Neurobiology of Stress 42 (2026) 100816

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr

Alterations of microRNAs across human and mouse limbic brain areas: molecular mechanisms and biological processes involved in major depressive disorder

Samantha Saleri [a,1], Moira Marizzoni [a,1], Patrizia Genini [b], Chiara Galbiati [a], Annamaria Cattaneo [a,b], Gustavo Turecki [c], Henning Grosskopf [d], Petra Steinacker [d], Markus Otto [d], Giulia Poggi [e,2], Christopher R Pryce [e,f], Naguib Mechawar [c], Nadia Cattane [a,*]

[a] Biological Psychiatry Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy
[b] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy
[c] McGill Group for Suicide Studies, Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, QC, Canada
[d] Department of Neurology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany
[e] Preclinical Laboratory, Department of Adult Psychiatry and Psychotherapy, University Hospital of Psychiatry and University of Zurich, Zurich, Switzerland
[f] Zurich Neuroscience Center, University of Zurich and ETH Zurich, Zurich, Switzerland

ARTICLE INFO

Keywords:
Depression
Stress
Limbic system
miRNAs
Cross-species preservation analyses

ABSTRACT

The etiology of major depressive disorder (MDD) is multifactorial with both genetic and environmental factors, such as adverse/stressful life events, contributing to risk. There is some evidence suggesting that microRNAs (miRNAs) mediate environmental-genetic interaction leading to the brain dysfunctions that underlie MDD. However, changes in miRNAs expression in human brain regions due to stress and associated with MDD are unclear. To increase the evidence in this regard, miRNA sequencing was performed on tissue samples of subgenual anterior cingulate cortex (sgACC) obtained from depressed patients and control subjects, as well on tissue samples of medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) from mice exposed to chronic social stress (CSS) and control animals. DESeq2 was applied to identify differentially expressed miRNAs (DEMs) and weighted co-expression network preservation analysis to uncover conserved molecular mechanisms between species. Finally, pathways obtained from DESeq2 and preservation analyses were overlapped to robustly identify MDD-related processes across bioinformatic approaches.Eighteen DEMs were identified in the human sgACC, 11 in the mPFC and 9 in the BLA of mice. The human sgACC DEMs were involved mainly in intracellular signaling and immune system-related pathways. The mouse mPFC and BLA DEMs were mainly involved in, respectively, intracellular signaling and nervous system functions. Preservation patterns between humans and mice indicated an over-representation of processes related to cellular signaling. Transcriptional regulation by MECP2 and Protein Kinase A signaling were the two pathways consistently altered across species, brain regions, and bioinformatic approaches. Although further studies are needed, they could represent a novel target for intervention strategies and confirm the dysregulation of intracellular signaling, immune, neuronal and synaptic functions in MDD.

1. Introduction

Major depressive disorder (MDD) is the leading cause of worldwide disability (Dion-Albert et al., 2022). According to the World Health Organization, 4% of the global population experience depression, with a prevalence around 5% among adults (https://www.who.int/news-room/fact-sheets/detail/depression). The biological processes and molecular mechanisms underlying MDD are unknown.

* Corresponding author
E-mail address: ncattane@fatebenefratelli.eu (N. Cattane).
[1] These authors equally contributed to this work.
[2] Current affiliation: Department of Psychiatry and Psychotherapy & Institute of Anatomy, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany.

https://doi.org/10.1016/j.ynstr.2026.100816
Received 12 November 2025; Received in revised form 12 March 2026; Accepted 10 April 2026
Available online 20 April 2026

Table 1
Demographic and clinical features of human post-mortem brain samples. Data are reported as mean ± standard deviation (SD) or percentage (%) for depressed patients and control subjects. P-values were generated using unpaired *t*-test. MDD: major depressive disorder; PMI: post-mortem interval; SNRI: serotonin-norepinephrine reuptake inhibitor; SSRI: selective serotonin reuptake inhibitor; TCA: tricyclic antidepressant.

	MDD patients	Controls	p-value
N	28	23	
Axis I diagnosis	MDD (28)	0	
Sex female (N %)	14 (50.0%)	9 (39.1%)	0.622
Age years (mean, ±SD)	44.68 (± 17.18)	55.74 (± 16.89)	**0.025**
PMI hours (mean, ±SD)	60.89 (± 15.01)	53.61 (± 18.80)	0.140
Tissue pH (mean, ±SD)	6.34 (± 0.20)	6.22 (± 0.24)	0.059
Substance dependence	4	3	
Cause of death	Accidental (2); Natural (3); Suicide (23)	Accidental (11); Natural (12)	
Refrigeration Delay hours (mean, ±SD)	13.56 (± 6.83)	10.61 (± 10.42)	**0.011**
Medication prescribed in the last 3 months	Antidepressants (9) (SSRI (4), SNRI (2), Unknown antidepressants (3)); Atypical Antipsychotic (2); Alzheimer patch (1), Benzodiazepine (3); Opioid (2)	Benzodiazepine and other hypnotics (3); Cholinesterase inhibitor (1); Morphine (1); TCA (1)	

Etio-pathophysiological hypotheses include dysregulation of specific neurotransmitters and their receptors, oxidative stress, glucocorticoid dysregulation, disturbed energy metabolism, gut microbiome dysbiosis, and immune/inflammatory changes (Bakunina et al., 2015; Cui et al., 2024; Osimo et al., 2020; Ruiz et al., 2022; Suda and Matsuda, 2022). In addition, the etiology of MDD is multifactorial including both genetic and environmental factors, with adverse/stressful life events among the latter (Chand and Arif, 2025). By acting through the modulation of the previously mentioned pathways, stressful events can affect brain structure and function, and depending on the stressor, they can impact on different circuits, including the limbic brain areas (McEwen et al., 2016). Alterations in the limbic system, which regulates emotions, behavior, motivation and memory (Rajmohan and Mohandas, 2007), have indeed been observed in MDD and involved both the basolateral amygdala (BLA) and the subgenual anterior cingulate cortex (sgACC), as demonstrated by neuroimaging and post-mortem brain studies (Goldstein-Piekarski et al., 2022; Spellman and Liston, 2020).

Environment-gene interactions can be mediated by epigenetic mechanisms through the regulation of gene expression by environmentally-responsive microRNAs (miRNAs), namely, small non-coding RNAs that influence messenger RNA (mRNA) translation and degradation (O'Brien et al., 2018). Recently, miRNAs have gained attention as potential mediators of altered mRNA expression levels in MDD (Roy et al., 2020), with their proposed role involving the regulation of neuronal diversity, morphology and structure. Recent studies have found that miRNAs also regulate the molecular composition of synaptic components such as dendritic spines, as well as the formation of pre-synaptic proteins and glial pathways that influence neurotransmission (Gao, Y.N. et al., 2022). Therefore, miRNAs might represent the epigenetic mechanisms linking external adverse stimuli to changes in brain functions observed in MDD patients (Ortega et al., 2021). Indeed, beyond the well-established etiological factors of MDD, of particular interest is the abnormal fluctuation of miRNA levels in the brain and in peripheral tissues of MDD patients versus controls, since they have emerged as key regulators of post-transcriptional gene silencing. Through this intricate mechanism, a single miRNA can modulate the expression of multiple genes, significantly impacting a wide range of biological pathways, including those involved in MDD and previously mentioned. Given that approximately 70% of known miRNAs are expressed in the brain and contribute to key neurological processes, their potential as biomarkers and therapeutic targets for MDD is promising.

MiRNAs act as critical epigenetic mediators between environmental factors, including stress exposure, and the development of MDD. Specifically, stress triggers altered miRNA expression, which subsequently affects MDD-related pathways, previously described, leading to long-term brain structural changes (Ding et al., 2023).

Based on this hypothesis, it becomes important to investigate the associations among stress, MDD and miRNA expression profiles in the limbic brain areas. Therefore, in the present study, a hypothesis-free approach was applied to investigate for altered expression levels of the miRNome in the sgACC of MDD patients versus control subjects and in the medial prefrontal cortex (mPFC, to some extent the mouse analogue of the sgACC) and the BLA of mice exposed to chronic social stress (CSS) versus control mice. The paradigm of CSS is based on psychosocial stress, a major risk factor for MDD (Azzinnari et al., 2014; Pizzagalli, 2014).

Rather than focusing on candidate miRNA/miRNAs, we opted for a hypothesis-free (unbiased) approach, which allows for the comprehensive, data-driven exploration of biological systems without pre-existing hypotheses, leading to the discovery of novel biomarkers, pathways, and mechanisms.

We first identified region-specific changes in miRNA expression levels across the three brain areas. Then, we investigated the functional roles of the dysregulated miRNAs and assessed their convergence in human and mouse limbic areas. Finally, we performed cross-species preservation analysis of miRNAs to discover conserved molecular mechanisms linked to human MDD and mouse CSS.

2. Materials and methods

2.1. Human post-mortem brain samples

Human sgACC tissue samples were provided by the Douglas-Bell Canada Brain Bank (DBCBB). All brains are donated to the Suicide section of the DBCBB by familial consent through the Quebec Coroner's Office. Human brain samples were obtained from MDD patients (N = 28) and matched sudden-death controls who have died suddenly, with no agonal period, due to work-related accidents, cardiovascular arrest or as passengers in car accidents (N = 23). All subjects were adults of French-Canadian origin and the two groups were matched according to sex, age, post-mortem interval (PMI) and tissue pH (measured from cerebellar tissue upon reception of the brain at the DBCBB). Inclusion criteria for cases and controls were I) absence of organic brain disease, II) death not caused by direct brain lesion (for instance, suicide by gunshot to the brain), and III) negative evidence of chronic inflammatory illness. Inclusion criteria only for cases were I) current (6-month) diagnosis of MDD, II) no current or lifetime history of manic and/or hypomanic episodes, III) no current or lifetime history of psychotic disorders and, IV) no evidence of psychotropic treatment. Brains underwent a process known as psychological autopsy to retrieve phenotypic information. These proxy-based interviews were supplemented with information from archival material obtained from hospitals, the Coroner's office and social services. Following the interviews, clinical vignettes were produced and assessed by a panel of clinicians to generate DSM-IV diagnostic criteria. These psychological autopsies provided sociodemographic characteristics, social developmental history, DSM-IV axis I diagnostic information and behavioral traits. This information was obtained through a variety of detailed questionnaires, such as SCID-I (Axis I disorders), SCID-II (personality disorders), family antecedent of psychiatric history, history of suicidal behavior, Brown-Goodwin

Assessment for Lifetime History of Aggression (BGLHA), Barratt Impulsiveness Scale (BIS-11), Buss-Durkee Hostility Inventory (BDHI), Temperament and Character Inventory (TCI), and Childhood Experience of Care and Abuse Questionnaire (CECA). Detailed information on substance abuse and dependence and on medication prescription was also obtained. Moreover, toxicological assessments were conducted, allowing verification of compliance to prescribed medication. The sociodemographic and clinical characteristics of cases and controls are presented in Table 1.

2.2. Chronic social stress in mice

Mice were males of the C57BL/6J strain and were aged 10-12 weeks at experiment onset. They were maintained as littermate pairs, which were allocated to either control (CON, N = 14 mice) or CSS (N = 14 mice) treatment groups according to a pseudo-random schedule. The CSS procedure leads reproducibly to increases in responsiveness to aversion and decreases in responsiveness to rewards (Madur et al., 2023; Poggi et al., 2025). Resident mice were singly caged with aggressive, ex-breeder CD-1 males; a transparent, perforated divider was placed along the length of each home cage. On each of 15 days, a BL/6 CSS (intruder) mouse was placed in the same compartment as an unfamiliar CD-1 mouse for physical attack during a cumulative total of 30-60 s or 10 min maximum, whichever occurred sooner. Thereafter, the two mice were placed either side of the divider, and remained in distal (visual, olfactory, auditory) contact for 24 h. It is essential that the stressor of CSS is not confounded by bite wounds: therefore, in addition to restricting daily attacks to 60 s, the lower incisor teeth of CD-1 mice were checked/trimmed every 3 days. Control mice were kept as littermate pairs and handled for weighing on each of the 15 days.

On the day after completion of the CSS/CON procedures, mice were deeply anaesthetized (pentobarbital) and perfused with 15 mL of cold PBS during 1 min to rinse out blood. The brain was removed from the skull and snap-frozen in isopentane at −40 °C and stored at −80 °C until further processing. Working at −20 °C, brains were sectioned coronally at 1 mm thickness using a stainless-steel brain matrix (model MMCS-1, Plastics One) and single-edge blades. With a mouse brain atlas as reference (https://mouse.brain-map.org/static/atlas), from the section at bregma +2.0 to +1.0 mm the medial prefrontal cortex (mPFC; infralimbic cortex + ventral prelimbic cortex), and from the section at −2.0 to −1.0 the amygdala, was microdissected bilaterally using a brain punch (Ø = 1 mm, model 57397, Stoelting Europe). The tissue mass of both punches per region was obtained rapidly and the samples were stored at −80 °C.

2.3. RNA isolation and miRNA sequencing

Total RNA was isolated from the human fresh frozen dissected sgACC tissue samples and from the mouse brain punches (1 mm) obtained from mPFC and BLA, using the miRNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Per sample of extractant, 100 ng total RNA was processed. Briefly, libraries were prepared for sequencing using the QIAseq® miRNA Library Kit (Qiagen, Hilden, Germany) following the standard protocol. For the quality control of cDNA libraries, Qubit™ dsDNA HS and the BioAnalyzer High Sensitivity DNA Chip were used (Agilent Biotechnology AG, Waldbronn, Germany). Then, the cDNA libraries were pooled using an equal amount of cDNA per library of each sample. Sequencing was conducted on an Illumina MiSeq System (Illumina Inc., San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 Cycles) with a configuration of sequencing depth of 2-3 million reads per sample and a single-end, 75 bp read length.

2.4. Data quality control and processing

The quality of raw sequencing data was assessed using FastQC software (v0.11.9, https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapter trimming was performed with Cutadapt (v4.4), removing (i) bases with a quality score below 20) ii) 3' (AACTGTAGGCACCATCAAT) and 5' (GTTCAGAGTTCTACAGTCCGACGATC) adapters, allowing a maximum error rate of 10%. One sample among the group of depressed patients was removed due to low quality.

Genomes for *Homo sapiens* (GRCh38, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001405.26/) and *Mus musculus* (GRCm39, https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001635.27/) were indexed with Bowtie1 (v1.2.3). For miRNA identification, mature and hairpin miRNA sequences from both *Homo sapiens* and *Mus musculus* were retrieved from miRBase and used as inputs. For human data, sequences from *Homo sapiens* database were used as the primary reference to identify known miRNAs, while those from *Mus musculus* database were included to improve the detection of conserved or novel miRNAs not annotated in human. Trimmed reads were collapsed and aligned to the genome using mapper.pl function from miRDeep2 (v2.0.1.3). Reads shorter than 18 nucleotides were discarded. Known and novel miRNAs were quantified using miRDeep2.pl function. The same pipeline was applied to mouse data, with mouse miRNAs providing the primary annotation reference, with human miRNAs included to support the detection of conserved or incompletely annotated miRNAs.

2.5. Differential expression analysis

Differential expression (DE) of miRNAs was assessed using the negative binomial model (Wald test) implemented in DESeq2 R package (v1.30.1) (Love et al., 2014).

For human samples, comparisons were performed between sgACC samples from MDD patients and controls, both by analyzing MDD as a single group and by stratifying patients based on antidepressant treatment. Covariates included post-mortem interval (PMI) and batch.

For mouse samples, comparisons were performed on mPFC and BLA samples from CSS mice and controls, with batch included as a covariate. Before analyses, 25% of the least expressed miRNAs across all samples were removed (Aass et al., 2022). To account for unwanted variation, the factors estimated by the RUVg function (RUVseq, v1.36.0) (Risso et al., 2014) were included in the model. Differentially expressed miRNAs (DEMs) were identified with a $|\text{fold-change (FC)}| > 1.2$ and an adjusted p-value (q) < 0.1.

2.6. WGCNA preservation analysis

Weighted co-expression network preservation analysis (WGCNA) was performed using the BioNERO R package (v1.10.3) (Almeida-Silva and Venancio, 2022). The human sgACC dataset was used as the training set, while mouse mPFC and BLA served as two independent test datasets. Prior to network construction, mouse miRNA sequences were assigned their corresponding human ortholog names using RNAcentral annotations (https://rnacentral.org). Before analyses, 25% of the least expressed miRNAs across all samples were removed (Aass et al., 2022). Outlier samples were identified and removed using the standardized connectivity Z.K method (Oldham et al., 2012). To mitigate the impact of hidden confounding factors and prevent spurious correlations, we performed principal component (PC)-based correction (Parsana et al., 2019). A signed hybrid network was then built using Pearson correlation. The soft-thresholding power (β) was selected based on scale-free topology, by identifying the lowest power > 0.8. Modules of co-expressed miRNAs were identified through hierarchical clustering, with a 'module_merging_threshold' of 0.8 and a 'min_module_size' of 30. The preservation of modules in the test datasets was assessed using the 'module_preservation' function from BioNERO, employing WGCNA's algorithm with 1000 permutations. Module preservation was assessed

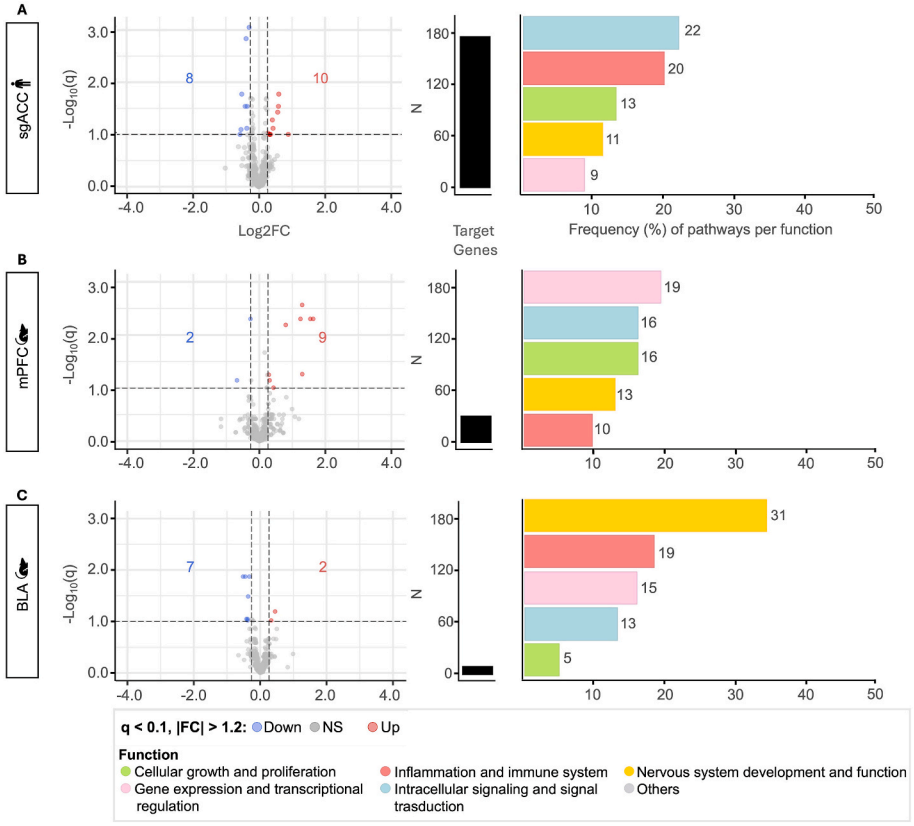

Fig. 1. Differential expression analysis of miRNAs
Volcano plots of DEMs (|FC| > 1.2, q < 0.1) in the MDD patient vs control sgACC samples (**A**), and in the CSS vs control mouse mPFC samples (**B**) and BLA samples (**C**). Histograms show the number of predicted target genes for sgACC (**A**), mPFC (**B**) and BLA samples (**C**). Bar plots show the percentage of biological pathways associated with MDD (sgACC, **A**) or CSS (mPFC and BLA, **B** and **C** respectively), classified according to their main function into cellular growth and proliferation, intracellular signaling and signal transduction, gene expression and transcriptional regulation, inflammation and immune system, nervous system development and functions, and "other" pathways.

using the Zsummary and medianRank statistics. Modules were considered highly preserved if they showed a Zsummary > 10 and a low medianRank. Modules with a Zsummary between 2 and 10 were classified as moderately preserved, while those with Zsummary < 2 were considered not preserved, regardless of their medianRank. Only highly or moderately preserved modules with a low medianRank were retained for further pathway analyses.

2.7. Target gene identification and pathway analyses

MiRWalk version 3 (http://mirwalk.umm.uni-heidelberg.de) was applied to identify miRNA target genes (Sticht et al., 2018) using DEMs and the list of miRNAs within the most preserved module of the WGCNA as input. A minimum score of 0.95 was applied to ensure high confidence in miRNA-target interactions. Only genes with predicted binding sites in the 3′ UTR were considered. Additionally, only validated genes annotated in all three databases (TargetScan, miRDB, and miRTarBase) were included. Lists of identified validated target genes were analyzed using the "Core Analysis" of the IPA software (Ingenuity System Inc, USA http://www.ingenuity.com) to understand the key biological processes they modulated. Significantly modulated pathways (p-value < 0.05) were categorized according to their primary biological function into the following groups: cellular growth and proliferation, gene expression and transcriptional regulation, inflammation and immune system, intracellular signaling and signal transduction and nervous system development and functions. Pathways involved in cancer or not related with brain functions were merged into "other" pathways.

3. Results

3.1. Altered miRNA modulation of cellular signaling and immune-related pathways in depressed human sgACC and the effect of antidepressant treatment

First, we were interested in the identification of miRNomic alterations in the sgACC of MDD patients compared to controls. Of the 1277 miRNAs detected in the sgACC miRNome, 18 (8 down- and 10 up-regulated) were differentially expressed in MDD patients compared to controls (Fig. 1A; Supplementary Table 1). MiRWalk analysis revealed that these DEMs targeted 173 validated genes (Supplementary Table 2) involved in the modulation of 321 significant pathways associated with intracellular signaling (22%), immune system (20%), cellular growth (13%), nervous system (11%), gene expression (9%) or other pathways (25%) (Fig. 1A; Supplementary Table 3).

Among the top significant biological processes involved in intracellular signaling and signal transduction, we identified *Proteasomal PSMD10 Signaling Pathway* (-log (p-value) = 7.70), *Activin Inhibin Signaling Pathway* (-log (p-value) = 5.67), *PIP3 activates AKT signaling* (-log (p-value) = 4.96) and *Antiproliferative Role of Somatostatin Receptor 2* (-log (p-value) = 4.82).

Among the most significant immune-related pathways, we found *PDGF Signaling* (-log (p-value) = 5.52), *PI3K/AKT Signaling* (-log (p-value) = 4.05), *Role of JAK1 and JAK3 in γc Cytokine Signaling* (-log (p-value) = 3.93), *Interleukin (IL)-4 and IL-13 signaling* (-log (p-value) = 3.83) and *IL-7 signaling* (-log (p-value) = 3.77).

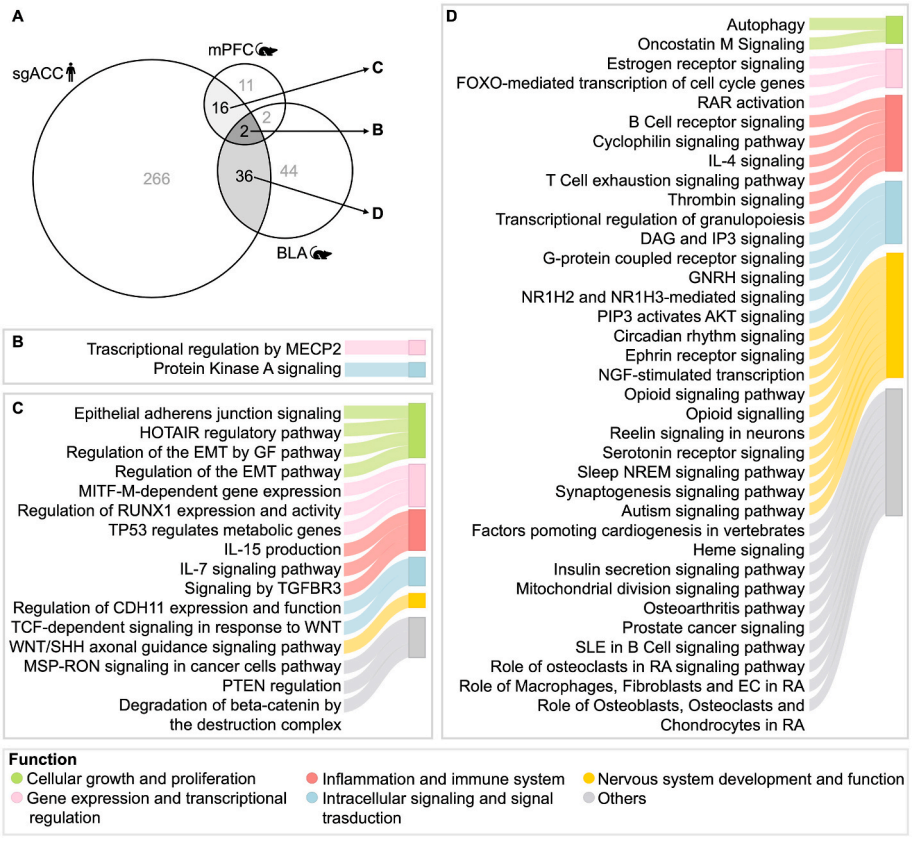

Fig. 2. Biological pathways which are common to specific brain tissues from MDD patients and CSS mice (**A**) Venn diagram of significant pathways (p-value < 0.05) across human and mouse brain regions. Sankey plots of shared pathways between (**B**) all three regions, (**C**) human sgACC and mouse mPFC, and (**D**) human sgACC and mouse BLA. Pathways were classified based on their main function into cellular growth and proliferation, intracellular signaling and signal transduction, gene expression and transcriptional regulation, inflammation and immune system, nervous system development and functions, and "other" pathways.

Considering that antidepressant treatment has been shown able to induce alterations in miRNA expression profiles, we then examined miRNomic changes in the sgACC of MDD patients, receiving or not receiving the antidepressant treatment, compared to controls. In the sgACC, antidepressant-treated MDD patients exhibited 44 DEMs (32 down- and 12 up-regulated) targeting 405 genes and 389 pathways, whereas untreated patients showed 55 DEMs (27 down- and 28 up-regulated) targeting 399 genes and 283 pathways (Supplementary Fig. 1; Supplementary Tables 1 and 2). Most of the pathways altered in both antidepressant-treated and untreated MDD patients overlapped (252 out of 389 for treated, and 252 out of 283 for untreated subjects), suggesting that these changes were more likely associated with MDD rather than the effect of antidepressants. Indeed, among the 137 pathways uniquely modulated in antidepressant-treated patients, most were associated with the immune system (26%), including *IL-7 signaling pathway* (-log (p-value) = 5.50), *PI3K/AKT Signaling* (-log (p-value) = 5.20) and *IL-15 production* (-log (p-value) = 4.43), and intracellular signaling and signal transduction (19%) as *Proteasomal PSMD10 Signaling Pathway* (-log (p-value) = 8.63), *GADD45 signaling* (-log (p-value) = 6.25) and *PIP3 activates AKT signaling* (-log (p-value) = 5.92) (Supplementary Fig. 1; Supplementary Table 3).

3.2. Altered miRNA modulation of gene expression, cellular signaling and growth pathways in CSS mouse mPFC

Following the same experimental approach applied in human samples, alterations in miRNA expression levels were investigated in CSS-exposed mice. In the mPFC, among the 856 miRNAs detected, 11 were differentially modulated by CSS exposure, 2 downregulated and 9 upregulated (Fig. 1B; Supplementary Table 1). MiRWalk analysis identified 30 genes targeted by the 11 DEMs (Supplementary Table 2). These genes were involved in the modulation of 31 pathways, 19% related to gene expression, 16% to intracellular signaling and growth, 13% to nervous system, 10% to immune system and 26% to "other" pathways (Fig. 1B; Supplementary Table 3). The most significant pathways ranked for their biological functions were *Transcriptional Regulation by MECP2* (-log (p-value) = 2.88), *TCF dependent signaling in response to WNT* (-log (p-value) = 2.60) and *Regulation of the Epithelial-Mesenchymal Transition Pathway* (-log (p-value) = 2.30).

3.3. Altered miRNA modulation of nervous system-related pathways in CSS mouse BLA

In the BLA, of the 884 miRNAs identified, 9 were differentially expressed in CSS-exposed compared to control mice, 7 downregulated and 2 upregulated (Fig. 1C; Supplementary Table 1). MiRWalk analysis identified 18 target genes (Supplementary Table 2) modulating 84 significant pathways, 31% related to nervous system, including neurotransmission- and neurodevelopment-related pathways, 19% to immune system, 15% to gene expression, 13% to intracellular signaling, 5% to cellular growth and 17% to "other" pathways (Fig. 1C; Supplementary Table 3). Among the top significant pathways related to the nervous system functioning, we found *WNT/Ca + pathway* (-log (p-value) = 3.68), *NGF-stimulated transcription* (-log (p-value) = 2.83), *Signaling by NOTCH2* (-log (p-value) = 2.72), *Sphingosine and Sphingosine-1-phosphate Metabolism* (-log (p-value) = 2.69),

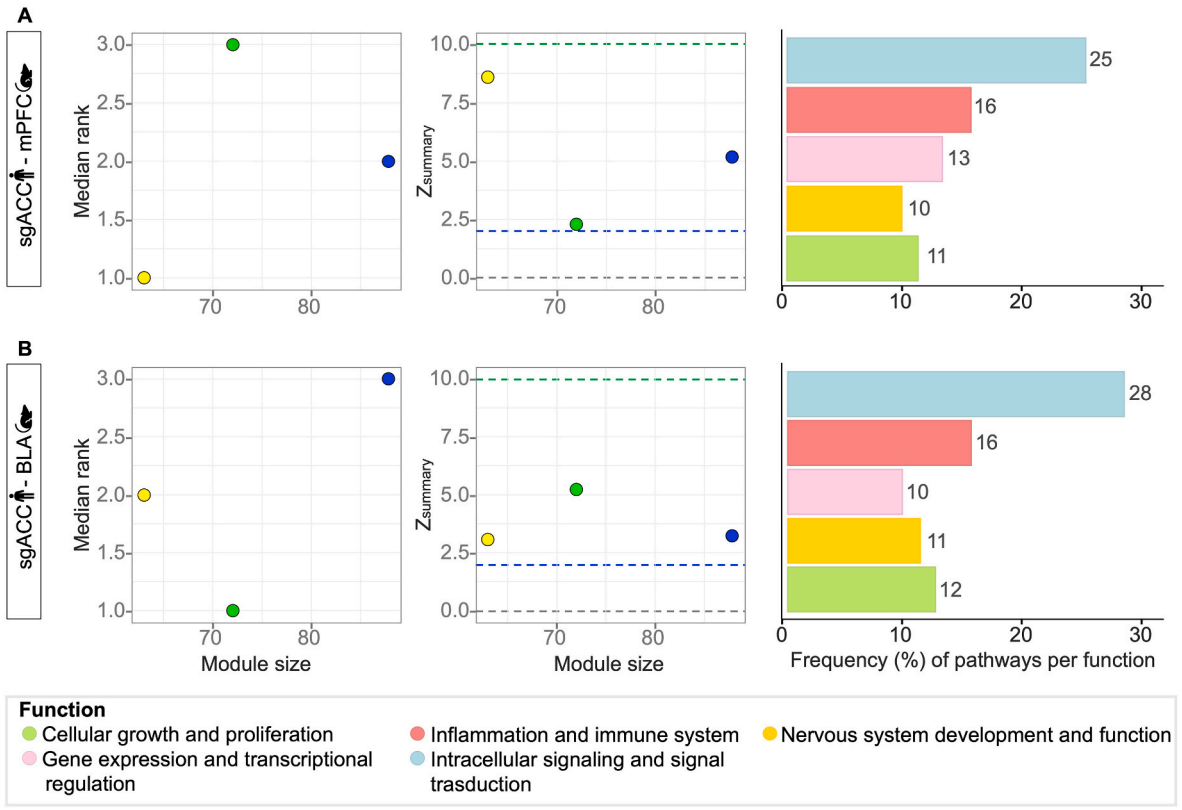

Fig. 3. WGCNA preservation analysis
Three main modules of co-expressed miRNAs (blue, green and yellow) showing the medianRank score (on the left) and the preservation score (Zsummary score) (in the middle) in the comparison between (**A**) human sgACC and mouse mPFC, and between (**B**) human sgACC and BLA. Bar plot (on the right) shows the percentage of significant biological pathways (p-value < 0.05) identified through the pathway enrichment of the most preserved module, classified according to their main biological functions.

Glutaminergic Receptor Signaling Pathway (Enhanced) (-log (p-value) = 2.67) and *Serotonin Receptor Signaling* (-log (p-value) = 2.58).

3.4. Converging biological pathways associated with differential miRNA expression in human and mouse limbic areas

To identify common pathways associated with DEMs in both the human and mouse limbic brain regions, the 321 significant pathways identified in the sgACC of all MDD patients were overlapped with the 31 and 84 pathways modulated in the mPFC and BLA, respectively, of CSS mice (Fig. 2A). Two pathways overlapped across the three tissues, *Transcriptional regulation by MECP2* and *Protein Kinase A signaling* (Fig. 2B). An additional 16 common pathways were shared between human sgACC and mouse mPFC, which included biological processes mainly involved in cellular growth and proliferation, such as *Regulation of the epithelial-mesenchymal transition (EMT) pathway*, *Epithelial adherens junction signaling*, *Regulation of the EMT by growth factors (GF) pathway* and *HOTAIR regulatory pathway* (Fig. 2C). In addition, 36 pathways were common between human sgACC and mouse BLA, including several involved in immune-related functions, such as *Cyclophilin signaling pathway*, *IL-4 signaling*, *B cell receptor signaling*, *T Cell exhaustion signaling pathway* and *Thrombin signaling*, and in neurotransmission, including *NGF-stimulated transcription*, *Serotonin receptor signaling*, *Synaptogenesis signaling pathway*, *Circadian rhythm signaling*, *Opioid signaling pathway*, *Reelin signaling in neurons* and *Opioid signaling* (Fig. 2D).

3.5. Preservation analysis to identify conserved modules in human and mouse

With the aim to dissect those biological processes regulated by DEMs and conserved between mouse and human, WGCNA was applied using the human sgACC as the training dataset and the mouse mPFC and BLA as the test datasets. Three main modules of co-expressed miRNAs were identified in the training network. In the comparison between human sgACC and mouse mPFC, one module was robustly preserved, showing the lowest medianRank (medianRank = 1.00) and a high preservation score (Zsummary = 8.94) (Fig. 3A), while comparing human sgACC and mouse BLA, another module emerged as preserved, with a medianRank of 1.00 and a Zsummary of 5.21 (Fig. 3B). Pathway enrichment of the module conserved between human sgACC and mouse mPFC indicated a strong overrepresentation of processes related to intracellular signaling (25%), with additional contributions from immune system (16%), gene expression (13%), nervous system-related pathways (10%), cellular growth (11%) and "other" functions (36%) (Fig. 3A; Supplementary Table 4**)**. The module preserved between human sgACC and mouse BLA included biological processes mainly involved in intracellular signaling (28%), immune system (16%), cellular growth (12%), nervous system-related pathways (11%), gene expression (10%), and "other" functions (36%) (Fig. 3B; Supplementary Table 4).

3.6. Integration of differential expression analysis with the co-expression networks for biological pathway prioritization

Next, by intersecting the results from the differential expression pathway analysis with those obtained from the two most preserved modules, we aimed to identify conserved biological pathways across bioinformatic methods and the two species. *Transcriptional regulation by MECP2* and *Protein Kinase A signaling* were consistently conserved across all three brain regions (sgACC, mPFC, and BLA) and detected by both analytic bioinformatics approaches (Fig. 4). Thirteen pathways were common only to human sgACC and mouse mPFC and included: cellular growth-related pathways, such as *HOTAIR regulatory pathway*,

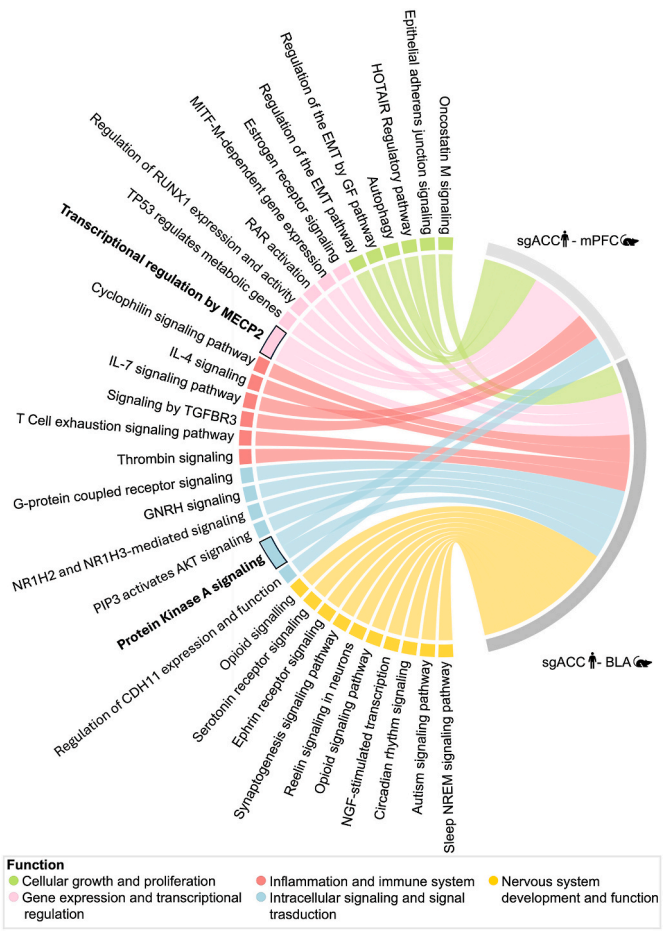

Fig. 4. Integration of differential expression analysis and co-expression networks

The cord diagram shows the connection among the pathways of the three limbic brain regions (human sgACC, mouse mPFC and BLA). Each pathway is represented by a fragment on the outer part of the circular layout and by the colour corresponding to its biological function. Pathways were classified according to their main function into cellular growth and proliferation, intracellular signaling and signal transduction, gene expression and transcriptional regulation, inflammation and immune system and nervous system development. We excluded "other" pathways, such as those not related to brain functions, from the representation.

Regulation of the EMT Pathway, *Regulation of the EMT by GF Pathway*, *Epithelial adherens junction signaling;* intracellular signaling-related processes, such as *Regulation of CDH11 expression and function*; pathways involved in gene expression, such as *TP53 regulates metabolic genes*, *MITF-M-dependent gene expression*, *Regulation of RUNX1 expression and activity*; and inflammation and immune system-related pathways, such as *Signaling by TGFBR3* and *IL-7 signaling pathway*. For human sgACC and mouse BLA, we found 24 shared pathways including those related to the nervous system, such as *Serotonin receptor signaling*, *Synaptogenesis signaling pathway*, *Circadian rhythm signaling*; immune system, as *IL-4 Signaling*, gene expression, such as *Estrogen receptor signaling*; and intracellular signaling such as *NR1H2 and NR1H3-mediated signaling* and *PIP3 activates AKT signaling* (Fig. 4).

4. Discussion

In the current study, a miRNomic approach was applied to investigate for miRNA signatures in the sgACC of MDD patients and in the mPFC and BLA of CSS-exposed mice compared to the respective controls. The key finding is the identification of conserved MDD/CSS miRNA signatures and associated biological processes across species.

4.1. Species- and region-specific miRNA changes and affected biological pathways

Firstly, we identified region-specific changes in miRNA expression levels (across the two species x three brain regions) associated with MDD/CSS. Analyses of the human sgACC revealed a dysregulation of miR-34a-5p, miR-17-5p, and miR-142-3p, consistent with their involvement in stress-related neurobiological alterations in MDD (Chauhan and Jain, 2025; Mizohata et al., 2021; Van der Auwera et al., 2019). In the mouse mPFC, we identified several DEMs, including miR-34b-5p, miR-34c-5p, miR-375-3p, miR-200a-3p, miR-322-5p, miR-204-5p, which in recent studies have been suggested as regulators of genes in pathways underlying the regulation of stress-related behavior, such as fear memory, anxiety, and impulsivity (Andolina et al., 2016; Chen et al., 2025; Guan et al., 2024; Liao et al., 2025; McKibben and Dwivedi, 2021; Zhan et al., 2021). Among DEMs identified in the BLA of CSS mice, we only confirmed the differential expression of mmu-miR-872-5p, reported to be modulated by chronic stress in the ventral tegmental area and PFC of chronic mild stress-exposed rats (Zurawek et al., 2017).

Next, we investigated the functional roles of such dysregulated miRNAs. In the human sgACC, we observed the modulation of intracellular signaling- and immune-related pathways. Regarding intracellular signaling processes, previous studies have shown alterations in *Proteosomal PSMD10 Signaling pathway* and *PIP3 activates PSMD10 Signaling pathway*, known to activate the PI3K/AKT/mTOR signaling pathway, which is often inhibited in MDD patients, contributing to reduced neurogenesis and synaptic dysfunction (Chen et al., 2024). Collectively, these findings suggest that a dysregulation of intracellular signaling pathways may contribute to the synaptic and neurogenic alterations observed in MDD (Li et al., 2023; Sahu et al., 2019; Zhang et al., 2017).

Also for immune-related pathways, our results show the modulation of several biological processes linked to cytokine production, such as *Role of JAK1 and JAK3 in γc Cytokine signaling, Interleukin-4 and 13 signaling* and *IL-7 signaling*, all in line with previous studies (Du et al., 2024; Gong et al., 2025; Strawbridge et al., 2023; Yin et al., 2024). Indeed, one major hypothesis underlying MDD is that it involves an overactive immune response, characterized by the upregulation of inflammatory mediators that can activate different components of the immune system, including B and T lymphocytes, leading to mood, emotional and cognitive dysfunctions (Yin et al., 2024).

Stratifying the human sgACC MDD cohort by antidepressant treatment and comparing these findings with the overall miRNA profile of all MDD patients revealed that the most functional modulation was driven by MDD rather than drug-related effects. This was suggested by the huge number of overlapping pathways between antidepressant treated and untreated patients. Of note, we found a modulation of IL-15 and serotonin receptor–associated mechanisms, consistent with previous evidence indicating that this cytokine, involved in both innate and adaptive immunity, may also influence brain functions. Experimental evidence has indicated that IL-15 can modulate both GABAergic and serotonergic transmission, potentially contributing to the regulation of affective and cognitive processes (Pan et al., 2013). In patients with moderate MDD, increased IL-15 levels were observed after treatment with the antidepressant escitalopram (Bleibel et al., 2025). In addition, a reduction in serotonin uptake and a depressive-like phenotype have been found in IL15Rα knockout mice (Wu et al., 2011). These results suggest that IL-15 could interact with serotonergic signaling, potentially contributing to the complex neuroimmune mechanisms involved in MDD and in the antidepressant response. However, the molecular basis of this

interaction remains to be clarified, and further studies are warranted.

Several highly relevant studies have been conducted in post-mortem brains obtained from MDD (Nagy et al., 2020) and post-traumatic stress disorder (PTSD) patients by comparing proteomics, transcriptomics and miRNomics profiles of different brain regions, by taking into account cell-type-specific contributions (Daskalakis et al., 2024; Hwang et al., 2025; Wang et al., 2025). Interestingly, the results coming from these studies are in line with our results, since they pointed to the dysregulation of immune function, neuronal and synaptic regulation, including GABAergic transmission, and the glucocorticoid signalling in both MDD and PTSD, suggesting shared molecular pathology (Daskalakis et al., 2024; Hwang et al., 2025; Nagy et al., 2020; Wang et al., 2025).

The results of pathway analyses conducted in the mPFC and BLA of CSS-exposed mice are in line with the preclinical literature data showing that CSS exposure induced alterations in inflammatory processes, G-protein coupled receptors and the nervous system functioning, with expression of genes important in dopamine function in the latter (Azzinnari et al., 2014; Poggi et al., 2025). Accordingly, we found alterations of pathways involved in gene expression, intracellular signaling and growth in mPFC, and involved in neurotransmission (i.e. *NGF-stimulated transcription, Glutaminergic* and *Serotonin Receptor Signaling),* as well as in neurodevelopment, such as *WNT/Ca + pathway,* in BLA. Interestingly, our findings could support recent results suggesting the existence of persistent neuronal adaptations in the PFC and BLA of socially defeated mice (Colyn et al., 2019).

4.2. Common biological processes among human and mouse limbic brain regions

Then, we investigated the functional convergence of dysregulated miRNAs in the limbic regions of both humans and mice as we were interested in identifying common biological process(es) among the three datasets (human sgACC, mouse mPFC and mouse BLA). Overall, we found that human sgACC shared pathways involved in cellular growth and gene expression with the mouse mPFC, while pathways associated with nervous system development and immune response were shared with the mouse BLA. Notably, *Transcriptional regulation by MECP2* and *Protein Kinase A signaling* emerged as the common biological processes across all datasets, suggesting possible shared molecular mechanisms through which MDD/CSS-related miRNA alterations might influence MDD/CSS-relevant neurobiological processes across species. MECP2 (methyl CpG binding protein 2) is a transcriptional regulator, highly abundant in the brain, which modulates gene expression in response to environmental stimuli (Gulmez Karaca et al., 2019), and although mainly studied in neurodevelopmental disorders, above all in Rett syndrome, altered expression of this gene has been also reported in stress-related disorders, including MDD (Abellan-Alvaro et al., 2021; Sanchez-Lafuente et al., 2022). In fact, MECP2 regulates several physiological functions implicated in neuronal development and adult synaptic plasticity, such as the transcriptional activation of brain derived neurotrophic factor (BDNF) and reelin (RELN), well-known genes involved in mood disorders. Interestingly, MECP2 phosphorylation at serine 421 in the rodent hippocampus is essential for ketamine's sustained antidepressant effect, and decreased blood level of MECP2 protein has been reported for MDD (Su et al., 2015). In addition, differential expression levels of MECP2 were observed between stress-resilient and susceptible mice following chronic social defeat stress (Huang et al., 2022), although it is important to note that the CSS procedure used in the current study does not yield sub-groups of resilient and susceptible mice. Regarding the other common pathway, *Protein Kinase A signaling*, protein kinase A (PKA) is a central component of the adenylyl cyclase-cyclic AMP (AC-cAMP) pathway, that plays a crucial role in synaptic plasticity, gene transcription, and the hypothalamic-pituitary-adrenal (HPA) axis, which is a major contributor to the body's stress response. Abnormalities in PKA activity and its downstream signaling have been observed in individuals affected by MDD, and it has been proposed that targeting this pathway with antidepressant drugs may offer therapeutic benefits (Gao, F. et al., 2022).

4.3. Integration of differential expression data with co-expression network analysis

To refine these findings, we integrated differential expression data with co-expression network analysis to prioritize pathways across species.

This analysis alone highlighted conserved biological processes related to intracellular signaling and the immune system, including *PDGF Signaling* and *Proteasomal PSMD10 Signaling Pathway*, which have already been associated with MDD (Li et al., 2023; Sahu et al., 2019). Crucially, it also revealed that two pathways, *Transcriptional regulation by MECP2* and *Protein Kinase A* signaling were consistently conserved across species, brain regions and both approaches, supporting the relevance of these biological systems as potential targets for further investigation in the context of MDD.

4.4. Limitations of the study

Despite its strength, this study also has limitations that need to be acknowledged. Firstly, the relatively modest sample sizes for both human and animal analyses. Second, the target-gene validation analysis did not take into account the gene expression modulation, making it impossible to determine whether a specific pathway was activated or inhibited. Therefore, further studies are needed to better clarify if *Transcriptional regulation by MECP2* and *Protein Kinase A signaling* are activated or inhibited in depressed patients and in CSS mice. Third, with this sample, it was not possible to assess potential changes caused specifically by suicide and to classify subjects by specific antidepressant compounds or pharmacological classes, as sample sizes within each category would be insufficient for reliable inference**.** Lastly, although females were included in the human MDD cohort, they were not included in the mouse CSS cohort, given that the CSS paradigm is only effective in males. However, the main function of such an animal paradigm is to investigate for causality between chronic psychosocial stress and neurobiological states, to augment, substantially, any descriptive association between a stress-related neuropsychiatric condition and neurobiological factors in human subjects. When viewed in this context, the incorporation of cause-effect animal data for one sex, male or female, constitutes a major attribute of the study. In mice, natural territorial aggression, and therefore psychosocial stress, only pertains between males, hence the CSS paradigm is male-specific. As such, it enables the causal study of stress effects on miRNAs in specific brain regions, but not of whether there are sex differences therein. Various paradigms have been developed to induce aggression by resident male mice towards female mice (Takahashi A et al., 2017; Harris AZ et al., 2018; Yohn CN et al., 2019).

5. Conclusions

Overall, our results identify the modulation of intracellular signaling- and immune system-related pathways in the sgACC of MDD patients and of intracellular signaling and nervous system-related processes in the mPFC and BLA, respectively, of CSS mice. In parallel, the cross-species preservation analysis revealed a predominant modulation of pathways involved in intracellular signaling. The interfacing of DESeq2 and WGCNA-based analyses identified that *Transcriptional regulation by MECP2* and *Protein Kinase A signaling* were the two pathways conserved across all three brain regions (human sgACC, mouse mPFC and BLA) and detected by both analytic approaches. Combining analyses between human sgACC and mouse mPFC revealed the involvement of cellular growth-related pathways, whereas those between human sgACC and mouse BLA suggested the key roles of neurotransmission pathways.

Although future studies are necessary, our findings suggest that genes associated with the pathways, *Transcriptional regulation by MECP2* and *Protein Kinase A signaling*, are of interest in terms of targeting by novel intervention strategies. Finally, our findings derived from the study of miRNomics profiles in different brain regions and in two species confirm the dysregulation of intracellular signaling, immune, neuronal and synaptic functions in MDD.

Funding

This study was supported by ERA-NET Neuron 2019 (MOOD-MARKER) to M.O., C.P., N.M. and N.C. This study was also supported by Ricerca Corrente (Italian Ministry of Health, MoH) to N.C., M.M. and A. C.

CRediT authorship contribution statement

Samantha Saleri: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Moira Marizzoni:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Patrizia Genini:** Investigation, Methodology, Writing – review & editing. **Chiara Galbiati:** Investigation, Methodology, Writing – review & editing. **Annamaria Cattaneo:** Conceptualization, Investigation, Writing – review & editing. **Gustavo Turecki:** Conceptualization, Investigation, Writing – review & editing. **Henning Grosskopf:** Conceptualization, Investigation, Writing – review & editing. **Petra Steinacker:** Conceptualization, Investigation, Writing – review & editing. **Markus Otto:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Giulia Poggi:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Christopher R Pryce:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Naguib Mechawar:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. **Nadia Cattane:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

NA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ynstr.2026.100816.

Data availability

Data will be made available on request.

References

Aass, K.R., Nedal, T.M.V., Tryggestad, S.S., Haukas, E., Slordahl, T.S., Waage, A., Standal, T., Mjelle, R., 2022. Paired miRNA- and messenger RNA-sequencing identifies novel miRNA-mRNA interactions in multiple myeloma. Sci. Rep. 12 (1), 12147.

Abellan-Alvaro, M., Stork, O., Agustin-Pavon, C., Santos, M., 2021. MeCP2 haplodeficiency and early-life stress interaction on anxiety-like behavior in adolescent female mice. J. Neurodev. Disord. 13 (1), 59.

Almeida-Silva, F., Venancio, T.M., 2022. BioNERO: an all-in-one R/Bioconductor package for comprehensive and easy biological network reconstruction. Funct. Integr. Genom. 22 (1), 131–136.

Andolina, D., Di Segni, M., Bisicchia, E., D'Alessandro, F., Cestari, V., Ventura, A., Concepcion, C., Puglisi-Allegra, S., Ventura, R., 2016. Effects of lack of microRNA-34 on the neural circuitry underlying the stress response and anxiety. Neuropharmacology 107, 305–316.

Azzinnari, D., Sigrist, H., Staehli, S., Palme, R., Hildebrandt, T., Leparc, G., Hengerer, B., Seifritz, E., Pryce, C.R., 2014. Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function. Neuropharmacology 85, 328–341.

Bakunina, N., Pariante, C.M., Zunszain, P.A., 2015. Immune mechanisms linked to depression via oxidative stress and neuroprogression. Immunology 144 (3), 365–373.

Bleibel, L., Sokolowska, P., Henrykowska, G., Owczarek, J., Wiktorowska-Owczarek, A., 2025. Unveiling the anti-inflammatory effects of antidepressants: a systematic review of human studies over the last decade. Pharmaceuticals 18 (6).

Chand, S.P., Arif, H., 2025. Depression, StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Hasan Arif Declares No Relevant Financial Relationships with Ineligible Companies.

Chauhan, A., Jain, C.K., 2025. Exploring gene regulatory interaction networks and predicting therapeutic molecules among major depressive disorder and bipolar disorder: a bioinformatics approach. J. Affect. Disord. 375, 64–74.

Chen, X., Gan, Y., Zhang, K., Wu, Y., Li, Y., Lan, T., Zhuang, X., Chen, S., Yu, S., 2025. MicroRNA-204-5p deficiency within the vmPFC Region contributes to neuroinflammation and behavioral disorders via the JAK2/STAT3 signaling pathway in rats. Adv. Sci. 12 (10), e2403080.

Chen, Y., Guan, W., Wang, M.L., Lin, X.Y., 2024. PI3K-AKT/mTOR signaling in psychiatric disorders: a valuable target to stimulate or suppress? The international journal of neuropsychopharmacology 27 (2).

Colyn, L., Venzala, E., Marco, S., Perez-Otano, I., Tordera, R.M., 2019. Chronic social defeat stress induces sustained synaptic structural changes in the prefrontal cortex and amygdala. Behav. Brain Res. 373, 112079.

Cui, L., Li, S., Wang, S., Wu, X., Liu, Y., Yu, W., Wang, Y., Tang, Y., Xia, M., Li, B., 2024. Major depressive disorder: hypothesis, mechanism, prevention and treatment. Signal Transduct. Targeted Ther. 9 (1), 30.

Daskalakis, N.P., Iatrou, A., Chatzinakos, C., Jajoo, A., Snijders, C., Wylie, D., DiPietro, C. P., Tsatsani, I., Chen, C.Y., Pernia, C.D., Soliva-Estruch, M., Arasappan, D., Bharadwaj, R.A., Collado-Torres, L., Wuchty, S., Alvarez, V.E., Dammer, E.B., Deep-Soboslay, A., Duong, D.M., Eagles, N., Huber, B.R., Huuki, L., Holstein, V.L., Logue, M.W., Lugenbuhl, J.F., Maihofer, A.X., Miller, M.W., Nievergelt, C.M., Pertea, G., Ross, D., Sendi, M.S.E., Sun, B.B., Tao, R., Tooke, J., Wolf, E.J., Zeier, Z., , Consortium, P.W.G.o.P.G., Berretta, S., Champagne, F.A., Hyde, T., Seyfried, N.T., Shin, J.H., Weinberger, D.R., Nemeroff, C.B., Kleinman, J.E., Ressler, K.J., Consortium, P.W.G.o.P.G., 2024. Systems biology dissection of PTSD and MDD across brain regions, cell types, and blood. Science 384 (6698) eadh3707.

Ding, R., Su, D., Zhao, Q., Wang, Y., Wang, J.Y., Lv, S., Ji, X., 2023. The role of microRNAs in depression. Front. Pharmacol. 14, 1129186.

Dion-Albert, L., Cadoret, A., Doney, E., Kaufmann, F.N., Dudek, K.A., Daigle, B., Parise, L. F., Cathomas, F., Samba, N., Hudson, N., Lebel, M., Signature, C., Campbell, M., Turecki, G., Mechawar, N., Menard, C., 2022. Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. Nat. Commun. 13 (1), 164.

Du, Y., Dou, Y., Wang, M., Wang, Y., Yan, Y., Fan, H., Fan, N., Yang, X., Ma, X., 2024. Efficacy and acceptability of anti-inflammatory agents in major depressive disorder: a systematic review and meta-analysis. Front. Psychiatr. 15, 1407529.

Gao, F., Yang, S., Wang, J., Zhu, G., 2022. cAMP-PKA cascade: an outdated topic for depression? Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 150, 113030.

Gao, Y.N., Zhang, Y.Q., Wang, H., Deng, Y.L., Li, N.M., 2022. A new player in depression: MiRNAs as modulators of altered synaptic plasticity. Int. J. Mol. Sci. 23 (9).

Goldstein-Piekarski, A.N., Ball, T.M., Samara, Z., Staveland, B.R., Keller, A.S., Fleming, S. L., Grisanzio, K.A., Holt-Gosselin, B., Stetz, P., Ma, J., Williams, L.M., 2022. Mapping neural circuit biotypes to symptoms and behavioral dimensions of depression and anxiety. Biol. Psychiatry 91 (6), 561–571.

Gong, H., Su, W.J., Deng, S.L., Luo, J., Du, Z.L., Luo, Y., Lv, K.Y., Zhu, D.M., Fan, X.T., 2025. Anti-inflammatory interventions for the treatment and prevention of depression among older adults: a systematic review and meta-analysis. Transl. Psychiatry 15 (1), 114.

Guan, W., Wu, X.Y., Jin, X., Sheng, X.M., Fan, Y., 2024. miR-204-5p plays a critical role in the pathogenesis of depression and anti-depression action of venlafaxine in the hippocampus of mice. Curr. Med. Chem. 31 (22), 3412–3425.

Gulmez Karaca, K., Brito, D.V.C., Oliveira, A.M.M., 2019. MeCP2: a critical regulator of chromatin in neurodevelopment and adult brain function. Int. J. Mol. Sci. 20 (18).

Harris, A.Z., Atsak, P., Bretton, Z.H., Holt, E.S., Alam, R., Morton, M.P., Abbas, A.I., Leonardo, E.D., Bolkan, S.S., Hen, R., Gordon, J.A., 2018. A novel method for chronic social defeat stress in female mice. Neuropsychopharmacology 43 (6), 1276–1283.

Huang, L., He, L., Ma, R., Ding, W., Zhou, C., Lin, S., Zhang, J.C., Chen, J., Yao, W., 2022. The role of MeCP2 and the BDNF/TrkB signaling pathway in the stress resilience of mice subjected to CSDS. Psychopharmacology 239 (9), 2921–2929.

Hwang, A., Skarica, M., Xu, S., Coudriet, J., Lee, C.Y., Lin, L., Terwilliger, R., Sliby, A.N., Wang, J., Nguyen, T., Li, H., Wu, M., Dai, Y., Duan, Z., Srinivasan, S.S., Zhang, X., Lin, Y., Cruz, D., Deans, P.J.M., Traumatic Stress Brain Research, G., Huber, B.R., Levey, D., Glausier, J.R., Lewis, D.A., Gelernter, J., Holtzheimer, P.E., Friedman, M. J., Gerstein, M., Sestan, N., Brennand, K.J., Xu, K., Zhao, H., Krystal, J.H., Young, K. A., Williamson, D.E., Che, A., Zhang, J., Girgenti, M.J., 2025. Single-cell transcriptomic and chromatin dynamics of the human brain in PTSD. Nature 643 (8072), 744–754.

Li, H.H., Liu, Y., Chen, H.S., Wang, J., Li, Y.K., Zhao, Y., Sun, R., He, J.G., Wang, F., Chen, J.G., 2023. PDGF-BB-Dependent neurogenesis buffers depressive-like behaviors by inhibition of GABAergic projection from medial septum to dentate gyrus. Adv. Sci. 10 (22), e2301110.
Liao, J., Liu, J., Zhou, Y., Shi, L., Chen, Y.J., Guo, S., Zhang, C.Y., Liu, X.Y., Tao, W.Q., Xiang, J.J., Yang, L., Liu, G., Wang, W., Kuang, L., Ran, L.Y., 2025. L1CAM(+) extracellular vesicles derived from the serum of adolescents with major depressive disorder induce depression-like phenotypes in adolescent mice. J. Affect. Disord. 375, 180–191.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15 (12), 550.
Madur, L., Ineichen, C., Bergamini, G., Greter, A., Poggi, G., Cuomo-Haymour, N., Sigrist, H., Sych, Y., Paterna, J.C., Bornemann, K.D., Viollet, C., Fernandez-Albert, F., Alanis-Lobato, G., Hengerer, B., Pryce, C.R., 2023. Stress deficits in reward behaviour are associated with and replicated by dysregulated amygdala-nucleus accumbens pathway function in mice. Commun. Biol. 6 (1), 422.
McEwen, B.S., Nasca, C., Gray, J.D., 2016. Stress effects on neuronal structure: hippocampus, Amygdala, and prefrontal cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 41 (1), 3–23.
McKibben, L.A., Dwivedi, Y., 2021. MicroRNA regulates early-life stress-induced depressive behavior via serotonin signaling in a sex-dependent manner in the prefrontal cortex of rats. Biological psychiatry global open science 1 (3), 180–189.
Mizohata, Y., Toda, H., Koga, M., Saito, T., Fujita, M., Kobayashi, T., Hatakeyama, S., Morimoto, Y., 2021. Neural extracellular vesicle-derived miR-17 in blood as a potential biomarker of subthreshold depression. Hum. Cell 34 (4), 1087–1092.
Nagy, C., Maitra, M., Tanti, A., Suderman, M., Theroux, J.F., Davoli, M.A., Perlman, K., Yerko, V., Wang, Y.C., Tripathy, S.J., Pavlidis, P., Mechawar, N., Ragoussis, J., Turecki, G., 2020. Single-nucleus transcriptomics of the prefrontal cortex in major depressive disorder implicates oligodendrocyte precursor cells and excitatory neurons. Nat. Neurosci. 23 (6), 771–781.
O'Brien, J., Hayder, H., Zayed, Y., Peng, C., 2018. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 9, 402.
Oldham, M.C., Langfelder, P., Horvath, S., 2012. Network methods for describing sample relationships in genomic datasets: application to Huntington's disease. BMC Syst. Biol. 6, 63.
Ortega, M.A., Alvarez-Mon, M.A., Garcia-Montero, C., Fraile-Martinez, O., Lahera, G., Monserrat, J., Munoz-Merida, L., Mora, F., Rodriguez-Jimenez, R., Fernandez-Rojo, S., Quintero, J., Alvarez-Mon, M., 2021. MicroRNAs as critical biomarkers of major depressive disorder: a comprehensive perspective. Biomedicines 9 (11).
Osimo, E.F., Pillinger, T., Rodriguez, I.M., Khandaker, G.M., Pariante, C.M., Howes, O.D., 2020. Inflammatory markers in depression: a meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. Brain Behav. Immun. 87, 901–909.
Pan, W., Wu, X., He, Y., Hsuchou, H., Huang, E.Y., Mishra, P.K., Kastin, A.J., 2013. Brain interleukin-15 in neuroinflammation and behavior. Neurosci. Biobehav. Rev. 37 (2), 184–192.
Parsana, P., Ruberman, C., Jaffe, A.E., Schatz, M.C., Battle, A., Leek, J.T., 2019. Addressing confounding artifacts in reconstruction of gene co-expression networks. Genome Biol. 20 (1), 94.
Pizzagalli, D.A., 2014. Depression, stress, and anhedonia: toward a synthesis and integrated model. Annu. Rev. Clin. Psychol. 10, 393–423.
Poggi, G., Portales, A., Robert, M., Hofer, C., Schmid, S., Kukelova, D., Sigrist, H., Just, S., Hengerer, B., Pryce, C.R., 2025. Chronic social stress induces generalized hypersensitivity to aversion: a mouse model with translational validity for understanding and treating negative valence disorders. Neuropharmacology 273, 110430.
Rajmohan, V., Mohandas, E., 2007. The limbic system. Indian J. Psychiatry 49 (2), 132–139.
Risso, D., Ngai, J., Speed, T.P., Dudoit, S., 2014. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat. Biotechnol. 32 (9), 896–902.
Roy, B., Yoshino, Y., Allen, L., Prall, K., Schell, G., Dwivedi, Y., 2020. Exploiting circulating MicroRNAs as biomarkers in psychiatric disorders. Mol. Diagn. Ther. 24 (3), 279–298.
Ruiz, N.A.L., Del Angel, D.S., Brizuela, N.O., Peraza, A.V., Olguin, H.J., Soto, M.P., Guzman, D.C., 2022. Inflammatory process and immune System in major depressive disorder. The international journal of neuropsychopharmacology 25 (1), 46–53.
Sahu, I., Nanaware, P., Mane, M., Mulla, S.W., Roy, S., Venkatraman, P., 2019. Role of a 19S proteasome Subunit- PSMD10(Gankyrin) in neurogenesis of human neural progenitor cells. Int. J. Stem Cells 12 (3), 463–473.
Sanchez-Lafuente, C.L., Kalynchuk, L.E., Caruncho, H.J., Ausio, J., 2022. The role of MeCP2 in regulating synaptic plasticity in the context of stress and depression. Cells 11 (4).
Spellman, T., Liston, C., 2020. Toward circuit mechanisms of pathophysiology in depression. Am. J. Psychiatry 177 (5), 381–390.
Sticht, C., De La Torre, C., Parveen, A., Gretz, N., 2018. miRWalk: an online resource for prediction of microRNA binding sites. PLoS One 13 (10), e0206239.
Strawbridge, R., Izurieta, E., Day, E., Tee, H., Young, K., Tong, C.C., Young, A.H., Cleare, A.J., 2023. Peripheral inflammatory effects of different interventions for treatment-resistant depression: a systematic review. Neuroscience applied 2, 101014.
Su, M., Hong, J., Zhao, Y., Liu, S., Xue, X., 2015. MeCP2 controls hippocampal brain-derived neurotrophic factor expression via homeostatic interactions with microRNA-132 in rats with depression. Mol. Med. Rep. 12 (4), 5399–5406.
Suda, K., Matsuda, K., 2022. How microbes affect depression: underlying mechanisms via the gut-brain axis and the modulating role of probiotics. Int. J. Mol. Sci. 23 (3).
Takahashi, A., Chung, J.R., Zhang, S., Zhang, H., Grossman, Y., Aleyasin, H., Flanigan, M. E., Pfau, M.L., Menard, C., Dumitriu, D., Hodes, G.E., McEwen, B.S., Nestler, E.J., Han, M.H., Russo, S.J., 2017. Establishment of a repeated social defeat stress model in female mice. Sci. Rep. 7 (1), 12838.
Van der Auwera, S., Ameling, S., Wittfeld, K., d'Harcourt Rowold, E., Nauck, M., Volzke, H., Suhre, K., Najafi-Shoushtari, H., Methew, J., Ramachandran, V., Bulow, R., Volker, U., Grabe, H.J., 2019. Association of childhood traumatization and neuropsychiatric outcomes with altered plasma micro RNA-levels. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 44 (12), 2030–2037.
Wang, J., Liu, Y., Li, H., Nguyen, T.P., Soto-Vargas, J.L., Wilson, R., Wang, W., Lam, T.T., Zhang, C., Lin, C., Traumatic Stress Brain Research, G., Lewis, D.A., Glausier, J., Holtzheimer, P.E., Friedman, M.J., Williams, K.R., Picciotto, M.R., Nairn, A.C., Krystal, J.H., Duman, R.S., Young, K.A., Zhao, H., Girgenti, M.J., 2025. A multi-omic approach implicates novel protein dysregulation in post-traumatic stress disorder. Genome Med. 17 (1), 43.
Wu, X., Hsuchou, H., Kastin, A.J., He, Y., Khan, R.S., Stone, K.P., Cash, M.S., Pan, W., 2011. Interleukin-15 affects serotonin system and exerts antidepressive effects through IL15Ralpha receptor. Psychoneuroendocrinology 36 (2), 266–278.
Yin, Y., Ju, T., Zeng, D., Duan, F., Zhu, Y., Liu, J., Li, Y., Lu, W., 2024. "Inflamed" depression: a review of the interactions between depression and inflammation and current anti-inflammatory strategies for depression. Pharmacol. Res. 207, 107322.
Yohn, C.N., Dieterich, A., Bazer, A.S., Maita, I., Giedraitis, M., Samuels, B.A., 2019. Chronic non-discriminatory social defeat is an effective chronic stress paradigm for both male and female mice. Neuropsychopharmacology 44 (13), 2220–2229.
Zhan, Y., Han, J., Xia, J., Wang, X., 2021. Berberine suppresses mice depression behaviors and promotes hippocampal neurons growth through regulating the miR-34b-5p/miR-470-5p/BDNF axis. Neuropsychiatric Dis. Treat. 17, 613–626.
Zhang, J.C., Yao, W., Qu, Y., Nakamura, M., Dong, C., Yang, C., Ren, Q., Ma, M., Han, M., Shirayama, Y., Hayashi-Takagi, A., Hashimoto, K., 2017. Increased EphA4-ephexin1 signaling in the medial prefrontal cortex plays a role in depression-like phenotype. Sci. Rep. 7 (1), 7133.
Zurawek, D., Kusmider, M., Faron-Gorecka, A., Gruca, P., Pabian, P., Solich, J., Kolasa, M., Papp, M., Dziedzicka-Wasylewska, M., 2017. Reciprocal MicroRNA expression in mesocortical circuit and its interplay with serotonin transporter define resilient rats in the chronic mild stress. Mol. Neurobiol. 54 (8), 5741–5751.