most in MDD pathophysiology [42,82]. Li et al. [42] showed upregulated miR-182 and miR-132 levels along with lower serum BDNF concentration in depressive patients as compared to healthy controls. Similarly, the Self-Rating depression scale displayed an inverse correlation with serum BDNF [42], showcasing it as a marker of MDD [83]. A more recent study by Fang et al. [82] showed miRNA alterations in control, MDD and MDD treatment group (citalopram). The results showed greater plasma concentration of miR-124 in the treatment free group compared to citalopram-treated and control groups. Contrary to this study, He et al. [84] showed a downregulation of peripheral blood mononuclear cells miR-124 following antidepressant treatment. Nevertheless, previous studies on miR-124 have linked it to synaptic plasticity and MDD pathology [85,86] highlighting its putative role in depression. miR-132 levels, on the other hand, were initially greater in the treatment-free group but decreased after citalopram treatment, implicating miR-132 as a marker for symptomatic change in depression [82].

Decreased glucocorticoid receptors and elevated cortisol levels in CSF and plasma have been heavily noted in depressive patients [87]. Results from Uchida et al. [88] implicate a role for miR-18a in these effects as miR-18a inhibited glucocorticoid receptor translation in neuron cell cultures; this expression was upregulated in MDD rats compared to the controls. A similar report showed miR-18a and miR-124a reduced glucocorticoid receptor protein expression in NS1 cells by luciferase reporter assays [80].

3.3 Histone modifications

The chromatin structure is formed upon the assembly of nucleosomes because of DNA forming tight coils around histone octamers. The spatial structure around the chromatin determines gene activity as genes surrounding spaced out nucleosomes results in active transcription, whereas genes around compact nucleosomes are silenced. The spatial activity of nucleosomes is determined by a series of processes including posttranslational, covalent modifications of histones on the N-terminal of specific amino acids [89]. Modifications include methylation, acetylation, ubiquitination, and phosphorylation just to name a few (see Fig. 2B).

The open state of chromatin is monitored by histone acetylation, catalyzed by histone acetyltransferases. These modifications can also be removed by HDACs, which favor a close chromatin state (Fig. 2Bi). In general, acetylation leads to greater gene expression. On the other hand, histone methyltransferases are responsible for the addition of methyl groups to chromatin, which generally leads to a closed state and prevents gene expression (Fig. 2Bii). Methylation can be reversed by histone demethylases which removes methyl groups and leads to a more relaxed chromatin structure. The enzymes that regulate the addition and removal of covalent modifications are known as “writers” or “erasers” and are highlighted above. “Reader” proteins monitor changes to gene transcription and chromatin structure by binding to the histone modifications [89].

Environmental stimuli such as stress can trigger the remodeling of histones. This occurs primarily through intracellular cascades, which regulate neural activity and synaptic transmission. The cascade monitors the activity of a diverse range of transcription factors such as CREB and Fos proteins. These factors prompt downstream changes in chromatin structure by specifically binding to response elements in the regulatory regions of genes. Secondly, the signaling cascade generated following the exposure to environmental stimuli can also influence chromatin regulatory proteins

[41]. All of this leads to changes in gene expression and take place chiefly through acetylation or methylation.

There are relatively few publications dissecting the role of histone modification on MDD. Some studies have demonstrated the role of HDAC inhibitors and antidepressants in animal models of depression [90–92]. Covington et al. [90] explored the effect of chronic stress on histone acetylation in the nucleus accumbens (NAc), an area in the brain's limbic circuit in chronic social defeat mouse models. The researchers found a transient decrease but a subsequent increase in histone acetylation (H3K14ac) in the NAc after chronic social defeat stress with a concomitant decrease in HDAC2. The same results were also seen in post-mortem tissue analysis of depressed patients [90]. Furthermore, the authors also infused an inhibitor of HDAC (MS-275) which showed antidepressant effects in the stress model by rescuing altered expression of genes and pointing towards a therapeutic role of specific HDAC inhibitors in MDD. A similar study by the same authors [93] showed HDAC5 expression was also repressed in the NAc of affected social defeat stress model mice. Upon imipramine treatment, however, HDAC5 activity was seen to increase. The study also indicated mice lacking HDAC5 were more prone to MDD compared to healthy controls. The above results highlight the role of histone acetylation in adaptive stress and MDD.

A post-mortem analysis in the pre-frontal cortex (PFC) of MDD patients revealed an increase in the enrichment of H3K4me3 [94]. This modification is connected to the *SYN2* promoter and affects transcriptional activity of the gene, which is responsible for the expression of neuronal phosphoproteins that are part of the synaptic vesicle machinery and play a role in synaptic plasticity and neurotransmitter release [95]. Previous studies have indicated aberrant expression of the *SYN1*, *SYN2* and *SYN3* genes in various psychiatric disorders [96,97] and may explain some of the depressive symptoms seen in MDD. Post-mortem PFC studies in MDD patients treated with antidepressants have also shown abnormal repressive histone signatures (H3K4me3 and H3K37me3) in *BDNF* genes; as seen in the methylation studies described above [91,98]. This common epigenetic etiology further points towards *BDNF* as a promising biomarker of MDD.

Downregulation of histone methyltransferases G9a and G9a-like protein which catalyzes H3K9me2 occurs in the NAc in chronic social defeat stress models in susceptible mice [99]. Overexpression of G9a showed antidepressant-like effects as seen after fluoxetine administration [100]. Fluoxetine treatment also reduced *CAMK2* expression in NAc by inducing H3K9me2 modulation and repressing histone acetylation at *CAMK2*

promoter. Similar effects were seen in the NAc of MDD humans treated with antidepressants, highlighting the role of stress induction, which triggers methylation resulting in pathological behavior as observed in depression [100]. Antidepressants, on the other hand, have an ameliorative effect of shifting the maladaptive gene modulation to the healthy state by inducing mechanisms at gene loci. H3K9me2 can also affect *Ras* regulation of susceptible mice. Histone methylation triggers increased expression of *Ras*, leading to extracellular-signal-regulated kinase (ERK) signaling and downstream CREB activity [99] and ultimately may be responsible for some of the depressive symptoms seen in MDD.

The above studies have highlighted the role of some key epigenetic mechanisms that may lead to prevalence for MDD. Early developmental stressors can trigger epigenetic functions ranging from DNA methylation, miRNA's modulation, and histone modification, all of which have an impact on downstream gene loci which brings about maladaptive behavioral pattern changes. With more research in the field of epigenetics and biomarkers, key signature patterns may be better elucidated and used for early-stage screening for MDD.



4. Proteomics and depression

As the name suggests, proteomics is the large-scale study and investigation of the proteome in an organism. At the “Omics” level, proteomics takes place following gene transcription and is generally attributed towards identifying the level of protein expression at a distinct site. Varying levels of proteins have been implicated in MDD, including inflammatory, growth factor, neurotransmitter, and endocannabinoid system related proteins and enzymes.

4.1 Inflammatory markers

Smith [97] proposed the macrophage theory of depression, based on the premise that excessive inflammatory factors (e.g., cytokines) produced by macrophages underlie symptoms of depression and contribute to its causality [101]. This theory has been supported by multiple studies that have shown a strong relationship between inflammation and depression and has been reviewed widely in the literature [101–103].

Single-nucleotide polymorphisms (SNPs) for genes that code for inflammation are associated with MDD [102–104]. These findings corroborate the evolutionary imperative of inflammation in depression. Studies indicate a

bidirectional relationship between inflammation and psychological stressors that result in psychiatric conditions such as depression [105]. Danese et al. [106,107] reported that individuals exposed to psychological stressors such as abuse or maltreatment as children were more prone to develop depression in adulthood. This has been strongly correlated with HPA axis dysregulation and enhancement of proinflammatory processes [105]. Consistent with these findings, many medical illnesses show heightened rates of inflammation and a greater tendency for comorbid depression than the general population [108,109]. Cancer patients, for example, have an abundance of proinflammatory cytokines and are approximately 50% more likely to develop depression compared to the general population [110,111]. Similarly, patients with diseases such as coronary heart disease, obesity, and type II diabetes have all exhibited biomarkers of inflammation and concurrent depression symptoms [112–114].

As noted above, the etiology of depression is heterogenous and inflammation is only one of many factors that mediate the development of MDD. A growing body of evidence implicates inflammatory factors in the pathophysiology of depression [115–119]. The inflammatory hypothesis of MDD highlights the adverse effects of certain pro-inflammatory cytokines on the peripheral immune system, neurotransmitter metabolism, neuroendocrine function and brain activity; all of these factors play a role in depression [120]. Some of the relevant proinflammatory factors are discussed below according to stringent evidence-based meta-analysis.

4.1.1 Cytokines in depression

Cytokines are a group of small proteins that play a central role in the body's defense towards pathogens and have been implicated in depression more than other inflammatory factors. Pro-inflammatory cytokines, Interleukin-1 (IL-1), Interleukin-6 (IL-6), tumor necrosis factor (TNF)- α and C-reactive protein are most extensively observed in major depression and have been reported to play a primary role as reported in multiple meta-analyses [121–125]. Alterations to cytokine concentrations have been seen in depressed individuals. Lindqvist et al. [126] demonstrated a significantly higher CSF IL-6 levels in suicide attempts compared to healthy subjects. Contrary to the hypothesis, other findings have reported no change in CSF cytokine concentrations between MDD and healthy patients [127,128], challenging the causal role of inflammation in depression. Methodological constraints, such as the challenge of extracting CSF from patients with MDD may explain the ambiguity in these studies. Further research with robust methodology will help to clarify this claim.

4.1.2 Interferon- α (IFN- α)

IFN- α is administered therapeutically on a chronic basis for specific cancers and viral infections [129] and has been tested as a treatment for COVID-19, where it showed great efficacy in viral clearance [130]. Adversely, IFN- α administration induces depressive symptoms in 30–50% of treated patients along with other behavioral alterations such as anxiety, sleep changes, anhedonia, and fatigue [131–133]. As a result, IFN- α induced depression is used to study inflammatory changes that lead to behavioral symptoms, as seen in MDD. There tends to be an increase in proinflammatory cytokines in the serum and CSF, including IL-1, IL-6, IL-8, TNF- α and their soluble receptors, all of which were generated upon IFN- α treatment [131–133]. Increased inflammation prior to treatment due to psychosocial stressors is a risk factor for developing MDD [133,134]. In individuals who develop depression, the cytokines mentioned above accumulate at a higher rate than in patients who are not at risk [135]. The exact mechanistic action of IFN- α -induced depression is not yet clear; however, prior factors including previous inflammation may play a correlational role in the depressive etiology. Furthermore, IFN- α might be acting through the central nervous system (CNS) to alter neurotransmitters in the brain, which may ultimately lead to MDD.

The role of psychosocial stress and cytokine-related depression has been briefly mentioned above. Many of these stressors are the result of a maladaptive childhood, physical or sexual abuse. This has an impact on the neuroendocrine and immune systems that trigger the emergence of proinflammatory phenotypes during adulthood [136,137]. A study by Pace et al. [138], which implemented the Trier Social Stress Test to trigger a stress response showed an upregulation of IL-6 and nuclear factor kappa B (NF- κ B) DNA-binding in men with a history of early life stress and this correlated with MDD severity. NF- κ B seems to increase upon stress and correlates with plasma IL-1 β , IL-6 and IL-10 mRNA levels [139], suggesting that NF- κ B could potentially be used as a risk biomarker for early depression. Furthermore, childhood trauma seems to elicit an increase in IL-6 and C-reactive protein levels. This may be due to a high HPA axis reactivity during adulthood [140,141]. This has also been observed in several MDD studies [142–144] and is associated with an increase in plasma cortisol levels [143].

A mechanistic approach to stress can be explained by the immune system that can detect danger signals even in the absence of pathogens [145]. This is regulated through the release of danger-associated molecular patterns (DAMPs), including heat shock protein-72, uric acid, and ATP [145,146].

Stress induction results in “sterile inflammation” of DAMPs, which stimulate the inflammasome in the presence of lipopolysaccharide (LPS). This triggers the activation of caspase-1, which cleaves IL-1 β and IL-18 precursors into the mature forms [145], setting up a positive feedback mechanism upon which additional inflammatory cytokines are released during stress (See Fig. 3A). Although glucocorticoids neutralize the effects of inflammation, chronic stress can lead to prolonged vulnerability towards inflammatory cytokines upon which glucocorticoid resistance can emerge [147]. Intervention at the level of caspase-1, through caspase-1 inhibitor ac-YVAD-cmk can attenuate the stress-induced levels of IL-1 β , IL-18, and IL-6 [145]. The DAMP-inflammasome pathway of stress-induced inflammation may explain the emergence of depressive inflammatory biomarkers in relation to chronic stress. Interference of this signaling pathway could provide a useful tool to alleviate inflammation-related depressive symptoms.

4.1.3 Neurotransmitter systems, kynurenine pathway and depression

Cytokines influence the production of neurotransmitters [133], the most widely studied being serotonin and its transporter, which is responsible for serotonin reuptake into presynaptic neurons. Administration of proinflammatory cytokines, including IL-1 β through LPS, increases 5-hydroxytryptamine (i.e., serotonin receptor; 5-HT) turnover in the brain [148]. However, these alterations in 5-HT correspond with a greater tendency for more tenacious depressive symptoms. This behavior seems to be mediated by the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO), which plays a role as a molecular mediator of comorbid inflammation-induced depression. One possible explanation for this may be through the catabolism of tryptophan through the kynurenine pathway. The amino acid tryptophan is transformed into kynurenine by IDO [149]. Kynurenine is further metabolized into kynurenic acid (KA) and quinolinic acid (QA). Out of these two metabolites, KA behaves as a neuroprotective *N*-methyl-D-aspartate (NMDA) antagonist [150], whereas QA is an NMDA agonist that is implicated with a neurotoxic function that may contribute towards depression [151] (See Fig. 3B). The activation of IDO and the balance between KA and QA seems to contribute towards neurodegeneration and depressive symptoms [152,153]; whereas blockage of IDO normalized the kynurenine/tryptophan ratio [154]. Furthermore, patients with IFN- α -induced depression showed elevated levels of tryptophan metabolites (KA & QA), which negatively correlated with peripheral tryptophan and serotonin concentrations [155]. This was observed in the

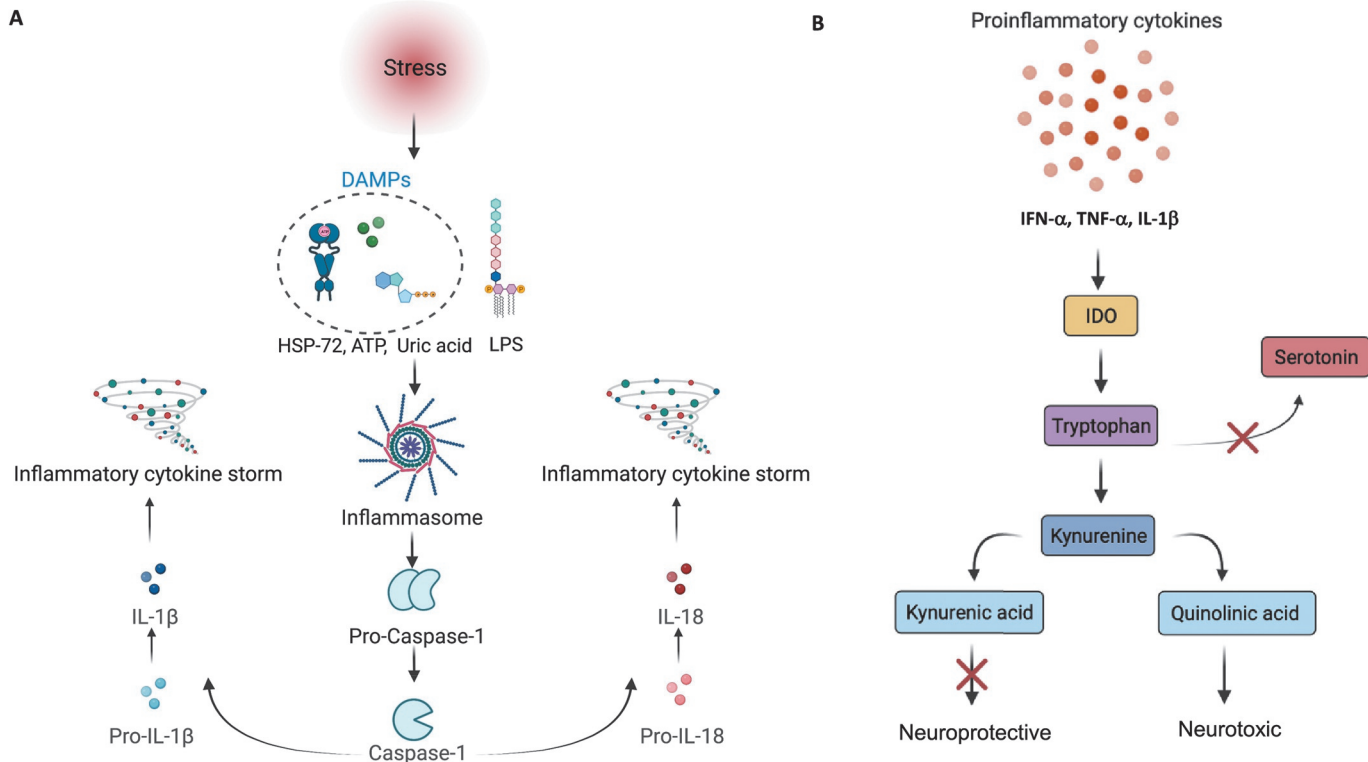


Fig. 3 (A) Mechanistic illustration of stress mediated positive feedback inflammation through DAMPs and cytokines. Chronic stress can lead to the development of inflammation-related biomarkers that have been evaluated for depression pathophysiology. (B) Inflammatory cytokines promote increased serotonin turnover in the brain through IDO and kynurenine pathway. In addition, increased quinolinic acid to kynurenic acid ratio increases neurotoxicity. Both these mechanisms can contribute towards depressive symptomology [35].

blood and CSF of IFN- α -treated patients with depression, who showed general increased levels of kynurenine and QA [152].

4.2 Growth factors

Recently, a growing body of evidence has highlighted the role of growth factors, including BDNF, fibroblast growth factor (FGF) and, vascular endothelial growth factor (VEGF) in the pathophysiology of MDD. Furthermore, expression of these growth factors tends to show normalization upon antidepressant intake. Not only are these present in the brain but are also readily available in the periphery, making them promising candidates as MDD biomarkers [156,157].

4.2.1 BDNF

The neurotrophic effects of BDNF have been discussed above [158,159]. Primarily, BDNF signaling occurs through the tropomyosin receptor kinase B (TrkB) which produces its neurotrophic effects. Abnormal levels of TrkB and BDNF have been reported in patients with MDD. Lowered peripheral BDNF concentrations has been largely linked towards MDD and meta-analyses have confirmed this despite the heterogeneity of results in the field [160,161]. The issue lies in the differences between available assays which makes BDNF difficult to quantify as a biomarker for psychiatric disorders [160]. Regardless of this disparity, post-mortem analysis of brain tissue from suicide victims has demonstrated a reduction in BDNF and TrkB expression in the PFC and hippocampus [162]. One explanation for these changes can perhaps be attributed towards rs6265 SNP from valine to methionine substitution at codon 66 (val66met). Meta-analyses have confirmed the Val66Met SNP influences downstream BDNF concentrations, which may have an impact on neural activation during MDD [163,164]. Homozygous valine groups at rs6265 tend to show reduced neural activation in brain areas linked to cognitive appraisal of emotional scenes, whereas allelic variation of a methionine group showed the opposite effect [165] and, in fact, increased susceptibility to depression [166]. Furthermore, post-mortem analysis of the patients displayed lower BDNF levels in the anterior cingulate cortex and caudal brainstem compared to healthy controls [166], providing further evidence for the connection between the methionine BDNF allele and the consequent lowered BDNF concentration in MDD.

Decreased BDNF concentrations in patients with depression tend to normalize following selective serotonin reuptake inhibitors (SSRI) treatment [167] or with electroconvulsive therapy (ECT) [168]. Treatment-

resistant patients, on the other hand, tend to show low levels of BDNF and MEK1 mRNA expression levels [169]. Such a profile may serve as a novel means to predict patients with treatment-resistant depression. Antidepressants, such as imipramine and fluoxetine [170], as well as psychoactive compounds like ketamine [171,172], induce autophosphorylation of TrkB (BDNF receptor). This effect was independent of any changes in other neurotrophins [173,174]. Inhibition of TrkB blocked these effects [175]. This highlights the mediating role of TrkB on BDNF, which results in antidepressive effects. Long term antidepressant treatments trigger TrkB-mediated activation of phospholipase-Cgamma1 [174]. This increased CREB phosphorylation resulting in the upregulation of BDNF, VEGF, and VGF nerve growth factor in the hippocampus ultimately contributing towards neuronal plasticity, neurogenesis, and increased hippocampal function [174,176]. This highlights the ameliorating effects of antidepressants in MDD through affecting BDNF expression levels.

While antidepressants serve as a practical tool to aid with depression, exercise can also benefit in alleviating depressive symptoms by affecting BDNF expression [177]. Indeed, exercise in an enriched atmosphere and on a voluntary basis, increases BDNF expression in the hippocampus [178,179]; an effect associated with enhancement of hippocampal neurogenesis [180]. This in turn promotes cognition, plasticity, learning and memory [181]. These outcomes help alleviate depressive symptoms.

4.2.2 FGF2

Fibroblast growth factor-2 (FGF2) is a key neurotrophic factor that plays a role in early neural induction, CNS patterning (e.g., neural plate formation) and the development of functional neural circuits, such as the spinal cord and neocortex [182]. As a result, FGF2 coordinates hippocampal neurogenesis, synaptic growth and formation, all of which regulate learning, memory, and injury response [183,184].

Second to BDNF, FGF2 is also an important growth factor that is heavily implicated in depression. An experiment by Turner et al. [185] revealed a reduction in the expression of FGF2 mRNA with its primary receptor, FGFR1, in the hippocampus and dentate gyrus following social defeat stress. Injection of FGF2, however, diminished depressive symptoms in rats [186] and improved cell proliferation in the PFC [187]; a pathway that may explain the antidepressant effect of FGF2. FGF2 treatment in the olfactory bulbectomy model of depression in rats also reduced neuronal cell death, increased hippocampal neurogenesis and improved depressive-like symptoms [188].

Furthermore, short-term or long-term treatment with olanzapine and fluoxetine upregulated FGF2 mRNA in the rat pre-frontal cortex [189]. FGF2 immunoreactivity in the hippocampal and cerebral cortex astrocytes was also improved [190]. From a mechanistic point of view, antidepressant-induced expression of FGF2 consists of ERK dependent early growth response 1 (EGR1) in the astrocytes. Activation of epidermal growth factor receptor and FGFR is induced by the antidepressant, amitriptyline, leading to ERK1/2 phosphorylation, which boosts EGR1 expression, consequently increasing the level of FGF2 [191]. In fact, inhibition of FGFR in the chronic unpredictable stress rat model or FGF2 knockout mice showed inadequate antidepressant effects, highlighting the modular role of FGF2 in governing antidepressant effects [187,192]. Activation of the ERK/mitogen-activated protein kinase pathway by FGF2 may explain this mediating role via subsequent increases in glucocorticoid receptor expression that may ameliorate depressive neuropathology [193]. Alternatively, FGF2 elevated the phosphorylation on ERK and protein kinase B by binding to FGFR1. This increased the expression of B-cell lymphoma 2 which in-turn reduced caspase-3 levels, resulting in a neuroprotective outcome that mediates antidepressant-like effects [194].

Post-mortem analysis of human brains disclosed lower levels of FGF2 of MDD patients in the dorsolateral PFC and the anterior cingulate cortex [195]. However, these results were not confirmed by other investigations. For example, Goswami et al. [196] evidenced that in post-mortem brain from depressed patients there were no changes in FGF2 expression. This was specific to the PFC Broadman's area 10, suggesting FGF2 expression may vary spatially across the brain. Currently, the literature reports mixed results concerning FGF2 expression in MDD patients [200–202]. However, a meta-analysis by Wu et al. [197] revealed significantly higher peripheral FGF2 levels in MDD patients compared to controls and no changes in FGF2 in the CNS. These results must be interpreted with caution as it only includes a small number of studies. Furthermore, there is no indication of FGF2 changes with and without treatment. Future research in this field with robust clinical studies is needed to form an accurate conclusion of the expression levels of FGF2 in MDD.

4.2.3 VEGF

Vascular endothelial growth factor (VEGF) is primarily responsible for angiogenesis, vascular permeability in embryogenesis and growth. VEGF is also a strong neurotrophic factor and mediates neurogenesis, neuronal survival, hippocampal proliferation, glia survival and migration [198].

Peripheral VEGF concentrations seem to negatively correlate with depressive mood [199]. In a study by Isung et al. [200] participants who attempted suicide and who met the criteria for a mood disorder showed lower levels of VEGF in CSF than healthy controls. While lower VEGF levels do not necessarily contribute towards depression, studies have indicated reductions in VEGF correlate negatively with stress that normally coincides with depression [201,202]. Alternative findings have shown polymorphic variations of the VEGF gene may predispose the susceptibility of developing treatment-resistant depression [203]. The neurotrophic effects of VEGF may also explain the depressive symptoms in the elderly due to disruptions to angiogenesis and neurogenesis mechanisms, which have been implicated in age-related depression [204].

Newton et al. [205] revealed that ECT potently up-regulated the VEGF gene. ECT also induced cell-proliferation in the hippocampus 48 hours post-treatment. The effect was blocked by inhibition of the VEGF-Flk-1 receptor by SU5416 [206]. Compared to ECT, various antidepressants seem to only stimulate distinct transient amplifying neuronal progenitor cells. Whereas, ECT acts on a much broader scale by modulating both amplifying neuronal progenitor cells and quiescent neuronal precursor cell [206,207]. This supports evidence that ECT therapy is more efficient than antidepressants to treat MDD; perhaps due to a greater quiescent and amplifying neuronal precursor/progenitor cell proliferation mediated by VEGF signaling.

VEGF results from peripheral blood studies in MDD patients, however, show heterogeneous results [156]. These inconsistent results may be due to heterogeneous study design, physical illness, depression subtypes, length of illness, and sample source. Ping-Tao et al. [208] conducted a meta-analysis to highlight the role of peripheral VEGF in depression. The results revealed a significant increase ($P < 0.01$) in VEGF in the blood of patients with MDD than healthy controls. Elevated blood VEGF may therefore prove to be an important biomarker in patients with MDD.

4.2.4 Endocannabinoids (ECBs)

The endocannabinoid (ECB) system contains the two endogenous ligands, anandamide (aka N-arachidonylethanolamide; AEA) [209] and 2-arachidonoylglycerol [210], which are synthesized and released on demand through the cleavage of post-synaptic neuron membrane phospholipids. These ECBs act on G-protein coupled cannabinoid receptor type 1 (CB₁) and 2 and are rapidly inactivated by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase [211,212]. While cannabinoid receptor type 2 are concentrated peripherally on various tissues, CB₁ isoforms are

predominantly expressed in the brain [213]. ECBs act as retrograde messengers and modulate the HPA axis and neuroendocrine systems [213]. More specifically, ECBs regulate neurotransmitters. Upon depolarization from postsynaptic neurons, ECBs act in retrograde and bind to CB₁ receptors in the presynaptic nerve terminals. This inhibits the release of neurotransmitters [214] (See Fig. 4). ECBs modulate a plethora of physiological processes in the brain including the regulation of body temperature, pain, sleep arousal, feeding behavior, memory, motor function, as well as emotional processes like anxiety and depression [215].

A growing body of evidence has implicated the role of the ECB system in emotion regulation. Hungund et al. [216] highlighted the involvement of the ECB system in the pathophysiology of depression and suicide [215]. The study showed an upregulation of CB₁ receptors in the dorsolateral prefrontal cortex in a post-mortem analysis of depressed suicide victims. In addition, a series of studies in a review by Pinna [216] highlighted an upregulation in CB₁-mediated G-protein activation, FAAH expression and serum AEA concentrations in suicide patients or attempters compared to controls. These outcomes may infer an increase in cannabinoidergic signaling in depressed patients. By identifying these patterns before they worsen, depression treatment and suicide-prevention strategies could be effectively implemented.

Similarly, Monteleone et al. [217] investigated the role of ECB genes in the susceptibility to MDD and bipolar disorder [217]. In this study, the authors analyzed single nucleotide polymorphisms of the CB₁-receptor gene (*CNR1*) and FAAH. Compared to controls, the MDD group demonstrated a significant difference in *CNR1* 1359G/A genotypes and alleles; increasing numbers of AC and GG genotypes and A alleles were observed. *FAAH* genotypes showed a cDNA 385C to A change that also significantly differed among the groups. Despite being preliminary, these findings indicate that *CNR1* 1359 G/A and *FAAH* cDNA 385C to A changes may make a person more susceptible to MDD and bipolar disorder.

Psychosocial stress is one of the many factors that contributes towards depression and the activation of the ECB system [218]. The ECB system plays a role in the modulation of the inflammatory and neuroendocrine response to stress. *CNR1*KO mice and CB₁ antagonists in humans increase the risk of anxiety, anhedonic and helpless behavior, which is all mediated by chronic stress [219]. Hill et al. [220] further demonstrated the effect of stress in a Trier social stress paradigm where basal serum concentrations of AEA and 2-arachidonoylglycerol were significantly reduced in women with MDD compared to controls. These findings suggest that MDD accompanied with stress may lead to reductions in peripheral ECB signaling.

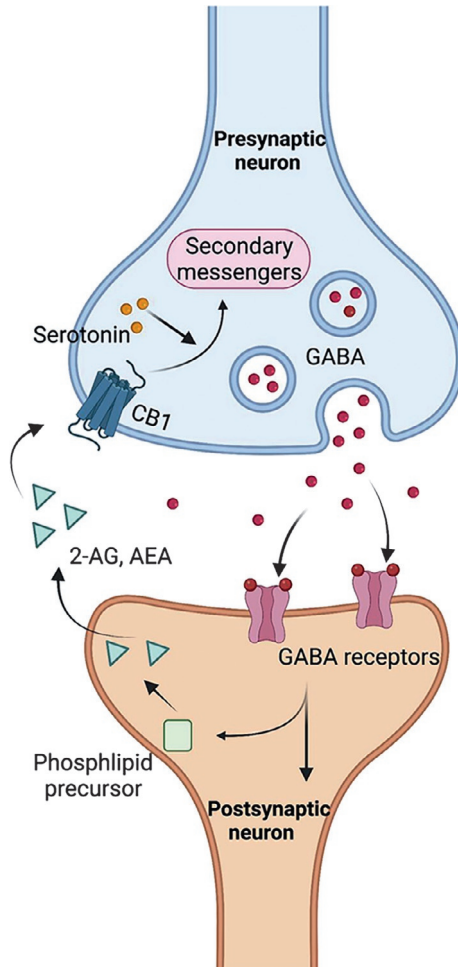


Fig. 4 Schematic representation of the physiological role of the endocannabinoid (ECB) system. Endocannabinoids (2-AG, AEA) act on G-protein coupled CB₁ receptors to modulate the HPA axis and neuroendocrine systems. Inhibitory neurotransmission is regulated by ECBs; upon cleavage of phospholipid precursors, ECBs act in retrograde and bind to CB₁ receptors in the presynaptic nerve terminals, this propagates the release of GABA neurotransmitters, which bind to post-synaptic GABA receptors to mediate inhibitory signals. The serotonin system is implicated in the fine-tuning of the coupling process of CB₁ receptors to G-protein second messengers in order to generate the pre-synaptic cellular cascade. This schematic representation provides possible targets for antidepressant interventions targeting the dysregulated ECB systems [35].

Several reports have implicated the serotonergic system's role in fine-tuning the coupling process of CB₁ receptors to G-protein second messengers in order to generate the presynaptic cellular cascade [221,222] (See Fig. 4). In fact, defects in the serotonergic system resulted in

CB₁-GTP γ S signaling cascade desensitization, as well as attenuation of CB₁ agonist related behavioral paradigms [222]. This suggests a parallel between the effects of serotonin and ECBs. Studies have showed ECB analogues exhibit antidepressive-like effects similar to that of SSRIs [223,224]. Palmitoylethanolamide (PEA) has shown to be an effective candidate in this regard. PEA is an ECB-like modulator that is synthesized by the enzyme N-acyl phosphatidylethanolamine phospholipase D and metabolized by FAAH as well as N-acetyethanolamine acid amide hydrolase. PEA functions as an endogenous modulator of the transcription factor/nuclear receptor, peroxisome proliferator-activated receptor (PPAR)- α . The latter targets downstream genes that regulate a plethora of conditions including inflammation, neuronal differentiation, oxidative stress and neurodegeneration [216]. Upon stress, FAAH is activated immediately, resulting in reduced PEA levels [225]. PEA was used as a candidate add-on for patients treated with SSRIs in a recent randomized controlled trial. PEA-treated patients showed significant improvement in depressive symptoms. Furthermore, the citalopram plus PEA treated group showed greater decrease in Hamilton depression scores over time compared to the citalopram only group [223]. However, this study lacked a PEA only group to see the “true” effects of the ECB-like compound by itself and long-term effects over 6 weeks were missing.

In preclinical studies, the mechanisms for the antidepressant and anti-fear effects of PEA were investigated by Locci and Pinna [226]. The authors administered PEA in a mouse model of stress-induced behavioral alterations reminiscent of PTSD and depression in humans [226,227], including prolonged social isolation [228–230]. Mice were exposed to contextual fear conditioning training followed by a reactivation session. Immediately after reactivation of fear memories, PEA was administered to block reconsolidation processes that resulted in a facilitation of fear extinction [231]. PEA treatment also resulted in antidepressive and anxiolytic-like effects. Similar effects were seen with PPAR- α synthetic agonists and these behavioral effects were prevented by both PPAR- α deletion and PPAR- α selective antagonist treatment. Furthermore, in a 4-week social isolation paradigm in mice modeling for PTSD/depression, PPAR- α was epigenetically modified due to stress exposure [232] with an increase in methylated cytosine at the PPAR- α gene, resulting in lower PPAR- α expression and neurosteroid biosynthesis [233]. PPAR- α hypermethylation was associated with an increase in pro-inflammatory markers and aggressive behavior, indicating a potential epigenetic mechanistic explanation for the development of behavioral dysfunction underlying PTSD or depression.

These results point towards a novel role for PPAR- α and PEA in the treatment of mental health conditions. Intriguingly, decreased PEA and the analogue oleylethanolamide levels have been seen in PTSD patients in a manner that inversely correlated with symptom severity [234].

In another study, socially isolated mice were treated with a non-sedating dose of analogues of the neurosteroid, allopregnanolone (e.g., ganaxolone, BR297, BR351) and was compared to the anti-aggressive effects produced by PEA administration. In both cases the compounds resulted in a dose-dependent reduction of aggressive behavior [227,231]. Furthermore, 100% of mice responded to the PEA treatment as opposed to the SSRI (S-fluoxetine) group, which showed elevated non-responder rate in this preclinical study. The allopregnanolone analogues also showed longer antiaggressive effect compared to the SSRI group. These results show promise in the treatment of PTSD and SSRI non-responder individuals. Future research translating towards MDD may yield similar outcomes.

Conversely, PEA levels have also shown to be elevated similar to AEA in suicide attempters when compared to controls and after controlling for cannabis use [235,236]. This suggests a parallel in mechanisms that drives PEA levels and may depend on specific environmental and biological factors. As a result, PEA as a biomarker of MDD or as an antidepressant must be interpreted in relation to the situational context of the psychopathology.

In a study using MDD mouse models, more promising results were observed. ECBs exhibited anti-depressive-like effects following the forced swim test in mice and produced a “U”-shaped response curve in the marble-burying behavior test [222]. When ECBs were co-administered with fluoxetine, the antidepressant-like effect was potentiated further on both paradigms. Furthermore, AM251, a CB₁ antagonist blocked the antidepressant-like effects of fluoxetine on mice in the forced swim test and marble-burying behavior paradigms. Similar outcomes were seen for ECB analogues, inferring interplay between ECB and the serotonergic system in the dysregulation of depressive behavior. Further studies in these fields will help confirm if specific ECBs may be suitable as antidepressants.



5. Metabolomics and depression

Metabolomics is the study of small molecules, also known as metabolites. These molecules can be present in cells, tissues and fluids of an organism. The interaction of these molecules within a biological context is what forms the metabolome. A wide variety of metabolites have been implicated in MDD but for the scope of this review, we will focus explicitly on the

metabolites with strong evidence for depression. Lipids including polyunsaturated fatty acids and cholesterol show promising evidence as potential biomarkers. The HPA axis, cortisol and corticotropin-releasing hormone as part of the endocrine system also show positive signs as MDD-related biomarkers.

5.1 Lipids

5.1.1 *Polyunsaturated fatty acids (PUFA)*

Polyunsaturated fatty acids (PUFA) are fundamental nutrients for humans. They consist of omega-6 and omega-3 fatty acids (FA) and once absorbed they incorporate into the cell membranes of long-chain active PUFA elements, including arachidonic acid for omega-6 and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for omega-3. Omega-6 acids play a pro-inflammatory role in vivo while omega-3 acids regulate anti-inflammatory processes [237]. These units play an essential physiological role for cellular processes; omega-3 acids, for example, increase the fluidity of cell membranes and are responsible for the regulation and maintenance of neuronal development and transmission [238]. Mammalian cells cannot directly synthesize PUFA from smaller molecules. As a result, consumption of these nutrients is essential for the regulation of cellular physiology as well as to prevent diseases, such as cardiovascular, autoimmune, inflammatory, diabetes and obesity [239]. Reduced levels of omega-3-PUFA has been consistently reported in depression, often in comorbidity with the above conditions [239].

There has been an increase in the ratio of omega-6 to omega-3 PUFA due to alterations in fatty acid composition in the western diet over the last 150 years [240,241]. The increased consumption of omega-6 FA and reduced intake of omega-3 has been correlated with reductions in pro-inflammatory properties and an increase in depressive disorders associated with cardiovascular diseases [242]. Multiple epidemiological studies have highlighted an inverse correlation between oily fish consumption and the incidence of depression [243–245]. Although oily fish contains PUFA, it is unclear if these inverse relationships are specific to omega-3. On the contrary, in spite of the greater intake of fish, some countries have shown an increase in depressive symptoms [241], suggesting the influence of factors such as lifestyle or low quality of diet that may counteract the protective effects of PUFA within oily fish. In fact, cross-sectional studies showed that after controlling for lifestyle, the relationship between depression and omega-3 intake was no longer significant [246,247]. This may suggest the association between omega-3 FA and depression might result from

collinearity with dietary and lifestyle factors. Regardless of this, multiple studies have implicated omega-3 FA in MDD. Pregnant women with lower concentration of omega-3 FA or high omega-6/omega-3 ratio revealed greater levels of depressive symptoms during pregnancy and a higher prevalence of post-partum depression [248,249]. These effects occurred after adjusting for confounding lifestyle factors.

Meta-analytical studies have disclosed contradictory findings both in support of and against a key role of omega-3 FA and depression [250,251]. Many of the contradictions of PUFA and depression in the literature arise from methodological limitations, in particular, heterogenous study samples (see [237] for a review). Publication bias, variability of depression and omega-3 assessments, duration of the trial and the differential effects of MDD pathology in comorbid conditions can all influence viability of the analysis. However, the potential for omega-3 FA as antidepressants has been implicated in some of these meta-analyses. When focusing on the specific type of omega-3 FA, EPA rather than DHA showed a positive effect on depressive symptoms [252,253]. A meta-analysis of clinical trials concluded that supplements containing EPA $\geq 60\%$ of total EPA and DHA in a dose ranging between 200 and 2200 mg/d of EPA in abundance of DHA resulted in ameliorative effects against depression [253]. This suggests EPA containing omega-3 FAs could potentially serve as novel antidepressants. The beneficial effects of omega-3 FA has especially been linked to severe MDD symptoms [254].

Multiple mechanisms of action have been proposed for the therapeutic effects of omega-3 FA on depression, including monoamine deficiency or HPA axis hyperactivation [241]. Since omega-3 FA are primarily anti-inflammatory factors, the present review will highlight this effect on MDD. The inflammatory equilibrium between omega-3 and omega-6 PUFA is derived from eicosanoids; These are small signaling molecules that function as messengers in the central and peripheral nervous systems to control inflammation [255]. During an inflammatory cascade omega-3 FA are responsible for neutralizing omega-6 arachidonic acid-derived eicosanoids [256]. This means the balance of omega-3 and omega-6 PUFAs, based on an individual's dietary intake, regulates eicosanoid-controlled functions. An imbalance of the two PUFA in the diet can result in a shift in the ratio of omega-6 to omega-3 FA and result in inflammation-induced MDD (see Fig. 3). In fact, multiple studies showed DHA-deficient rats displayed increased concentrations of IL-6 and TNF α ; these effects were reversed upon normalization of DHA [257,258]. This provides evidence for DHA as a potential monitoring biomarker for MDD.

5.1.2 Cholesterol

Cholesterol is a lipid molecule synthesized from sterol. It is responsible for the regulation of membrane fluidity of the plasma membrane. This, in turn, influences the regulation of membrane-bound proteins and ion channels, as well as synaptic transmission. It is also required for the formation of synapses, dendrites, and axonal guidance. Dysfunction in any of these can disrupt neurotransmission and reduce synaptic plasticity [255]. In addition, cholesterol is an important precursor for steroid hormones, vitamin D and bile acids. The level of cholesterol is tightly regulated within the body, whereby too much is associated with an increased risk of coronary heart disease and too little can affect neurotransmission, hormonal homeostasis and plasticity. Dysregulations of these functions is particularly implicated in depression pathophysiology.

In MDD, an overall decrease in total serum cholesterol has been reported as compared to healthy controls [259]. In addition, individuals with depression have shown a higher proportion of low-density lipoprotein (LDL) [260] and a reduction of high-density lipoprotein (HDL) [261]. Low circulating total cholesterol also correlates significantly with suicidality or suicidal ideation and has been reported in multiple studies [262–264]. It is also specific to MDD, as there was no significant link between suicidality and cholesterol levels in bipolar disorder patients [265]. With further rigorous research confirming specificity across alternative psychiatric conditions, cholesterol could be used as a potential biomarker for identifying high-risk MDD patients.

However, there have been contradictory reports in which depressed patients showed increased total cholesterol levels and no change in HDL concentrations [261,266]. Furthermore, there was no significant correlation with low serum cholesterol and MDD in elderly patients (>65) [267]. These findings may infer that alternative mechanisms in association with age can influence depression and cholesterol metabolism. Meta-analyses have also found conflicting links between serum lipoproteins levels and MDD in relation to the majority of the studies. Although total cholesterol and depression were inversely correlated, Shin et al. [268] reported an increase in HDL correlated with heightened depression symptoms, particularly in women. Alternatively, Persons and Fiedorowicz [269] disclosed that depression was associated with a decrease in LDL levels. To determine and identify cholesterol as a diagnostic tool for depression, more thoroughput studies with better consistency must be conducted.

The effects of cholesterol as an antidepressant remain elusive. Following psychological and/or pharmacological intervention, MDD patients showed an increase in serum cholesterol and amelioration of depressive symptoms

[270]. Although assessment of changes in cholesterol levels may help monitor treatment, the antidepressant potential of cholesterol remains tentative until a clearer relationship between cholesterol lipoproteins and MDD is established. However, cholesterol-reducing statins have shown promise in targeting cholesterol in MDD. Statins regulate the uptake of peripheral LDL and the reduction of cholesterol synthesis. Studies have highlighted that lovastatin and atorvastatin as adjuvants alongside fluoxetine and citalopram respectively, produced a greater antidepressant effect than the antidepressants alone [271,272]. In addition, atorvastatin also produced significant reductions in cholesterol and LDL, whereas HDL levels increased over time compared to the placebo group; these findings are consistent with the cholesterol pathway changes associated with MDD (see above).

One of the mechanisms of action suggested for cholesterol's role in depression involves the serotonin system. Reduced cholesterol concentrations in the cell membrane is linked with a lower serotonin receptor density in mice [260,273]. A study by Sun et al. [260] showed a reduction in cholesterol levels in the mPFC following chronic mild stress. The stress induced depressive-like behavior was reversed by supplementation of cholesterol through food. In addition, injection of a 5-HT receptor antagonist in the mPFC inhibited the effects of cholesterol treatment on depressive behavior, suggesting the pathway is specific to cholesterol. It is important to note that this is one of many cholesterol mechanisms implicated in MDD.

5.2 The endocrine pathway

5.2.1 The HPA axis

The Hypothalamus-Pituitary-Adrenal-Axis (HPA) constitutes the interaction between the hypothalamus, pituitary gland and the adrenal glands. The HPA axis is primarily responsible for the regulation of bodily stress. Upon stress detection, the hypothalamus secretes and releases corticotrophin-releasing hormone (CRH). CRH stimulates the production of proopiomelanocortin in the anterior pituitary gland. Proopiomelanocortin serves as the building block for adrenocorticotrophic hormone (ACTH). ACTH is responsible for the stimulation of the stress hormone cortisol from the adrenal glands (see Fig. 5) [274,275]. Cortisol mediates a variety of adaptive changes in response to stress including increased blood pressure, cardiac output, immune response and glucose circulation in the blood [276]. The HPA axis, CRH, ACTH and cortisol play an important role in dealing with stress; however, repeated stress can disrupt the physiological functioning of the system and lead to maladaptive psychiatric conditions (See Fig. 5). Overstimulated HPA axis disruptions are seen in ~50%–70% of patients

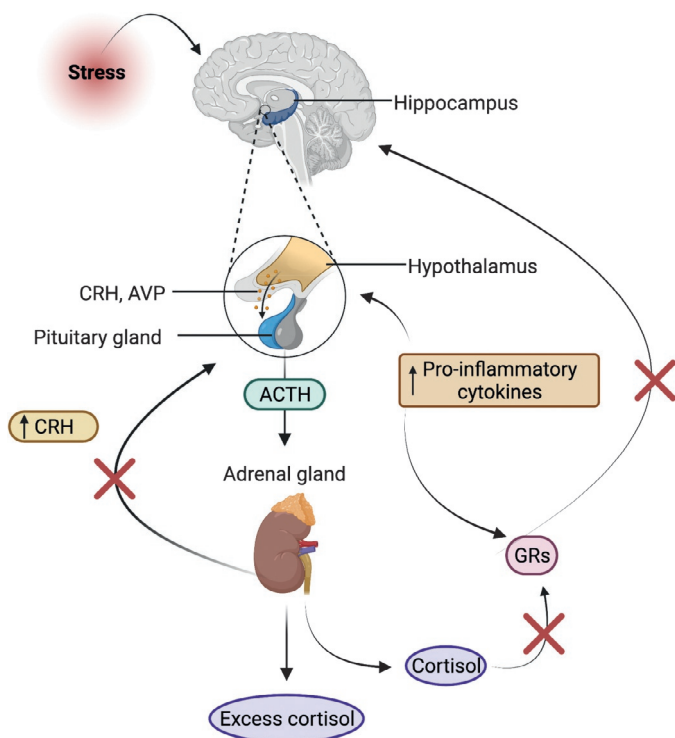


Fig. 5 Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis system during depression. Multiple pathways are implicated in HPA axis hyperactivity during chronic stress, including inflammation and glucocorticoid resistance. Stress triggers the release of corticotropin-releasing hormone (CRH) and vasopressin (AVP) from the hypothalamus. This in turn stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. ACTH triggers receptors on the surface of adrenal glands to secrete cortisol. Excess cortisol is released during depression due to the down-regulation of negative feedback pathways (marked with “X”). Excess cortisol and increased inflammation can desensitize glucocorticoid receptors (GRs) and contribute to HPA axis hyperactivity and pro-inflammatory neurotransmitter interference [35].

with MDD [277]. In addition, elevated peripheral CRH and cortisol has been heavily implicated in depression pathophysiology.

A plethora of studies have implicated hypercortisolemia in severe cases of depression [142]. Hypercortisolemia adversely impacts the limbic system, especially the CA3 hippocampal neurons. The damaged hippocampus (i.e., dendritic atrophy, neuronal apoptosis and reduced spinogenesis [278]) is less effective in inhibiting the HPA axis through glucocorticoid receptors as seen under normal physiological settings [279]. Inhibition of the negative feedback loops results in an even greater increase of detrimental HPA activity [238]. Cortisol-mediated damage to hippocampal cells can lead

to decreased neurogenesis and subsequent volume reductions and atrophy of the hippocampus as reported in patients with depression [176,280]. The directionality between HPA axis dysregulation and MDD is still unclear. However, numerous studies implicate that the HPA axis is heavily linked with depressive symptoms. As such the HPA axis must be monitored and tested routinely in depressed individuals.

The Dexamethasone Suppression Test (DST) together with the CRH test measures cortisol dysfunction in depression and is very specific in differentiating healthy subjects from individuals with a psychiatric illness, including MDD. The low sensitivity of the DST limits its usefulness in distinguishing between MDD and other psychiatric illnesses [281]. The DST/CRH test can be used instead as a state-dependent marker to detect elevated cortisol response and predict risk of MDD recurrence after antidepressant treatment [282]. An alternative method of examining elevated cortisol concentrations for clinical assessment is by using hair [283]. Hair cortisol levels are abundant and studies have showed that they reflect changed induced by exposure to chronic stress [284].

Higher concentrations of CRH have been seen in the CSF of depressed patients [285], perhaps due to a general increase in the number of CRH-secreting neurons in limbic brain regions [286]. Elevated CRH levels produce behavioral and physiological changes similar to those seen in MDD such as disrupted sleep, decreased appetite and decreased libido [287]. Similar effects have been seen in animal models following CRH injection [288]. Zobel et al. [289] demonstrated a reduction in depression and anxiety scores following CRH-1 receptor blockade. Comparable effects were observed in CRH1 receptor knockout mice in the presence of stressful stimuli [290]. These studies thus implicate CRH-1 receptor antagonism for possible clinical treatment in cases where there is inflated CRH activity and MDD. These studies are limited to animal models unfortunately. CRH antagonists as treatments for depression is one of general failure as indicated by multiple studies in the literature [291,292].

Social stress plays an important role in the activation of the CRH gene and the production of depression-related symptoms and behavior. Chronic social stress causes long-term demethylation of the CRH-gene in stress-prone mice, increasing its expression and eventually leading to depressive-like symptoms [54]. Resilient mice on the other hand failed to show epigenetic modification at the CRH-gene and also did not show a depressive phenotype. This suggests that some individuals may be more susceptible to a maladaptive stress response compared to others. Identifying those individuals more at risk at an early stage may prove fruitful for an intervention strategy as aimed at minimizing the deleterious effects following exposure to chronic stress [54].

Studies implicating genetic polymorphisms have revealed three particular CRH-1 SNPs (rs1876828, rs242939 and rs242941). Presence of these SNPs has been associated with an increased risk of developing major depression [293]. Before concluding this section, it is important to note that not all studies are homogenous in representing CRH with MDD. A quantitative summary by Stetler & Miller [142] displayed no significant increases in CRH in depressive patients compared to healthy controls. This may suggest a differential effect of CRH among individuals. Nevertheless, the above studies have highlighted the involvement of CRH, stress response, and depressive symptomology in MDD. Future studies assessing the directionality problem between cortisol, CRH and the HPA axis system may help identify better markers that are specific to depressive etiology.

5.3 The neurosteroid system in depression

Neurosteroids are endogenous ligands that modulate neuronal excitability by an allosteric potentiation of the gamma aminobutyric acid A (GABA_A) receptor [294]. Key physiological processes such as stress response [295], neuroinflammation [296], learning and memory [297] are regulated by these steroid hormones. Neurosteroids are synthesized explicitly within the brain [298] through a cholesterol precursor [299] resulting in the production of allopregnanolone (3 α ,5 α -tetrahydroprogesterone) and pregnanolone (3 α ,5 β -tetrahydroprogesterone) that act as positive modulators of GABA_A receptors [300,301] and regulate receptor plasticity [291,292]. Following sulfation, the latter neurosteroid results in pregnanolone sulphate that also inhibits tonic-mediated NMDA receptor neurotransmission [302,303]. Neurosteroid homeostasis is disrupted in individuals with MDD and post-partum depression [304–307]. In particular, allopregnanolone concentrations are reduced in individuals with major depression and antidepressant treatment both normalized CSF levels of allopregnanolone and reduced depressive symptoms [308,309]. These findings may infer the possibility of allopregnanolone's role as a biomarker and antidepressant in the treatment of depression (See [310] for a review). Animal models of depression induced by protracted stress exposure or by bulbectomy have likewise observed that allopregnanolone concentrations are decreased in several corticolimbic areas and SSRI (fluoxetine and sertraline) treatment normalized neurosteroid levels, through mechanisms that are independent from the serotonergic action of these drugs [228,230,311–313].

In women suffering from postpartum depression, allopregnanolone levels were found to be altered during the peripartum period, followed by a significant decrease in reproductive steroids during the postpartum period [314]. Serum levels of allopregnanolone in women with postpartum depression showed dramatic reductions compared to women without any mood disturbances [315]. Similar allopregnanolone alterations were also seen in depression during pregnancy [316]. Recently, a phase 3 clinical trial of brexanolone, a synthetic intravenous formulation of allopregnanolone induced a clinically significant, rapid and long-lasting antidepressant effect in women with postpartum depression [317]. This breakthrough resulted in the USA Food and Drug Administration (FDA) approval of brexanolone as the first specific treatment for postpartum depression. Although the antidepressive mechanism for brexanolone are unclear, one possible route is through the potentiation of inhibition of gamma amino-butyric acid (GABA) at GABA_A receptors [318].

Allopregnanolone serves to protect the innate immune system and brain by restoring GABAergic inhibition, modulating CRH signaling, and preventing pro-inflammatory toll-like receptor signaling [296]. The inflammatory theory of depression points towards elevated inflammatory markers in individuals with major depression and in women with postpartum depression [319]. Similarly, brexanolone infusion might be acting through a pathway that includes anti-inflammatory functions by inhibition of the LPS-mediated activation of toll-like receptor signaling, thus leading to decreased pro-inflammatory mediators [320]. The therapeutic effect of allopregnanolone may therefore be associated not only with potentiation of GABAergic neurotransmission but also with toll-like receptor signal inhibition; ameliorating the proinflammatory signaling as seen in MDD [321].



6. The gut microbiome and depression

The gut microbiota can be described as a virtual organ responsible for homeostatic maintenance in humans. It primarily consists of bacteria which *en masse* comprise a genomic makeup that exceeds the human genome by 150 times [322]. The gut microbiota and the brain makeup the microbiota-gut-brain axis. The brain and gut microbiota interact through a bidirectional pathway at the level of the neuroendocrine or metabolic systems; which in turn is regulated by multiple factors and mediators (See Fig. 6A). The gut microbiota can produce over one hundred different metabolites that directly influence local systems and organs, including the brain [325]. This in turn has been implicated in the aetiopathology towards depression.

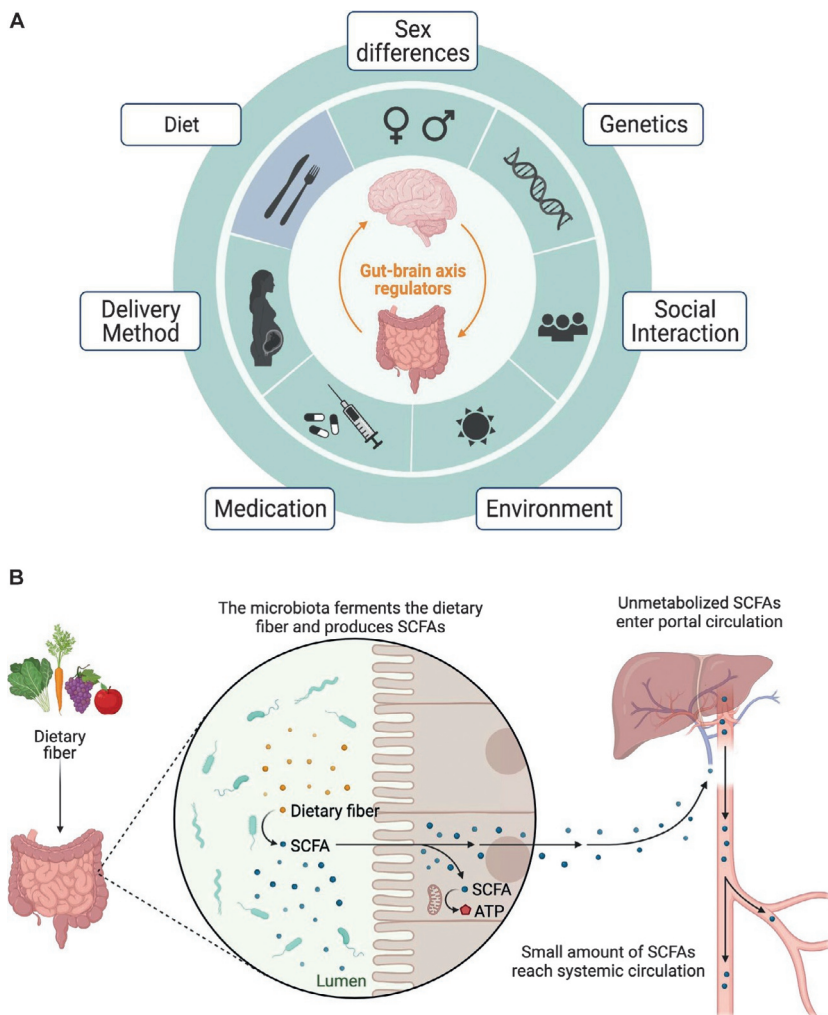


Fig. 6 (A) Factors that influence the microbiome gut-brain axis. Diet is the primary factor considered in this review [323]. (B) An illustration of short-chain fatty acid (SCFA) production and metabolism. The type of food consumed can influence the production of healthy or maladaptive SCFA and can dictate the shape of the microbiome towards dysbiosis; this in turn influences depressive mood and antidepressive properties [324].

6.1 Microbiome role in depression

Multiple studies have found significant differences between the gut microbiota of patients with MDD and healthy controls [326,327]. In general, patients with depression have shown a gut microbiota with lower microbial diversity and reduced alpha and beta diversity [328]. At the level of the phylum, patients with major depression showed a lower abundance of

Bacteroidales, *Firmicutes*, *Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, *Coprococcus* and *Dialister* [328–332]. On the other hand, *Actinobacteria*, *Fusobacteria* *Enterobacteriaceae*, *Lachnospiraceae*, *Prevotellaceae*, *Prevotella*, *Bacteroidetes*, *Proteobacteria*, *Bacteroides*, and *Flavonifractor* were all increased in the gut microbiota of patients with depression [328–331]. To summarize, the pattern highlighted in many of these studies revealed an increased abundance of harmful inflammatory bacteria, including *Enterobacteriaceae* and reduced concentrations of beneficial bacteria such as *Faecalibacterium*. A problem lies in the inconsistencies found between bacteria across studies. To form a defined consensus of bacteria implicated in MDD, consistent methodological approaches including diagnostic criteria or fecal method detection must be administered. In addition, factors such as age, diet, genetics and region must all be taken into account as they determine the composition of the gut microbiota across individuals [333–336]. The sections that follow will consider the potential bacterial metabolites that may be involved in major depression.

6.2 Neurotransmitter changes

The gut microbiota is responsible for the production and release of specific neurotransmitters, some of which have been heavily associated with depression. The disruption of serotonin metabolism and reuptake is believed to contribute to MDD pathophysiology [337]. The gut microbiota seems to produce over 90% of the body's serotonin and may thus contribute towards the serotonin deficiency that may facilitate onset of MDD [338] (see Fig. 3B). Studies have shown certain bacterial populations can sequester tryptophan, which is then converted to tryptamine [339]. Tryptophan is the precursor for serotonin synthesis in the brain. This process is consequently inhibited due to an altered microbiota, potentially leading to development of depressive symptoms. This effect was seen in sterile mice which displayed reduced plasma and brain serotonin levels compared to controls [340]. Furthermore, serotonin levels were normalized following recolonization of germ-free animals [341].

The catecholamine, dopamine is involved in depressive-like behaviors and symptoms [342]. There is evidence of the microbiota-gut-brain axis being involved in the primary dopamine pathways of the mesocorticolimbic system [343]. The interaction between the gut microbiota and dopamine happens through the HPA axis [344], the immune system [345] and the vagus nerve [346]. Stimulation of the latter through gut-innervating vagal sensory neurons replicated the effects of the dopaminergic system.

Increasing the overall level of dopamine in the brain [346]. These findings suggest a role for gut microbiota to synthesize dopamine that might be useful for treatment.

Another neurotransmitter that is associated with MDD is GABA. GABA concentrations have been found to be reduced in one-third of patients with major depression [332]. Altered gut microbiota in mice led to a disruption in the physiological levels of GABA. A healthy gut microbiota can in fact promote the production of GABA as observed in multiple studies [347]. GABA production by bacteria occurs through the enzyme glutamate decarboxylase, which irreversibly converts L-glutamate into GABA [348]. Genetic studies have shown the *gadB* gene which codes for glutamate decarboxylase was found in several bacterial species in the gut [349]. Capitalizing on these GABA-producing gut bacteria may be useful in alleviating depressive symptoms in patients. These studies point towards novel ways of improving imbalances in neurotransmitter-related depressive symptoms by reorganizing the microbiota.

6.3 Short-chain fatty acids

Short-chain fatty acids (SCFAs) also play a key role in communication between the brain and the gut. Fermentation of non-digestible carbohydrates by bacteria produces SCFAs [350] (See Fig. 6B). The lipophilicity of SCFAs allows them to cross the blood-brain-barrier and transmit signals to the brain. SCFAs can activate G-protein coupled receptors resulting in a downstream anti-inflammatory cascade [327]. Low concentrations of SCFAs are indicative of a “dysbiotic” gut microbiota; this could explain some of the findings of the inflammatory hypothesis for depression.

The therapeutic implications of SCFAs have been demonstrated in multiple animal studies. Kelly et al. [347] showed transplantation of fecal microbiota from MDD patients to sterile rats resulted in an upregulation of fecal SCFA levels compared to controls. The SCFA, sodium butyrate, also ameliorated depressive-behavior in rats [351]. Butyrate from bacteria has shown to regulate the synthesis of dopamine, adrenaline and noradrenaline by modifying the expression of the tyrosine hydroxylase gene [352]. These signs point towards an antidepressive-like property of SCFAs.

An imbalance of certain SCFAs may contribute towards the initial symptoms of depression. A reduction in the levels of acetate, for example, correspond to lower levels of butyric acid as seen in depressed individuals [353]. Acetate is responsible for protection against enteropathogenic infections and its dysregulation may lead to an increase in inflammatory cytokines, which

manifest depressive symptoms. Furthermore, acetate and propionate were found to be inversely related to depressive symptoms as measured by the Beck Depression Inventory [353]. Isocaproic acid levels in the feces were also significantly reduced [354]. Isovaleric acid on the other hand has exhibited a direct correlation with depressive symptoms and serum cortisol levels. Mechanistically, isovaleric acid interferes with neurotransmitter release in the synaptic cleft, which may explain its connection towards depression [355]. In general, the eubiosis of the bacterial microbiota community is shown to directly correspond to the expression of specific bacterial metabolites. The identification of these specific SCFAs may prove fruitful as novel biomarkers for depression and help in early intervention strategies.

6.4 The impact of diet

Diet noticeably influences the composition of the gut microbiome, a phenomena that has been replicated in multiple studies [334,356]. Foods high in fiber and complex sugars provide energy to beneficial bacteria, typically the microbes that produce butyrate [327]. In contrast, western diets have high consumption of fats and simple sugars and overconsumption of these foods drives the growth of maladaptive bacteria that produce neurotropic metabolites [357]. This means that the quality of diet can directly affect the severity of depressive symptoms. For example, a 12-week intervention using a slightly altered Mediterranean diet in adults with MDD led to the improvement of depressive symptoms [358]. The food groups that are rich in the Mediterranean diet, such as eggs, fish, nuts, dairy, fruits and vegetables correspond with anti-inflammatory and antidepressive qualities. This was observed in a large cross-sectional study where vitamin B12 and folate inversely correlated with MDD [359]. Adhering to the well-documented Mediterranean diet can influence the gut microbiota by increasing the abundance of bacteria that produce healthy SCFAs, which may consequently reduce depressive symptomology [360] (See Fig. 6B). Furthermore, a lack of PUFAs in the diet can contribute towards MDD symptoms as previously denoted in this chapter [361]. This was also confirmed by observations in patients with MDD who showed lower serum concentrations of omega-3 and omega-5 PUFAs [362]. Foods rich in omega-3 and omega-6 PUFAs can improve depressive symptoms. Similarly, diets rich in avocado, broccoli, berries and green tea help in the production of useful anti-inflammatory mediators. Whereas, proinflammatory foods including foods rich in gluten, sugars, preservatives and chemical additives may contribute towards the progression of inflammatory processes thereby facilitating symptoms of depression [363].

Along with dietary interventions as discussed above, interventions using prebiotic and probiotic supplementation can reconstruct the balance of the gut microbiota. Prebiotics consist of foods, such as fibers, that are not digestible by humans but have a beneficial effect on the host's microbiome [364]. Probiotics on the other hand consist of microbes that have a positive effect on the host's gut microbiota. Together, these products are termed "psychobiotics" when used to improve mental-health by targeting the microbiome [365]. Multiple studies have reported the positive effects these compounds have on mood [366]. The significant alleviation of depressive symptoms has also been reported in a meta-analysis [367] and thus the supplementation of these products as a preventive strategy in depression is very promising. This area of research in the field of developing new treatment for MDD is novel. As such, any inferences towards causality must be interpreted with caution. Future research translating animal studies to humans will provide a better understanding on the utility of psychobiotics in MDD treatment.

6.5 Conclusion

This review highlights the vast number of potential biomarkers for MDD and shows the value of a biopsychological approach in diagnosis and treating depression. However, many studies also point towards contradictory findings, hindering the possibility of a general consensus of biomarkers for depression. Much of the ambiguity arises from methodological differences across studies and biological differences among individuals. The heterogeneity of depression indicates that there is no all-for-one model. With advancements in research and improvements in psychological and biological measurements, we can identify robust biomarkers of depression. Moving forward, a holistic framework accounting for biological, psychological, and social factors among individuals can help identify specific markers that generalize to subgroups. This can be particularly useful in treatment-resistant depression.

Among the various neurobiological changes associated with major depression pathophysiology, it is rather difficult to state at the current time which biomarker candidates are among the most promising, however, hereinafter, we attempt to list at least three "best case" scenarios.

There is robust evidence that neurotropic factors, including BDNF, play a role in MDD pathophysiology. As detailed previously, not only their expression level is downregulated in major depression but there is also substantial evidence that normalized levels associate with improvement of

depression symptoms. Altered levels of BDNF and its receptor TrkB have been observed in MDD. Meta-analyses have largely confirmed this findings although not without contradictory results [160,161]. Post-mortem studies conducted in PFC and hippocampus have backed these results [162]. There is also plenty of evidence that downregulated BDNF concentrations are normalized by antidepressants in depressed individuals [167]. There are also interesting results connecting low BDNF levels in treatment-resistant depressed subjects [169]. Several non-pharmacological antidepressant therapies, including ECT and physical exercise have also been linked with the upregulation of BDNF signaling and improvement of neurogenesis and cognitive functions [177]. Most importantly, concentrations of neurotrophic factors expressed in blood appears to reflect changes occurring in brain, which makes them intriguing biomarker candidates for major depression [156,157].

The biosynthesis of neuroactive steroids, including the concentration of the GABAergic allopregnanolone has been impaired in major depression [304–306,368]. Intravenous allopregnanolone (i.e., brexanolone) has been approved by the FDA for the treatment of post-partum depression and the orally-available formulation of this endogenous modulator (i.e., zuranolone) has also demonstrated efficacy in several clinical trials of both major depression and post-partum depression [369,370]. These clinical trials were based on the supporting evidence of dysregulation in neurosteroids levels in preclinical and clinical studies of depression [313,371]. Therefore, it appears that allopregnanolone levels may be considered in the top list for neurobiological alterations that may help the diagnosis of major depression as well as for indicating treatment response in individuals with depression and other mood disorders [301,372].

Inflammatory markers should also be considered among the top potential biomarkers in major depression given a strong evidence suggesting their role in the pathophysiology of this debilitating disorder and the finding that both anti-inflammatory drugs, including anti-inflammatory non-steroidal agents or inhibitors of pro-inflammatory factors provide robust antidepressant effects both in basic research studies, using rodent models of depression, and in clinical studies. Excessive production of pro-inflammatory cytokines has been consistently associated with severity of depression symptoms [101]. A bidirectional relationship was assessed between elevated inflammation and protracted stress resulting in depression [105]. Of note, elevated pro-inflammatory cytokines that have been quantified in the peripheral immune system play a role in onset of depression as evidenced in various meta-analyses [121–125], suggesting that they can be assayed as future MDD biomarkers.

We also believe that future research on the role of the microbiome in the pathophysiology of major depression may profoundly and positively affect the field of biomarker discovery as well as positively influence the treatment of depression in unexpected ways.

Acknowledging the importance of these biomarkers can considerably improve misdiagnosis, interventions, treatment response to antidepressants and create a path for individual-specific treatments.

Conflict of interest

G.P. is a paid consultant to PureTech Health (Boston, MA, USA), GABA Therapeutics, and NeuroTrauma Sciences (Alpharetta, GA, USA). He has two patent applications, one on *N*-palmitoylethanolamine (PEA) and peroxisome proliferator-activated receptor alpha (PPAR- α) agonists US20180369171A1, pending, and one on allopregnanolone analogs US11266663B2 granted on March 8, 2022 in the treatment of neuropsychiatric disorders. The other authors declare no conflict of interest.

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