Pharmacological Research 219 (2025) 107894

Contents lists available at ScienceDirect

Pharmacological Research

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

Effect of ketamine and esketamine on RNA expression and its relevance for depression: A systematic review

Claudia Pisanu [a], Rosana Carvalho Silva [b], Mattia Meattini [b], Massimo Gennarelli [b,c], Bernhard T. Baune [d,e,f], Alessio Squassina [a,g], Alessandra Minelli [b,c,*]

[a] Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, 09124, Italy
[b] Department of Molecular and Translational Medicine, University of Brescia, Brescia 25123, Italy
[c] Genetics Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia 25125, Italy
[d] Department of Psychiatry and Psychotherapy, University of Münster, Münster 48149, Germany
[e] Department of Psychiatry, Melbourne Medical School, University of Melbourne, Parkville, VIC 3010, Australia
[f] The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC 3052, Australia
[g] Department of Psychiatry, Dalhousie University, Halifax, Nova Scotia B3H 2E2, Canada

ARTICLE INFO

Keywords:
RNA expression
Ketamine
Esketamine
Systematic review
Biomarkers
Depression
Transcription

ABSTRACT

Treatment-resistant depression (TRD) remains a challenge in psychiatry, necessitating novel therapeutic strategies beyond traditional monoaminergic antidepressants. Ketamine and its S-enantiomer esketamine have demonstrated rapid and robust antidepressant effects in TRD, probably through mechanisms involving glutamatergic modulation, neuroplasticity, and anti-inflammatory properties. However, the molecular underpinnings of these effects are not yet understood. This systematic review aimed to synthesize evidence from human and in vitro studies evaluating transcriptional changes associated with ketamine and esketamine treatment, to identify potential biomarkers and clarify molecular pathways relevant to their antidepressant properties. A systematic search conducted on PubMed and Scopus identified 12 studies assessing RNA expression following ketamine or esketamine treatment in patients with unipolar or bipolar depression or in human-derived cell models. Eligibility and quality were evaluated using PRISMA guidelines and a modified version of Downs and Black checklist. Twelve studies met inclusion criteria, only one of which explored the effect of esketamine, while all others focused on racemic ketamine. Five studies examined peripheral blood gene expression in patients with TRD, while seven assessed mRNA changes in vitro using human-derived cells. Transcriptome and candidate gene expression studies revealed modulation of genes and pathways related to glutamatergic signaling (GRM2, GRIN2D), immune regulation (STAT3, CCL22, IL6), and neuroplasticity (IGF2). No consistent peripheral biomarkers emerged, but transcriptional profiling revealed dynamic molecular responses to ketamine and esketamine. Ketamine and esketamine induce diverse transcriptional changes implicating neuroplastic, inflammatory, and metabolic pathways. Transcriptomic profiling offers a promising approach for uncovering biomarkers and mechanisms of antidepressant response, warranting further multi-omics, large-scale studies.

1. Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide, with a substantial proportion of patients developing treatment-resistant depression (TRD) despite adequate trials of conventional antidepressant therapies [1,2]. TRD presents an important pharmacological challenge, as traditional antidepressants, primarily targeting monoaminergic systems, often fail to provide a rapid or sustained response [1]. This resistance highlights an urgent need for novel therapeutic strategies that act in alternative neurobiological mechanisms, offering more rapid and effective symptom reduction and better treatment outcomes.

Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has emerged as a promising rapid-acting antidepressant, with effects often observed within hours of administration. Initially developed as an anesthetic in the 1970s, ketamine's antidepressant

* Correspondence to: Department of Molecular and Translational Medicine, Biology and Genetic Division, University of Brescia, Viale Europa 11, Brescia 25123, Italy
E-mail address: alessandra.minelli@unibs.it (A. Minelli).

https://doi.org/10.1016/j.phrs.2025.107894
Received 3 June 2025; Received in revised form 21 July 2025; Accepted 30 July 2025
Available online 31 July 2025

properties were identified decades later, with first trials using intravenous ketamine for depression published in the 2000s, stimulating extensive research into its mechanisms of action and therapeutic potential in TRD [3]. Evidence suggests ketamine modulates gene expression in pathways involved in neuroplasticity, inflammation, and metabolic regulation [4,5].

Esketamine, the S-enantiomer of racemic ketamine, was subsequently developed and approved for TRD in 2019 due to its rapid and robust antidepressant effects [3], while racemic ketamine may be used as an off-label treatment. In 2025, esketamine was also approved in the U.S. as monotherapy for TRD patients [6]. Esketamine has a higher affinity for the NMDA receptor and exhibits varying antidepressant effects compared to ketamine [7], with some findings suggesting that its effects on clinical symptoms scales are approximately half those of racemic ketamine [8]. Similar to ketamine, esketamine acts through glutamatergic modulation, enhancement of synaptic plasticity, and transcriptional changes. Esketamine also influences immune pathways, including interferon signaling, via signal transducer and activator of transcription 3 (STAT3) activation, and upregulates neurotrophic factors such as brain-derived neurotrophic factor (BDNF), reinforcing its role in synaptic function and neuroplasticity [9]. Despite clinical efficacy, the precise molecular mechanisms underlying esketamine's effects remain unclear.

Some studies, including systematic and umbrella reviews, have analyzed biomarkers related to ketamine and esketamine response. Pretreatment biomarkers can help predict which patients are most likely to respond, enabling more personalized and effective treatment decisions. Post-treatment biomarkers reflect the molecular effects of ketamine and can provide insights into its mechanism of action and treatment efficacy. Elucidating these biomarkers would support both precision medicine and a deeper understanding of ketamine's antidepressant effects. An umbrella review of 108 studies confirmed ketamine's anti-inflammatory effects but highlighted the absence of validated clinical biomarkers [10]. Frequently studied biomarkers included proteins related to the inflammatory and immune systems, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP), with evidence linking ketamine to changes in neutrophil-to-lymphocyte ratio and BDNF. Kumar and colleagues reviewed metabolomic biomarkers in TRD and healthy controls (HC), identifying ketamine-related changes in energy metabolism, acylcarnitines, kynurenine, and arginine pathways [11]. However, the findings were preliminary due to small sample sizes and high heterogeneity among the included studies. Medeiros and colleagues conducted a systematic review and meta-analysis of 56 studies examining over 460 peripheral biomarkers, concluding that baseline levels did not consistently predict response to ketamine [12]. Only BDNF showed a modest but significant post-treatment increase in responders. Overall, while several biomarkers show promise, none seems currently applicable for clinical use.

From a molecular perspective, ketamine appears to exert widespread effects across several biological systems. Studies have investigated inflammatory, neurotrophic, metabolic, and neurotransmission-related markers to elucidate its mechanisms. Despite promising findings, the complexity of these pathways and their interactions with environmental and genetic factors complicate the identification of robust biomarkers. Among the most explored targets are cytokines, including IL-6 and TNF-α, neurotrophic factors, such as BDNF, metabolic markers, and components of the kynurenine pathway. These reflect ketamine's heterogeneous biological effects and highlight the need for alternative and more integrative approaches.

Most previous studies have focused on ketamines's effects at the protein level, leaving a gap in understanding potential gene expression changes. Addressing this gap by highlighting transcriptional studies can offer a new perspective on ketamine's molecular impact and may help identify more reliable and predictive biomarkers. Transcriptional profiling represents a promising strategy to unravel ketamine's molecular impact, as it can detect earlier gene level changes that may precede biochemical alterations. Transcriptomics allows for comprehensive analysis of gene expression changes and offers insights into how ketamine and esketamine might modulate biological pathways relevant to antidepressant response. In particular, understanding how ketamine affects RNA expression in pathways related to neuroplasticity, inflammation, and metabolism may help identify novel treatment targets and predictive biomarkers. Transcription studies also offer the opportunity to uncover dynamic molecular responses to treatment that may be missed when focusing only on protein peripheral biomarkers. These studies can reflect short-term and long-term changes in gene activity following ketamine administration. By studying these dynamic alterations, transcription studies can help identify early signals of treatment response, uncover new therapeutic targets, and distinguish responders and non-responders more accurately.

Given ketamine's broad transcriptional effects in multiple biological pathways, a systematic synthesis of studies is needed to better understand its molecular mechanisms. We conducted a systematic review of the molecular effects of ketamine and esketamine on RNA expression, focusing on human studies and in vitro experiments using human-derived cells. This review aimed to identify transcriptional changes associated with ketamine and esketamine treatment with MDD episode, both in unipolar and bipolar disorder, and clarify potential molecular biomarkers and mechanisms underlying their antidepressant properties.

2. Materials and methods

2.1. Systematic search of the literature and eligibility criteria

A systematic search was conducted on PubMed and Scopus to retrieve studies in English, published from inception to February 10th, 2025. Details of the protocol were registered on PROSPERO, registration number CRD420250654037. The search was conducted using combinations of the terms ("ketamine " OR " esketamine " OR " s-ketamine") AND (mrna* OR microrna * OR mirna * OR transcript OR "gene expression" OR non-coding). References of relevant articles were reviewed for additional studies. We included studies 1) evaluating gene expression in peripheral blood from adults with unipolar or bipolar depression (according to any recognized diagnostic criteria, e.g. in accordance to any version of The Diagnostic and Statistical Manual of Mental Disorders, Research Diagnostic Criteria, or the International Classification of Diseases) treated with ketamine or esketamine; or 2) studies in which the effect of in vitro treatment with ketamine or esketamine on transcription was evaluated in cells derived from humans to investigate molecular mechanisms associated with their antidepressant effect. We excluded a) preclinical studies, b) reviews, c) case reports, d) studies in which gene expression was not measured or in-silico studies.

2.2. Study selection

A systematic review following the PRISMA guidelines (http://www.prisma-statement.org/) was conducted (Fig. 1). The screening of title and abstract of eligible articles, as well as the evaluation of the full text of potentially relevant articles, was carried out independently by two authors (CP and AM). Any disagreement was resolved through discussion with a third author (AS). From each included study the following information was collected: authors, year of publication, study design, clinical characteristics of participants, diagnosis and diagnostic criteria, assessed RNA markers, type of cells/biofluids, method of assessment, statistical analysis methods, and main results. Quality assessment of selected studies was conducted using a modified version [13] of the Downs and Black checklist [14] as in previous studies [15]. The checklist includes 27 questions that evaluate the overall quality of reporting, external validity, internal validity - study bias, internal validity - confounding and selection bias and power of the study. Since most included

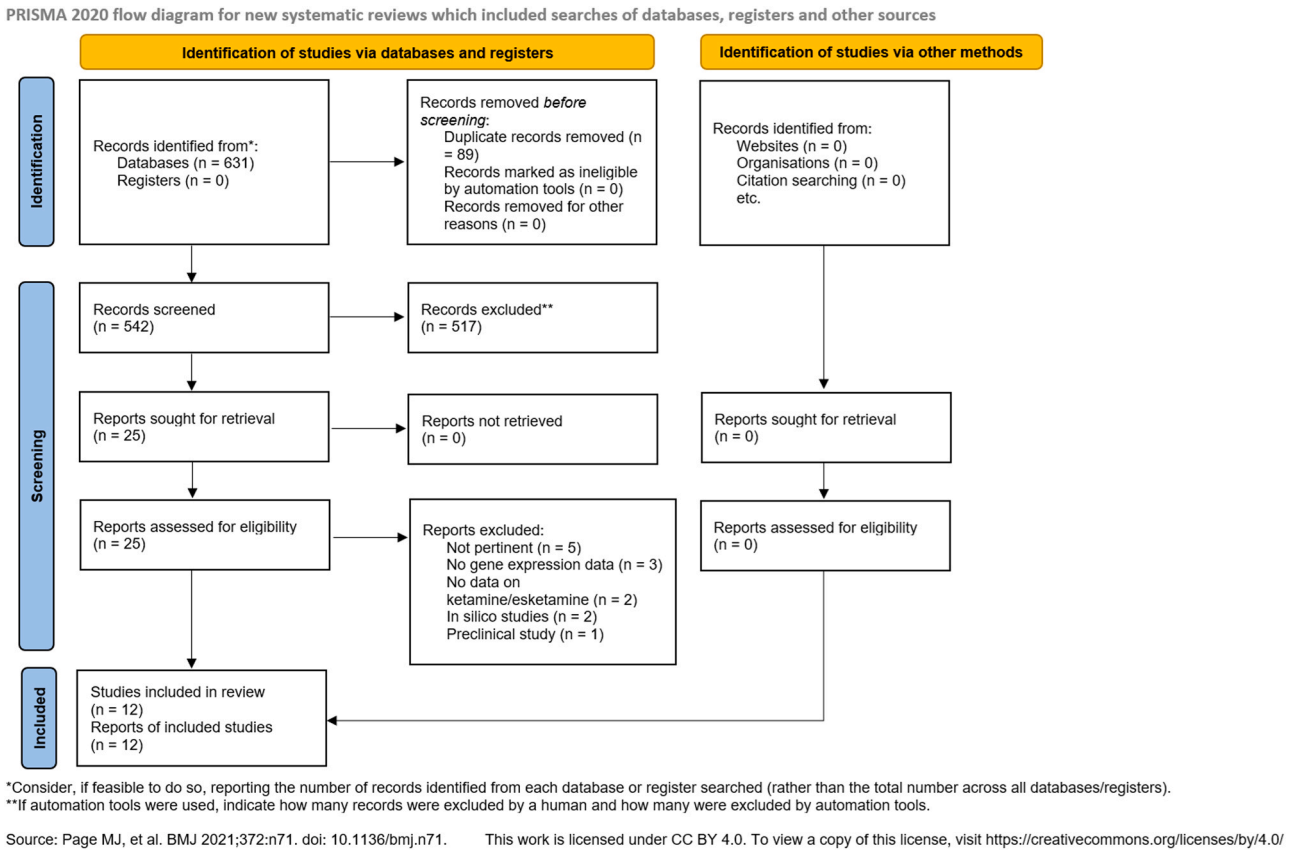

Fig. 1. PRISMA diagram.

articles were observational studies with a cross-sectional design, we excluded questions related to adverse events (#8), follow-up (#9, #17 and #26), blind (#14 and #15), randomization (#23 and #24), leading to a maximum score of 19 points.

3. Results

Our search retrieved a total of 631 articles and no additional study was retrieved through references (Fig. 1). After exclusion of duplicates (n = 89), 542 articles were reviewed. Of these, 517 were excluded after evaluation of title and abstract, while for 25 articles identified as potentially relevant, the full text was evaluated. Thirteen articles were excluded for the following reasons: not pertinent (n = 5), no gene expression data (n = 3), no data on ketamine or esketamine (n = 2), in silico (n = 2) or preclinical (n = 1) studies (Fig. 1). Twelve articles met the inclusion criteria and were included in this review. Among studies evaluating the association between blood RNA levels and ketamine response, three had a genome-wide design and evaluated whole blood transcriptome [5], miRNome [16] or both [17], while two had a candidate gene design and measured mRNAs [18] or lncRNAs [19] (Table 1 and Supplementary Table 1). All studies conducted in in vitro models measured mRNAs, two with a genome-wide design [4,9] and five with a candidate gene design [20–24] (Table 2 and Supplementary Table 2). Characteristics and results of included studies are described in the following sections.

3.1. Genome-wide studies evaluating the association between blood RNA levels and response to ketamine

Among studies that evaluated the association between RNA levels and response to ketamine in blood, Cathomas and coworkers conducted a transcriptomic analysis including 26 patients with TRD with different origins (Caucasian [n = 21], African-American [n = 1] or other origins [n = 4]) and 21 HC (Caucasian [n = 9], African-American [n = 7] or other origin [n = 5]) [5]. All patients had a major depressive episode of at least moderate severity according to the Clinical Global Impression - Severity scale (CGI-S) and were defined as having TRD based on lifetime history of non-response to at least two trials of an antidepressant, as assessed with the Antidepressant Treatment History Form [25,26]. Patients were naïve to ketamine and free of antidepressant and anxiolytic medications at recruitment. Eighteen patients were defined as responders based on a $\geq$ 50 % reduction in the Montgomery-Åsberg Depression Rating Scale (MADRS) score 24 h after a single ketamine infusion (0.5 mg/kg over 40 min) compared to baseline, while eight were defined as non-responders. Transcriptomic analyses were conducted both at baseline and 24 h after the infusion. In this study, no multiple testing correction was conducted, while a fold-change threshold was set at 1.3. The comparison between ketamine responders and non-responders at baseline identified 331 genes nominally differentially expressed and enriched in the cAMP-mediated signaling and neuropathic pain signaling in dorsal horn neurons pathways [5]. Among genes included in these pathways, the metabotropic glutamate receptor gene GRM2 and the ionotropic glutamate receptor gene GRIN2D were upregulated in responders compared to non-responders. In addition, genes associated with a change in the MADRS score were enriched for the cAMP signaling pathway [5]. Israeli-Elgali and colleagues measured whole genomemRNA and miRNAs in peripheral blood mononuclear cells (PBMC) from patients with TRD (defined according to unspecified criteria) and HC [17]. Patients were either treated with ketamine (n = 14), electroconvulsive therapy (ECT, n = 17) or required a change in the antidepressant treatment (n = 16). Participants treated with ketamine were randomized to two double-blind groups: intravenous (iv) 0.2 mg/kg ketamine and intranasal placebo, or intranasal 50 mg ketamine and iv placebo twice weekly, for three weeks. Response

Table 1
Studies investigating the association between blood RNA markers and the antidepressant effect of ketamine.

First author	Year	Sample	Drug	Outcome	RNA source	Targets and methods	Main findings	Ref
Cathomas	2022	26 patients with TRD (non-response to at least two trials of ADs assessed with the Antidepressant Treatment History Form [29], naïve to ketamine; 21 HC	Single racemic ketamine iv infusion (0.5 mg/kg)	Depression severity at baseline and 24 h post-infusion, assessed with MADRS	Whole blood	Transcriptome sequencing	331 genes nominally differentially expressed at baseline between R and NR, enriched in the cAMP-mediated signaling and neuropathic pain signaling in dorsal horn neurons pathways. Among these genes, GRM2 and GRIN2D were upregulated in R compared with NR.	[5]
Issler	2022	38 patients with TRD (lifetime history of non-response to at least two trials of ADs, 20 F, 18 M); 27 HC (16 F, 11 M)	Single racemic ketamine iv infusion (0.5 mg/kg)	Depression severity at baseline and 24 h post-infusion, assessed with QIDS	Whole blood	qPCR	Significant correlation between the change in the FEDORA lncRNA levels and the change in depression severity in female but not male patients with TRD	[19]
Israel-Elgali	2021	47 patients with TRD of which 14 treated with ketamine, 17 with ECT, 16 with ADs; 23 HC	Racemic ketamine iv 0.2 mg/kg and intranasal placebo; intranasal 50 mg racemic ketamine and iv placebo twice weekly for three weeks	Depression severity at baseline and 10 days post-treatment, assessed with HDRS	PBMCs	mRNA sequencing and miRNA NanoString assay	A decrease of FKBP5 levels among R but not NR was observed following treatment with ECT, while responders to ketamine or other ADs only showed a non-significant trend for FKBP5 reduction.	[17]
McGrory	2020	23 patients with a major depressive episode (unipolar or bipolar) according to DSM-IV and HDRS score $\geq$ 21 (12 receiving ketamine and 11 midazolam)	Single racemic ketamine iv infusion (0.5 mg/kg)	Depression severity at baseline and 4 h after the infusion, assessed with HDRS	Whole blood	Two mRNAs (VEGFA1 and PEDF) measured with real-time PCR	Ketamine but not midazolam significantly increased VEGFA levels but this change was not correlated with a change in the HDRS score	[18]
Gururajan	2016	40 patients with TRD (lack of response to two trials of ADs as assessed with a modified version of the Antidepressant Treatment History Form [23], of whom 16 treated with ketamine and 24 with ECT; 20 HC	Racemic ketamine iv (0.5 mg/kg once a week for up to 3 sessions)	Depression severity at baseline, 24 h and 1week post-infusion, assessed with HDRS	Whole blood	miRNA arrays (Exiqon)	No miRNAs were significantly associated with response to ketamine	[16]

Abbreviations: AD, antidepressants; ECT, electroconvulsive therapy; F, females; FKBP5, FKBP Prolyl Isomerase 5; GRIN2D, Glutamate Ionotropic Receptor NMDA Type Subunit 2D; GRM2, Glutamate Metabotropic Receptor 2; HC, healthy controls, HDRS, Hamilton Depression Rating Scale; iv, intravenous; lncRNA, long non-coding RNA; MADRS, Montgomery-Åsberg Depression Rating Scale; M, males; microRNA, miRNA; NR, non-responders; PBMC, peripheral blood mononuclear cells; qPCR, quantitative PCR; R, responders; QIDS, Quick Inventory of Depressive Symptomatology; TRD, treatment-resistant depression; VEGFA, vascular endothelial growth factor.

was defined as a $\geq$ 50 % reduction in the Hamilton Depression Rating Scale (HDRS) score at 10 days compared to baseline. A significant decrease of levels of the FKBP Prolyl Isomerase 5 (FKBP5) gene, that encodes a protein found to inhibit the glucocorticoid receptor by reducing its affinity to cortisol, was observed following treatment with ECT exclusively in responders, although ECT responders still maintained higher levels compared to controls [17]. Conversely, responders to ketamine or other antidepressants showed a non-significant decrease of FKBP5 levels. Gururajan and colleagues evaluated genome-wide miRNAs profiles in 16 patients with TRD treated with ketamine intravenously (0.5 mg/kg once a week for up to three sessions), 24 treated with ECT and 20 HC [16]. TRD was defined as lack of response to at least two adequate trials of antidepressant treatment assessed with a modified version of the Antidepressant Treatment History Form [26]. miRNAs were measured 24 h after the first infusion, while depression severity was assessed with the HDRS at baseline, 24 h and one week after the first infusion. Baseline miRNA expression was not significantly associated with response to ketamine either after 24 h or one week [16].

3.2. Candidate-gene studies conducted in blood

Among studies with a candidate-gene design, Issler and colleagues measured expression of the FEDORA lncRNA in whole blood from patients with TRD (defined as lifetime history of non-response to at least two trials of antidepressants) during a major depressive episode of at least moderate severity according to the CGI-S, assessed at baseline as well as 24 h after a single ketamine iv infusion (0.5 mg/kg) [19]. The authors showed that levels of FEDORA were higher in female but not male patients with TRD compared with HC before treatment, and that the change in FEDORA expression after treatment was correlated with the change in depression severity as measured by the Quick Inventory of Depressive Symptomatology (QIDS), exclusively in females [19]. Finally, McGrory and colleagues measured whole blood mRNA levels of vascular endothelial growth factor (VEGFA) and PEDF (SERPINF1) in patients with a major depressive episode (unipolar or bipolar) and an HDRS score $\geq$ 21, before and 4 h after treatment with a single ketamine or midazolam infusion [18]. The authors found that ketamine but not midazolam increased VEGFA levels, although this change was not correlated with the change in depression severity.

3.3. Studies conducted in in vitro models

Nowak and colleagues conducted the only in vitro study including cells derived from patients with MDD [22], rather than commercial lines or lines derived from HCs. Specifically, the study included patients with MDD hospitalized for a suicide attempt (n = 33) as well as gender and

Table 2
In vitro studies investigating transcriptional signatures of treatment with racemic ketamine, individual enantiomers, and their active metabolites.

First author	Year	Model	Drug	Targets and methods	Main findings	Ref
Jozwiak-Bebenista	2022	Astrocytes with induced ER stress (treatment with tunicamycin)	Esketamine, R-ketamine 10 μM for 24 h	Thirteen genes related to ER stress measured with real-time PCR	Treatment with esketamine and R-ketamine, but not with escitalopram or amitriptyline, nominally increased the expression of DDIT3 compared to cells treated with tunicamycin alone.	[21]
Nowak	2020	PBMCs derived from 33 patients with MDD hospitalized for a suicide attempt and 20 HC	Macrophage differentiation under treatment with racemic ketamine 0.1, 1 and 10 μM for 7 days	Genes related to inflammation measured with real-time PCR	Treatment with ketamine induced higher levels of CCL22 and TGM2 as well as higher levels of mTOR pathway associated genes (SGK1, EIF4B and FOXO1)	[22]
Grossert	2019	Human iPSC-derived NPCs	Racemic ketamine 1 μM for 24 h	Genome-wide mRNA sequencing; validation with real-time PCR	Upregulation of IGF2 and p11 24 h after treatment with ketamine	[4]
Ho	2019	HMC3 Human microglial cell line	Racemic ketamine, *2R–6R*-HNK, *2S–6S*-HNK (400 nM) for 24 h	Genome-wide mRNA sequencing; validation with real-time PCR	Modulation of genes included in the type I interferon pathway via activation of the STAT3 transcription factor	[9]
Ho	2018	U251-MG cells; human iPSC-derived astrocyte progenitors	Racemic ketamine, *2R–6R*-HNK, *2S–6S*-HNK (400 nM) for 24 h	GRIA1, GRIA2, GRIA4, ESR1, CYP2A6, CYP2B6 measured with real-time PCR	Upregulation of GRIA1, GRIA2 and GRIA4 AMPA receptor subunits, ERα, CYP2A6 and CYP2B6	[20]
Yuhas	2017	Human glioblastoma astroglial A172 cells and neuroblastoma SK-N-SH cells	Racemic ketamine (50–500 μM) for 24 h	Endothelial (eNOS) and neuronal (nNOS) NO synthase	Treatment with ketamine dose-dependently increased eNOS expression in astroglial A172 cells at 4, 8 and 24 h.	[23]
Yuhas	2015	Human glioblastoma astroglial A172 cells	Racemic ketamine (50–500 μM) for 24 h	IL−6, TNFα, IL1B and IL−8 measured with real-time PCR	Treatment with ketamine dose-dependently reduced IL6 and TNFα gene expression and increased IL1B expression.	[24]

Abbreviations: eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; HNK, hydroxynorketamine; IGF2, insulin-like growth factor 2; iPSC, induced pluripotent stem cells; MG, malignant glioblastoma; NPC, neural progenitor cells; STAT3, transcription 3

age-matched HC (n = 20) [22]. In this study, PBMCs derived from patients were cultured to induce macrophage differentiation under treatment with different concentrations of ketamine (0.1, 1 and 10 mM). Treatment with ketamine induced higher levels of CCL22 and TGM2 as well as higher levels of mTOR pathway associated genes (SGK1, EIF4B and FOXO1) [22]. Ho and colleagues tested the effect of a 24-hour treatment with ketamine and its two active metabolites *2 R, 6R*-hydroxynorketamine (HNK) and *2S,6S*-HNK (400 nM), with or without exposure to estradiol, on genome-wide mRNA expression in the HMC3 human microglial cell line [9]. The authors found that ketamine and its two metabolites modulated genes included in the type I interferon pathway via activation of the transcription 3 (STAT3) transcription factor [9]. Using a similar drug treatment protocol, the same authors had previously shown that treatment with ketamine or its metabolites increased mRNA expression of the GRIA1, GRIA2 and GRIA4, but not GRIA3 AMPA receptor subunits, in U251-malignant glioblastoma (MG) cells or in human iPSC-derived astrocytes [20]. Concomitant treatment with estradiol 0.1 nM showed an addictive effect on the induction of AMPA receptor subunits [20]. Similar results were observed for induction of estrogen receptor alpha (ERα) as well as CYP2A6 and CYP2B6, the main enzymes involved in ketamine metabolism [20]. Grossert and colleagues investigated transcriptional effects of treatment with ketamine in human induced pluripotent stem cells (iPSC)-derived neural progenitor cells (NPC) [4]. Treatment with ketamine 1 μM for 24 h induced a significant up- and downregulation of 31 and 29 genes, respectively. Among these, insulin-like growth factor 2 (IGF2) and p11 were validated with real-time PCR [4]. In the same study, IGF2 knockdown reduced the ability of ketamine to induce proliferation of NPCs, suggesting that the effect of ketamine on IGF2 expression might be involved in ketamine-induce proliferation. Among studies exploring specific hypothesis, Jozwiak-Bebenista and colleagues evaluated whether in vitro treatment with esketamine or R-ketamine 10 μM for 24 h modulated expression of 13 genes related to the unfolded protein response (UPR) pathway in a human astrocyte commercial line in which endoplasmic reticulum stress was induced by treatment with the antibiotic tunicamycin [21]. Concomitant treatment with esketamine and R-ketamine, but not with escitalopram or amitriptyline, increased the expression of DDIT3 compared to cells treated with tunicamycin alone. However, no multiple testing correction was applied in this study. Finally, two studies conducted by the same group evaluated the effect of ketamine (50–500 μM) on different candidate genes [23,24]. In the most recent study, the authors evaluated the effect of ketamine endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) in human glioblastoma astroglial A172 and neuroblastoma SK-N-SH cell lines, respectively [23]. Treatment with ketamine increased eNOS levels in a concentration-dependent manner, while no effect on the expression of nNOS was detected [23]. In the previous study, the authors found that treatment with ketamine (100–500 μM) at 24 h decreased dose-dependently IL6 and TNFα gene expression and increased IL1B gene expression in both unstimulated and cytokine-stimulated cells [24].

4. Discussion

This systematic review synthesized current evidence from human and in vitro studies regarding the transcriptional effects of ketamine and esketamine, aiming to clarify the molecular pathways underlying their antidepressant properties in MDD and bipolar disorders. A total of 12 studies were included. These studies explored the associations between ketamine or esketamine treatment and gene expression changes in patients with TRD or in in vitro models. Five studies examined blood samples, including three genome-wide [5,16,17] and two candidate gene studies [18,19], identifying differential expression in pathways such as cAMP signaling and highlighting genes like GRM2, GRIN2D, FKBP5, and FEDORA. Seven in vitro studies assessed ketamine's transcriptional effects in various cell types, showing modulation of genes

related to immune response, neuroplasticity, and metabolism, including IGF2, CCL22, TGM2, and STAT3-regulated interferon pathways [4,9,20–24]. These findings highlight the complex and heterogeneous molecular responses to ketamine across different biological models and systems.

Some studies highlighted the involvement of biological pathways related to the modulation of glutamatergic signaling mechanisms. For example, the comparison between ketamine responders and non-responders at baseline revealed differential expression of 331 genes enriched in cAMP-mediated signaling and neuropathic pain signaling in dorsal horn neurons, with GRM2 and GRIN2D upregulated in responders compared to non-responders [5]. In vitro findings showed increased expression of AMPA receptor subunits (GRIA1, GRIA2, GRIA4) in astrocytes and glioblastoma cells following treatment with ketamine, especially when co-administered with estradiol, which enhanced the effect [20]. These findings suggest a modulation of ketamine's effects on glutamatergic and plasticity-related genes.

Ketamine's immunomodulatory actions also emerged from several studies. A whole-blood transcriptomic analysis demonstrated that TRD patients exhibited baseline gene expression signatures indicative of interferon signaling pathway activation, with ketamine promoting distinct transcriptional modifications associated with clinical improvement [5]. In human microglial cells, ketamine and its active metabolites modulated genes involved in type I interferon signaling via STAT3 activation [9]. Further, ketamine increased CCL22 and TGM2 expression in PBMC-derived macrophages from patients with MDD and upregulated several mTOR-related genes (SGK1, EIF4B, FOXO1), indicating immune pathway activation in patient-derived cells [22]. Other in vitro studies showed that ketamine dose-dependently decreased IL6 and TNFα expression while increasing IL1B levels in glioblastoma and neuroblastoma cells, and enhanced eNOS gene expression in a concentration-dependent manner, suggesting potential effects on the immune and vascular systems [23,24].

Stress-related biological mechanisms were also implicated in ketamine's mechanism of action, including the FKBP5 gene, which encodes a protein that inhibits the glucocorticoid receptor. In blood, a genome-wide transcriptomic study showed a significant reduction in FKBP5 expression after electroconvulsive therapy in responders, while a non-significant decrease was observed after ketamine treatment [17]. Additionally, esketamine and R-ketamine modulated expression of 13 genes related to the unfolded protein response pathway in human astrocytes in which endoplasmic reticulum stress was pharmacologically induced [21].

Neurodevelopmental and metabolic genes were implicated in a study using iPSC-derived neural progenitor cells treated with ketamine, which led to upregulation of IGF2 and p11. IGF2 knockdown reduced ketamine-induced cell proliferation, suggesting IGF2 mediates neurogenic effects of ketamine [4]. Also, VEGFA expression increased significantly in whole blood following ketamine, but not midazolam, treatment in depressed patients, though this was not associated with changes in depression severity [18].

Hormonal and metabolic pathways also appeared to be influenced by ketamine. In cell models, ketamine-induced expression of CYP2A6 and CYP2B6, key enzymes in ketamine metabolism, was also potentiated by estradiol, further supporting hormone-dependent pharmacogenomic interactions [20].

The results should be interpreted in light of some limitations. First, the number of eligible studies was relatively small, with many studies employing small sample sizes and lacking correction for multiple testing. The diversity in study designs, patient populations, and gene expression quantification methods limited meta-analytic synthesis and the generalizability of the findings. Additionally, the use of in vitro models may not fully replicate in vivo neurobiological complexity. Many in vitro studies used commercial human cell lines or cells lines derived from HC, that might not fully reflect the molecular features of TRD. The validity of identifying predictive transcriptional biomarkers depends in part on the assumption that treatment response is biologically tractable and reflected in patient-derived cellular profiles. Therefore, the use of TRD patient-derived cells may offer greater translational relevance and should be used in future research. There are also methodological differences between different biological sampling strategies. Blood is easily accessible but may not fully reflect central nervous system processes. Neural or glial cell models can provide insight into how brain-specific responses work, but are limited by culture conditions. Biopsy tissue is rarely feasible in psychiatric populations. Similarly, genome-wide analyses are unbiased and can discover novel pathways but may lack statistical power in small samples. Candidate gene approaches provide targeted insights but may miss unexpected biological mechanisms. Using both strategies in larger groups may lead to more robust results. Another question is whether transcriptional changes induced by ketamine are associated with treatment response over time. Some evidence suggests gene expression modulation occurs rapidly, in parallel with clinical improvement, but it is unclear if these changes are sustained or reversible. Additionally, whether transcriptional changes vary with clinical improvement or are better detected by comparing extremes (full responders vs. non-responders) remains unresolved. Identifying robust biomarkers may require the discovery of key genes or pathways in extreme phenotypes, followed by validation in larger and more heterogenous patient populations. Another limitation is the underrepresentation of studies using esketamine, with most transcriptional analyses focusing on racemic ketamine. No human studies have investigated transcriptomic biomarkers associated with esketamine treatment in depression, and this should be explored in future studies. Furthermore, few studies examined long-term transcriptional changes or assessed whether gene expression modulation correlates with sustained clinical improvement. Sex differences and diagnostic heterogeneity were often not considered in the analyses, despite their known influence on treatment outcomes.

Nevertheless, this review has important strengths. It is, to our knowledge, the first systematic synthesis specifically focusing on human RNA effects of ketamine and esketamine, including studies performed in human peripheral blood and in vitro experiments using human-derived cells. By integrating genome-wide and candidate gene findings, we provide a broad and detailed overview of transcriptional pathways potentially involved in antidepressant response. This review also identified several negative or inconsistent findings that warrant consideration. Genome-wide studies identified nominal associations between response and genes involved in glutamatergic and cAMP signaling, such as GRM2, GRIN2D, and FKBP5, though these findings were limited by small sample sizes. Notably, baseline miRNA profiles did not predict ketamine response. Candidate-gene studies yielded sex-specific and mechanistic insights, including FEDORA lncRNA correlating with symptom improvement only in females, and ketamine-induced VEGFA increases that were not associated with clinical outcomes. In vitro models supported a role for pathways related to inflammation, mTOR, AMPA receptors, and estrogen signaling, with some specificity for esketamine over racemic formulations. However, no consistent transcriptional signature was identified across models. Overall, this systematic review supports the view that ketamine exerts extensive transcriptional influences by modulating networks involved inneuroplasticity, immune regulation, endocrine, and metabolic pathways. These findings should be interpreted with caution, particularly regarding the type of ketamine studied. All included studies evaluating blood RNA levels focused exclusively on racemic ketamine, while only one in vitro study examined esketamine. Since racemic ketamine contains both enantiomers, and research has shown that these enantiomers have distinct pharmacological profiles, their co-administration could influence each other's pharmacodynamics or pharmacokinetics, potentially affecting clinical outcomes [27,28]. Future studies should explore enantiomer-specific effects and specifically blood RNA levels in patients treated with intranasal esketamine.

Despite promising insights, the current evidence is limited by small

sample sizes, methodological heterogeneity, and the lack of replicated biomarkers. Future directions include storing clinical samples for transcriptional analyses, which could support the discovery of more clinically useful and reliable biomarkers, both pretreatment and post-treatment. Studies with larger cohorts, longitudinal designs, and integration of transcriptomic data with clinical, proteomic, and multi-omics markers are needed to better elucidate the molecular mechanisms of these antidepressants and to translate them into personalized treatment strategies.

CRediT authorship contribution statement

Alessandra Minelli: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Alessio Squassina:** Writing – review & editing, Methodology, Conceptualization. **Mattia Meattini:** Methodology. **Rosana Carvalho Silva:** Writing – original draft. **Bernhard T. Baune:** Writing – review & editing, Funding acquisition. **Massimo Gennarelli:** Visualization, Data curation. **Claudia Pisanu:** Writing – original draft, Methodology, Conceptualization.

Funding

Massimo Gennarelli and Alessandra Minelli are supported by the Italian Ministry of Health (IT-MoH) under Grant Ricerca Corrente 2025 (RC-2025). The assistant research position of Rosana Carvalho Silva is funded by Psych-STRATA project (European Union's Horizon Europe research and innovation programme under Grant Agreement No. 101,057,454).

Declaration of Competing Interest

All the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The grants declared in support of the following authors, Rosana Carvalho Silva, Massimo Gennarelli, and Alessandra Minelli, do not present any potential competing interests with the present work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2025.107894.

References

[1] D.H. Jaffe, B. Rive, T.R. Denee, The humanistic and economic burden of treatment-resistant depression in Europe: a cross-sectional study, BMC Psychiatry 19 (1) (2019) 247.
[2] S. Dodd, M. Bauer, A.F. Carvalho, H. Eyre, M. Fava, S. Kasper, S.H. Kennedy, J. P. Khoo, C.Lopez Jaramillo, G.S. Malhi, R.S. McIntyre, P.B. Mitchell, A.M.P. Castro, A. Ratheesh, E. Severus, T. Suppes, M.H. Trivedi, M.E. Thase, L.N. Yatham, A. H. Young, M. Berk, A clinical approach to treatment resistance in depressed patients: what to do when the usual treatments Don't work well enough? World J. Biol. Psychiatry 22 (7) (2021) 483–494.
[3] G.C. Medeiros, I. Demo, F.S. Goes, C.A. Zarate Jr., T.D. Gould, Personalized use of ketamine and esketamine for treatment-resistant depression, Transl. Psychiatry 14 (1) (2024) 481.
[4] A. Grossert, N.Z. Mehrjardi, S.J. Bailey, M.A. Lindsay, J. Hescheler, T. Saric, N. Teusch, Ketamine increases proliferation of human iPSC-Derived neuronal progenitor cells via Insulin-Like growth factor 2 and independent of the NMDA receptor, Cells 8 (10) (2019).
[5] F. Cathomas, L. Bevilacqua, A. Ramakrishnan, H. Kronman, S. Costi, M. Schneider, K.L. Chan, L. Li, E.J. Nestler, L. Shen, D.S. Charney, S.J. Russo, J.W. Murrough, Whole blood transcriptional signatures associated with rapid antidepressant response to ketamine in patients with treatment resistant depression, Transl. Psychiatry 12 (1) (2022) 12.
[6] FDA SPRAVATO, ⟨https://www.accessdata.fda.gov/drugsatfda_docs/label/2025/211243s016lbl.pdf, 2025⟩. (Accessed July 14 2025).
[7] E.J. Daly, J.B. Singh, M. Fedgchin, K. Cooper, P. Lim, R.C. Shelton, M.E. Thase, A. Winokur, L. Van Nueten, H. Manji, W.C. Drevets, Efficacy and safety of intranasal esketamine adjunctive to oral antidepressant therapy in Treatment-Resistant depression: a randomized clinical trial, JAMA Psychiatry 75 (2) (2018) 139–148.
[8] P. Zanos, R. Moaddel, P.J. Morris, L.M. Riggs, J.N. Highland, P. Georgiou, E.F. R. Pereira, E.X. Albuquerque, C.J. Thomas, C.A. Zarate Jr., T.D. Gould, Ketamine and ketamine metabolite pharmacology: insights into therapeutic mechanisms, Pharm. Rev. 70 (3) (2018) 621–660.
[9] M.F. Ho, C. Zhang, L. Zhang, H. Li, R.M. Weinshilboum, Ketamine and active ketamine metabolites regulate STAT3 and the type I interferon pathway in human microglia: molecular mechanisms linked to the antidepressant effects of ketamine, Front. Pharm. 10 (2019) 1302.
[10] S. Meshkat, R.C. Ho, B. Cao, K.M. Teopiz, J.D. Rosenblat, T.G. Rhee, J.D. Di Vincenzo, F. Ceban, M.Y. Jawad, R.S. McIntyre, Biomarkers of ketamine's antidepressant effect: an umbrella review, J. Affect Disord. 323 (2023) 598–606.
[11] R. Kumar, N.A. Nunez, N. Joshi, B. Joseph, A. Verde, A. Seshadri, A.B. Cuellar Barboza, L.J. Prokop, G.C. Medeiros, B. Singh, Metabolomic biomarkers for (R, S)-ketamine and (S)-ketamine in treatment-resistant depression and healthy controls: a systematic review, Bipolar Disord. 26 (4) (2024) 321–330.
[12] G.C. Medeiros, T.D. Gould, W.L. Prueitt, J. Nanavati, M.F. Grunebaum, N.B. Farber, B. Singh, S. Selvaraj, R. Machado-Vieira, E.D. Achtyes, S.V. Parikh, M.A. Frye, C. A. Zarate Jr., F.S. Goes, Blood-based biomarkers of antidepressant response to ketamine and esketamine: a systematic review and meta-analysis, Mol. Psychiatry 27 (9) (2022) 3658–3669.
[13] V. Korakakis, R. Whiteley, A. Tzavara, N. Malliaropoulos, The effectiveness of extracorporeal shockwave therapy in common lower limb conditions: a systematic review including quantification of patient-rated pain reduction, Br. J. Sports Med 52 (6) (2018) 387–407.
[14] S.H. Downs, N. Black, The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions, J. Epidemiol. Community Health 52 (6) (1998) 377–384.
[15] C. Pisanu, G. Severino, I. De Toma, M. Dierssen, P. Fusar-Poli, M. Gennarelli, P. Lio, E. Maffioletti, E. Maron, D. Mehta, A. Minelli, M.C. Potier, A. Serretti, D. Stacey, R. van Westrhenen, L. Xicota, P. European college of neuropsychopharmacology, N. Transcriptomics, B.T. Baune, A. Squassina, transcriptional biomarkers of response to pharmacological treatments in severe mental disorders: a systematic review, Eur. Neuropsychopharmacol. 55 (2022) 112–157.
[16] A. Gururajan, M.E. Naughton, K.A. Scott, R.M. O'Connor, G. Moloney, G. Clarke, J. Dowling, A. Walsh, F. Ismail, G. Shorten, L. Scott, D.M. McLoughlin, J.F. Cryan, T.G. Dinan, MicroRNAs as biomarkers for major depression: a role for let-7b and let-7c, Transl. Psychiatry 6 (8) (2016) e862.
[17] I. Israel-Elgali, L. Hertzberg, G. Shapira, A. Segev, I. Krieger, U. Nitzan, Y. Bloch, N. Pillar, O. Mayer, A. Weizman, D. Gurwitz, N. Shomron, Blood transcriptional response to treatment-resistant depression during electroconvulsive therapy, J. Psychiatr. Res 141 (2021) 92–103.
[18] C.L. McGrory, K.M. Ryan, B. Gallagher, D.M. McLoughlin, Vascular endothelial growth factor and pigment epithelial-derived factor in the peripheral response to ketamine, J. Affect Disord. 273 (2020) 380–383.
[19] O. Issler, Y.Y. van der Zee, A. Ramakrishnan, S. Xia, A.K. Zinsmaier, C. Tan, W. Li, C.J. Browne, D.M. Walker, M. Salery, A. Torres-Berrio, R. Futamura, J.E. Duffy, B. Labonte, M.J. Girgenti, C.A. Tamminga, J.L. Dupree, Y. Dong, J.W. Murrough, L. Shen, E.J. Nestler, The long noncoding RNA FEDORA is a cell type- and sex-specific regulator of depression, Sci. Adv. 8 (48) (2022) eabn9494.
[20] M.F. Ho, C. Correia, J.N. Ingle, R. Kaddurah-Daouk, L. Wang, S.H. Kaufmann, R. M. Weinshilboum, Ketamine and ketamine metabolites as novel estrogen receptor ligands: induction of cytochrome P450 and AMPA glutamate receptor gene expression, Biochem Pharm. 152 (2018) 279–292.
[21] M. Jozwiak-Bebenista, P. Sokolowska, M. Siatkowska, C.A. Panek, P. Komorowski, E. Kowalczyk, A. Wiktorowska-Owczarek, The importance of endoplasmic reticulum stress as a novel antidepressant drug target and its potential impact on CNS disorders, Pharmaceutics 14 (4) (2022).
[22] W. Nowak, L.N. Grendas, L.M. Sanmarco, I.G. Estecho, A.R. Arena, N. Eberhardt, D. E. Rodante, M.P. Aoki, F.M. Daray, E.A. Carrera Silva, A.E. Errasti, Pro-inflammatory monocyte profile in patients with major depressive disorder and suicide behaviour and how ketamine induces anti-inflammatory M2 macrophages by NMDAR and mTOR, EBioMedicine 50 (2019) 290–305.
[23] Y. Yuhas, S. Ashkenazi, E. Berent, A. Weizman, Ketamine upregulates eNOS expression in human astroglial A172 cells: possible role in its antidepressive properties, J. Neuroimmunol. 305 (2017) 75–81.
[24] Y. Yuhas, S. Ashkenazi, E. Berent, A. Weizman, Immunomodulatory activity of ketamine in human astroglial A172 cells: possible relevance to its rapid antidepressant activity, J. Neuroimmunol. 282 (2015) 33–38.
[25] H.A. Sackeim, The definition and meaning of treatment-resistant depression, J. Clin. Psychiatry 62 (16) (2001) 10–17.
[26] H.A. Sackeim, R.F. Haskett, B.H. Mulsant, M.E. Thase, J.J. Mann, H.M. Pettinati, R. M. Greenberg, R.R. Crowe, T.B. Cooper, J. Prudic, Continuation pharmacotherapy in the prevention of relapse following electroconvulsive therapy: a randomized controlled trial, JAMA 285 (10) (2001) 1299–1307.

[27] A. Bahji, G.H. Vazquez, C.A. Zarate, Jr., comparative efficacy of racemic ketamine and esketamine for depression: a systematic review and meta-analysis, J. Affect Disord. 278 (2021) 542–555.
[28] J. Bonaventura, S. Lam, M. Carlton, M.A. Boehm, J.L. Gomez, O. Solis, M. Sanchez-Soto, P.J. Morris, I. Fredriksson, C.J. Thomas, D.R. Sibley, Y. Shaham, C. A. Zarate Jr., M. Michaelides, Pharmacological and behavioral divergence of ketamine enantiomers: implications for abuse liability, Mol. Psychiatry 26 (11) (2021) 6704–6722.
[29] C.R. Conway, M.S. George, H.A. Sackeim, Toward an Evidence-Based, operational definition of Treatment-Resistant depression: when enough is enough, JAMA Psychiatry 74 (1) (2017) 9–10.