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BIOINFORMATIC INTERPRETATION OF TRACKING GENES OF MIRNA-LET 7B AND MIRNA-LET 7C IN DEPRESSION

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ABSTRACT

Depression, a psychiatric disorder, has a profound impact on both physical and mental health. Despite its extensive prevalence, the complexity of its underlying causes, remains largely elusive. Growing interest has focused on microRNAs , endogenous short-stranded non-coding RNAs that inhibit, gene translation and are abundant in brain tissues. This study, conducted as portion of a clinical trial involving patients with depression, examined the plasma levels of miRNA-let-7b and miRNA-let-7c, which were observed to be down-regulated, in depression but showed improvement following treatment.The study aimed, to identify molecular genes associated with depression to uncover potential pathological mechanisms through bioinformatics. Using the miRabel database, we tracked genes and pathways affected by miRNA let-7b and let-7c in the context of depression. Differentially expressed genes and pathways were identified, with 37 tracking genes implicated in long-term depression. Through these, 11 genes had the highest potential impact: MAPK3, PLA2G4F, PLA2G4B, PLA2G4C, GNAZ, CACNA1A, GNA12, GRID2, ITPR3, ITPR1, and GUCY1A3.
These 11 genes affected various pathways, including the CAMKK2 pathway, CREB1 phosphorylation via NMDA receptor-mediated RAS signaling, and the regulation of neuron apoptotic processes. These pathways, are involved in neural circuits activated by stress and environmental factors, leading to intracellular signal transduction cascades that contributes to neural plasticity, in addition to cell persistence or demise. Disparity in neuronal adaptably, can lead to depression. In summary, signal transduction cascades and neuronal adaptability are crucial during the pathophysiology of depression, and these pathways are tracked by miR-let-7b and miR-let-7c.

Key Words: Depression, miR-let-7b and miR-let-7c, CAMKK2 pathway and CREB

INTRODUCTION:

Depression is a familiar depressive illness, primarily marked by continuous grief and loss of attentiveness. Currently, Depression has emerged as a leading health threat in the 21st century, claiming millions of lives annually.[1] The primary age of onset is 20-30 years, with a higher prevalence in females than males.[2] Data from 2020 indicated that depression became the second most prevalent disease after heart problems. Despite the fact that the clinical curative rate for depression is elevated, the rates of medical therapy and recurrence are not promising due to patients' lack of awareness and reluctance to adhere to regular treatment. The precise foundation of depression persists unknown[1] and there is scarcity of objective biomarkers.[3] Bioinformatics analysis has been extensively used in genomic testing remaining for the previous several years, aiding in the identification of distinctively revealed genes and working pathways linked to the confirmation of depression.[4] MicroRNAs are short nucleotide sequences capable of modulating gene expression during translation, and recent research across various studies has underscored their potential as biomarkers in various diseases like depression.[5] MicroRNAs operate in a time-sensitive and tissue-certified fashion to control as well as adjust the post-transcriptional impression of selected mRNA molecules. These miRNAs are able to travel as contents of extra-cellular vacuoles linking central nervous system along with bloodstream, providing insights into central nervous system mechanisms via peripheral signals. Research indicates that microRNAs found peripherally exhibit dysregulation corresponding to the pathological characteristics observed in depression.[6] It was found that let-7b is expressed in mammalian brains, with its levels increasing during neural differentiation.[7]

It regulates NSC proliferation and differentiation and is crucial for stem cell self-renewal and cell cycle regulation.Experimental findings showed over-expression of let-7b leads to decreased NSC multiplication together with expanded disparity.And antisense knockdown of let-7b results in increased NSC proliferation.[7] miRNA-let-7C is linked to human depression. In a study by Roumans S et al,[8] 104 initially healthy participants were tracked over five years. Within this period, 52 developed major depressive disorder (MDD) and 52 did not. Researchers used qRT-PCR to measure baseline plasma amount regarding miR-17-5p, miR-134-5p, miR-144-5p, let-7b-5p, along with let-7c-5p. They discovered that those who later developed MDD had significantly lower baseline measure referring to let-7b-5p ($p = 0.02$). The decrease within let-7b-5p levels was more significant in individuals diagnosed with MDD within two years of baseline compared to those diagnosed between two to five years later. This study suggests a particular decreased blood amount regarding let-7b-5p, is linked by an increased chance about future depression.[8] Gururajan et al.[9] conducted a bioinformatic analysis that found let-7b and let-7c adjust 27 genes within PI3k-Akt-mTOR signaling pathway, which is known for maladjustment within depressed states. The expression levels of miR-16, miR-182, miR-451, along with miR-223 exists close to those in control subjects. The beginning levels of miRNA expression, infrequently anticipates medication feedback, in addition microRNAs were not influenced side by side with medication. Consequently, let-7b and let-7c have been identified like potential bio-markers for depressed states.[9]

Against this backdrop, our investigation directed towards identifcation of tracking genes and pathways of microRNA let-7b and miRNA let-7c that could be utilized for diagnosing depression and predicting treatment response.

METHODOLOGY:

This study was a proportion based on a randomized controlled trial directed at the Psychiatry Outpatient Department (OPD) of Mercy Teaching Hospital in Peshawar, starting with february to december 2021, involving patients with depression. Initially, blood samples were collected to analyze the expression of miRNA-let-7b and miRNA-let-7c. After three months of treatment, a second blood sample was taken from the same patients to remeasure the expression amount of these miRNAs, and the outcomes were then compared.

The secondary objective came about, towards relating the target genes of miRNA-let-7b and miRNA-let-7c using bioinformatic analysis through the miRabel software, which contains an extensive database of miRNAs, genes, and signaling pathways. MiRabel compiles human data based

on four major miRNA target prediction methods: miRanda, PITA, SVmicrO, and TargetScan. The application could be approached at miRabel.
Upon accessing the website, users can choose from powers to choose: miRNAs, genes, and signaling pathways. By selecting, the miRNAs accessory with entering specific miRNAs such as hsa-miR-let-7b and hsa-miR-let-7c, and choosing a relevant pathway like "long-term depression," users can compute the results to see a table of genes affected next to the chosen miRNAs along with their marked outcomes with regard to long-term depression. Items are sorted in descending order by their scores, with higher scores (e.g., 0.99) ranked above lower ones (e.g., 0.98). Each gene listed is linked to NCBI, Ensembl, and Gene Cards for detailed information, including gene name, ID, and associated pathways. The study correlates, these computational findings with existing literature on depression.

RESULTS:

Scores of miR-let 7b given by miRabel in Long-term depression

Table 1: The target genes regulated by miR-let-7b, accompanied by their scores assigned by miRabel, within the context of long-term depression

S.NO	Gene	hsa-let-7b
1)	PLA2G4F	0.998
2)	PLA2G4C	0.998
3)	ITPR3	0.998
4)	GNAZ	0.997
5)	MAPK3	0.997
6)	GNA12	0.996
7)	ITPR1	0.996
8)	GUCY1A3	0.996
9)	PLA2G4B	0.995
10)	CACNA1A	0.994
11)	GRID2	0.990
12)	PLCB3	0.989
13)	MAP2K2	0.989
14)	GUCY1A2	0.988
15)	GRIA1	0.988
16)	PLA2G4A	0.987
17)	PPP2R1B	0.986
18)	PLA2G4E	0.981
19)	PRKG1	0.980
20)	ITPR2	0.977
21)	PRKCA	0.962
22)	GNAS	0.962
23)	GNA13	0.958
24)	KRAS	0.910
25)	PLCB1	0.885
26)	GNAQ	0.806
27)	GRIA2	0.747
28)	ARAF	0.596
29)	GRIA3	0.563
30)	PRKCB	0.555
31)	LYN	0.502
32)	MAPK1	0.479
33)	PLCB2	0.423
34)	IGF1	0.417

35)	PLCB4	0.362
36)	NRAS	0.160
37)	IGF1R	0.115
	Total	**37 entries**

There are a total of 37 target genes, of miR-let-7b participating during long-term depression. In the group of above-mentioned genes, eleven genes exhibit the highest potential impact: MAPK3, PLA2G4F, PLA2G4B, PLA2G4C, GNAZ, CACNA1A, GNA12, GRID2, ITPR3, ITPR1, and GUCY1A3. Their potential impact score is 0.99, indicating a value close to 1.0.

Scores of miR-let 7c given by miRabel in Long-term depression

Table 2: The selected genes, regulated by miR-let-7c, along with their scores assigned by miRabel, within the context of long-term depression

S.NO	Gene	hsa-let-7c
1-	PLA2G4F	0.998
2-	ITPR3	0.998
3-	PLA2G4C	0.997
4-	GNAZ	0.997
5-	PLA2G4B	0.996
6-	GUCY1A3	0.996
7-	MAPK3	0.995
8-	ITPR1	0.995
9-	CACNA1A	0.995
10-	GNA12	0.995
11-	MAP2K2	0.992
12-	GRID2	0.989
13-	PLCB3	0.989
14-	GRIA1	0.988
15-	GUCY1A2	0.988
16-	PLA2G4A	0.987
17-	PPP2R1B	0.986
18-	ITPR2	0.982
19-	PLA2G4E	0.981
20-	PRKG1	0.980
21-	PRKCA	0.969
22-	GNAS	0.962
23-	GNA13	0.949
24-	KRAS	0.891
25-	PLCB1	0.863
26-	GNAQ	0.852
27-	GRIA2	0.739
28-	GRIA3	0.723
29-	ARAF	0.633
30-	MAPK1	0.632
31-	PRKCB	0.525
32-	LYN	0.503
33-	PLCB2	0.393
34-	IGF1	0.375
35-	PLCB4	0.360
36-	NRAS	0.197

37-	IGF1R	0.129
	Total	**37 entries**

There are 37 target genes, of miR-let-7c associated with long-term depression. Among these, eleven genes exhibit the greatest potential impact. These genes are MAPK3, PLA2G4F, PLA2G4B, MAP2K2, PLA2G4C, GNAZ, CACNA1A, GNA12, ITPR3, ITPR1, and GUCY1A3. Their potential impact score is 0.99, which is nearly 1.0.

Table 3: Target genes of **miR-let7b and miR-let7c** having a highest potential impact of **0.99**

S.NO	miR-let7b	miR-let7c	Pathways and processes involve
1-	MAPK3	MAPK3	It is implicated in 54 distinct processes, such as the ERK1 and ERK2 cascades, the MAPK cascade, Schwann cell maturation, adjustment of chemical synaptic communication, myelination, regulation of the stress-activated MAPK cascade, and sensory perception of pain.[10]
2-	PLA2G4F	PLA2G4F	It is involved in six different processes like arachidonic acid secretion, glycerophospholipid catabolic process and prostaglandin biosynthetic process.[11]
3-	PLA2G4B	PLA2G4B	The present gene make up a component of the cytosolic phospholipase A2 protein group. Phospholipase A2 enzymes dissolves the sn-2 bond of phospholipids, leaving lysophospholipids and fatty acids. This enzyme meet the requirements to correlate through mitochondria and premature endosomes. [12]
4-	GNAZ	GNAZ	It is involved in five different processes including G protein-associated receptor signaling path-way , G protein- associated serotonin receptor signaling path-way.[13]
5-	PLA2G4C	PLA2G4C	It is involved in arachidonic acid metabolic process, glycerophospholipid catabolic process and twelve other processes[14]

6-	CACNA1A	CACNA1A	It participates in chemical synaptic transmission, cellular response to amyloid beta, and six additional processes.[15]
7-	GNA12	GNA12	It is engaged in the G protein-associated receptor signaling path-way, modulation of TOR signaling, and fourteen additional processes.[16]
8-	ITPR3	ITPR3	It is involved in long-term synaptic potentiation, memory, and eleven additional processes.[17]
9-	ITPR1	ITPR1	It is involved in G protein-coupled receptor activation and approximately 126 other pathways.[18]
10-	GUCY1A3	GUCY1A3	It is concerned with retrograde trans-synaptic signaling along nitric oxide and ten other processes.[19]
11-	GRID2		Cerebellar granule cell differentiation, excitatory postsynaptic potential, excitatory synapse assembly, glutamate receptor signaling path-way, ionotropic glutamate receptor signaling path-way, chemical synaptic mediation, modulation of long-term synaptic depression, synaptic communication, glutamatergic pathways[20]
12-		MAP2K2	It is engaged in MAP kinase kinase movement, protein serine/threonine/tyrosine kinase action, and ten additional processes.[21]

This table shows that **miR-let7b and miR-let7c** have similar target genes with a highest potential impact of 0.99.

DISCUSSION:

There are 37 target genes, of miR-let-7b linked to long-term depression, with 11 showing the greatest potential impact: MAPK3, PLA2G4F, PLA2G4B, PLA2G4C, GNAZ, CACNA1A, GNA12, GRID2, ITPR3, ITPR1, and GUCY1A3. These genes have an impact score of 0.99, indicating a significant influence. Similarly, miR-let-7c targets 37 genes associated with long-term depression, with 11 genes exhibiting the highest potential impact: MAPK3, PLA2G4F, PLA2G4B, MAP2K2, PLA2G4C, GNAZ, CACNA1A, GNA12, ITPR3, ITPR1, and GUCY1A3, also with an impact score of 0.99.

Li, X et al.[22] conducted a study in 2023 on depressed illness, a complex psychosomatic disease accompanied by unclear pathogenesis potentially linked to various stressors. Previous research often focused on single distress-influenced depressed models, limiting the understanding of MDD's origins. This study induced, depressed-corresponding activities in rats using four established stress patterns: chronic uncertain mild tension, experienced weakness, chronic restraint pressure, and social defeat strain.[22]

Through protein analysis and metabolic analyses, of the hippocampus in these models, the researchers identified 529 proteins and 98 products of metabolism. Using Ingenuity Pathways inspection (IPA) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, they discovered distinctively adjusted path-ways and created a simplified design illustrating the AKT and MAPK communicating path-ways together with their interconnections.

The researchers identified the AKT, MAPK, and mTOR communicating routes significantly well known.[22] Eight proteins (p-AKT, p-ERK12, GluA1, p-MEK1, p-MEK2, p-P38, Syn1, and TrkB) within the AKT along with MAPK tracks, have been markedly modified in more than one depression pattern, whereas three proteins (p-mTOR, p-P70S6K, PSD95) within the mTOR routes occurred rarely. Consequently, the AKT as well as MAPK communicating tracks have being highlighted as tracks of depression. Foregoing researches, [23,24,25,26] have demonstrated that the CUMS, CRS, along with LH depression patterns reduce AKT communication in the hippocampus of mice and that CUMS activates the MAPK communicating track. This aligns with our findings, showing that miRNA-let-7b and miRNA-let-7c have an impact score of 0.997 and 0.995 on the MAPK3 gene, respectively. The MAPK3 gene, regulates the ERK1 and ERK2 cascades, the MAPK cascade, modulation of chemical synaptic transmission, and the regulation of the stress-activated MAPK cascade.

Eicosanoids, derived from arachidonic acid, are crucial lipid signaling mediators in the brain, playing significant roles in chronic inflammation within the central nervous system (CNS). This inflammation is linked to brain pathologies, like depression. Mood disorders, including depression, involve complex pathogeneses with neurotransmitter imbalances, endocrine dysfunction, and inflammatory responses.[27] In our study, we identified the genes PLA2G4F, PLA2G4B, and PLA2G4C as significant targets of miRNA-let7b and miRNA-let7c, each with an impact score of 0.99. These genes, are involved in processes such as arachidonic acid secretion and prostaglandin biosynthesis. Eicosanoids, are synthesized by cyclooxygenases (COX), producing pro-inflammatory prostaglandins and thromboxanes, and by lipoxygenases (LOXs), producing both pro-inflammatory leukotrienes and anti-inflammatory lipoxins. Maintaining a balance, between these metabolites is vital, as an imbalance may lead to chronic neuroinflammation.[27] Initial studies by Maes et al. [28,29] highlighted the involvement of inflammation during depression, finding raised strength of inflammatory markers particularly IL-1β, IL-6, TNF-α, along with others in depressed individuals.[30] Further research, confirmed increased IL-6, TNF-α, along with C-reactive protein levels, in blood specimens out of these depressed individuals, along with elevated IL-1β in plasma and cerebrospinal fluid correlated with depression severity.[31] A meta-analysis also revealed elevated concentrations of several inflammatory markers in depression, while anti-inflammatory markers like IL-4 and IL-10 were reduced, indicating immune system dysregulation.[32]

Serotonin receptors, abundant in the nervous system and periphery, are crucial for drug discovery, targeting conditions from migraines to neuropsychiatric disorders like schizophrenia and depression. Of the 14 serotonin receptors, 13 are G protein-associated receptors (GPCRs), implicated in approximately 40% regarding approved medicines.[33] Our study highlights the GNAZ , GNA12 and ITPR1 gene, a target of miRNA-let7b and miRNA-let7c with an impact score of 0.99, which is

connected with G-protein integrated serotonin receptor communicating tracks. GPCRs exists as the largest membrane protein family, featuring seven transmembrane α-helices linked by three extracellular and three intracellular loops, allowing precise responses to external and internal stimuli. They are heavily targeted in drug development, with around 35% of FDA-approved drugs acting through these receptors, and are considered promising targets for treating depression and other psychiatric diseases.[34] Long-term potentiation (LTP), a stretched out increase in synaptic strength, is believed towards the anatomical foundation of long-term memory. LTP induces substitution with respect to both cellular and molecular levels, as well as time-specified modifications in gene mesh-works.[35] ITPR3 gene is connected with process of Long-term potentiation of memory process. Through bioinformatic analysis, we found that ITPR3 gene is a target of miRNA-let7b and miRNA-let7c with an impact score of 0.99. Inositol 1,4,5-trisphosphate receptors (ITPRs) occurs as intra-cellular calcium release passages functioning with endoplasmic reticulum of nearly all cells.[36] Wu W et al.[37] analyzed anatomical expression data to pinpoint brain regions and cell types with high expression of candidate genes. They identified twelve genes, accompanied by persistent distinctive expression, including ITPR3, which was nearly all highly communicated in the supraoptic nucleus of the hypothalamus, an area producing vasopressin. Increased vasopressin levels have been linked to major depressive disorder (MDD) and psychomotor retardation. While inositol, supplementation in depression has yielded mixed results, further research is desired towards understanding particular interplay between ITPR3, vasopressin, inositol, calcium, and depression.[37] Xiao Y et al.[38] investigated synapse-connected communicating tracks coming out of protein-protein interactions (PPIs) by classifying protein phosphorylation sites by synaptic types (glutamatergic, GABAergic, cholinergic, and dopaminergic). They analyzed phosphoprotein substitute induced by persistent unpredictable mild strain (CUMS) and ketamine therapy within the medial prefrontal cortex (mPFC) as well as nucleus accumbens (NAc). They found that CUMS down-regulated CACNA1 (T1888), Gng10 (S8), and Adcy5 (S156) at glutamatergic synapses, meantime ketamine up-regulated CACNA1 (T1888; S2153) inside mPFC. At GABAergic synapses, CUMS down-regulated CACNA1 (T1888), Gng10 (S8), Adcy5 (S156), and Slc6a1 (T15), whereas ketamine up-regulated CACNA1 (T1888; S2153) as well as Plcl1 (S570) in the mPFC. These genes affect synaptic plasticity and postsynaptic excitability.[38] This goes with our bioinformatic findings that CACNA1 gene is involved in chemical synaptic transmission and is a target gene of miRNA-let7b and miRNA-let7c with an impact score of 0.99. Sabatini MJ et al.[39] studied gene expression alteration in the amygdala of differently reared monkeys (1 week separated, 1 month moved apart, maternally looked after). They found significant differences in GUCY1A3, which showed the most change between 1 week separated and maternally looked after groups, differed between 1 week and 1 month moved apart groups, was highly expressed inside amygdala, together with NO communicating cascade. Quantitative in situ hybridization, established significant down-regulation of GUCY1A3 mRNA in the 1 week separated group, especially within lateral along with basal nuclei. These findings suggest GUCY1A3 may influence socioemotional behaviors directed by the amygdala[39]. In our miRabel analysis, GUCY1A3 is affected by both miRNAs let-7b and let-7c with a score of 0.996 and is implicated during retrograde trans-synaptic communication along nitric oxide.

GRID2 is linked to the ionotropic glutamate receptor group moreover it is specifically expressed in Purkinje cells, where it plays crucial roles in the synthesis of synapsis, synaptic flexibility, and motor collaboration. Variants in glutamate-associated genes, including GRID2, seemed to be linked to responses to antipsychotic medications.[40] Another finding of our study is GRID2 gene a target gene of miRNA-let 7b and miRNA-let7c with a high impact score of 0.996. GRID1 and GRID2 genes encrypts GluD1 and GluD2 proteins, essential for synaptic organization and CNS development as tetrameric receptors. Variations in these genes, are associated with neurodevelopmental disorders. Allen JP et al.[41] explored human variants in GRID1 and GRID2, identifying pathogenic effects, of certain variants like GluD2-A654T, which constitutively activate receptors. Similarly, the SCHEMA schizophrenia M3 alternative form GluD1-A650T showed similar effects. They tested, multiple compounds with this including pentamidine which successfully prevented GluD2-T649A constitutive passages(IC50 50 nM). Such findings underscore critical areas sensitive towards differences in GRID

genes, elucidate the operative outcomes of GRID1 plus GRID2 alternative forms, and provide insights into their roles in normal and diseased conditions.[41]

CONCLUSION:

Our study suggests, these particular let-7b and let-7c could be valuable bio-markers for diagnosing depression. Bioinformatics tools, are essential for managing miRNA data, identifying miRNA targets, and further research is necessary to validate the potential of let-7b, let-7c, and additional microRNAs. These investigations, analyzing both blood and cerebrospinal fluid samples, aim to demonstrate the significant role, of microRNAs in various developmental processes and their relevance in diagnosing depression, predicting treatment responses, and understanding the underlying neuromolecular mechanisms.

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