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Exploring the Role of miRNAs and Dysregulated Gene Expression in Alzheimer's Disease: A Multi-Omics Study on Oxidative Stress, Inflammation, and Neuronal Dysfunction

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Abstract:	Alzheimer's disease is a progressive neurological disorder that leads to cognitive impairment and memory loss. It is characterized by synaptic loss, disrupted neuronal signaling, inflammation, and brain tissue damage. Alzheimer's disease is the primary cause of dementia globally, accounting for approximately 60% to 80% of cases. The objective of this study was to investigate the specific molecular mechanisms involved in Alzheimer's disease and identify potential targets for therapeutic interventions. To achieve this, we utilized RNA sequencing to analyze the expression of miRNAs and mRNAs in individuals with Alzheimer's disease compared to healthy individuals. By comparing the data, we identified genes and miRNAs that were differentially expressed and involved in the pathological processes of Alzheimer's disease. Functional enrichment analysis revealed that these dysregulated genes and miRNAs are associated with crucial biological pathways and processes related to synaptic function, neurodevelopment, inflammation, and neurodegeneration. Additionally, various signaling pathways, including neuroactive ligand-receptor interaction, cAMP signaling, MAPK signaling, and Alzheimer's disease pathways were identified to be enriched. The study specifically identified genes such as CALM3, PPP3CB, and ITPR1, which play a significant role in the Alzheimer's disease pathway. Dysregulation of these genes disrupts the balance and function of neurons, leading to their accumulation and eventual death in brain regions responsible for learning, memory, and cognition. Moreover, through network analysis, the study revealed the top five upregulated hub miRNAs, namely hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p, and hsa-miR-3180-5p. Interestingly, hsa-miR-1908-3p and hsa-miR-6754-3p were previously unreported in Alzheimer's disease and may have a role in its progression. Furthermore, genes like RASGRF1, SHANK1, and NPAS4, which regulate long-term memory processes, were found to be involved in Alzheimer's disease. However, their dysregulation leads to damage in the brain regions responsible for memory creation and retrieval, resulting in dementia or memory loss, which are often the initial symptoms. These findings provide valuable insights into the molecular landscape of Alzheimer's disease, shedding light on its underlying mechanisms. The study also uncovers novel miRNAs associated with Alzheimer's pathogenesis that were not previously reported.
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Alzheimer's disease is a neurodegenerative disorder causing progressive cognitive decline, memory loss, and neuronal dysfunction and characterized by molecular and cellular changes, including the accumulation of beta-amyloid protein outside neurons and the presence of twisted strands of tau protein within neurons. Understanding the molecular mechanisms and identifying inflammatory biomarkers and therapeutic targets are crucial for developing effective interventions in AD. Investigating the role of dysregulated miRNAs in AD pathogenesis shows promise in unraveling disease mechanisms and identifying novel therapeutic strategies.

The analysis revealed miRNAs and genes that show association with key biological pathways involved in synaptic function, neurodevelopment, inflammation, and neurodegeneration. The findings support the importance of understanding the fundamental molecular mechanisms of AD to identify potential therapeutic targets and biomarkers for diagnosis and treatment.

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Abstract

Alzheimer's disease is a progressive neurological disorder that leads to cognitive impairment and memory loss. It is characterized by synaptic loss, disrupted neuronal signaling, inflammation, and brain tissue damage. Alzheimer's disease is the primary cause of dementia globally, accounting for approximately 60% to 80% of cases. The objective of this study was to investigate the specific molecular mechanisms involved in Alzheimer's disease and identify potential targets for therapeutic interventions. To achieve this, we utilized RNA sequencing to analyze the expression of miRNAs and mRNAs in individuals with Alzheimer's disease compared to healthy individuals. By comparing the data, we identified genes and miRNAs that were differentially expressed and involved in the pathological processes of Alzheimer's disease. Functional enrichment analysis revealed that these dysregulated genes and miRNAs are associated with crucial biological pathways and processes related to synaptic function, neurodevelopment, inflammation, and neurodegeneration. Additionally, various signaling pathways, including neuroactive ligand-receptor interaction, cAMP signaling, MAPK signaling, and Alzheimer's disease pathways were identified to be enriched. The study specifically identified genes such as CALM3, PPP3CB, and ITPR1, which play a significant role in the Alzheimer's disease pathway. Dysregulation of these genes disrupts the balance and function of neurons, leading to their accumulation and eventual death in brain regions responsible for learning, memory, and cognition. Moreover, through network analysis, the study revealed the top five upregulated hub miRNAs, namely hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p, and hsa-miR-3180-5p. Interestingly, hsa-miR-1908-3p and hsa-miR-6754-3p were previously unreported in Alzheimer's disease and may have a role in its progression. Furthermore, genes like RASGRF1, SHANK1, and NPAS4, which regulate long-term memory processes, were found to be involved in Alzheimer's disease. However, their dysregulation leads to damage in the brain regions responsible for memory creation and retrieval, resulting in dementia or memory loss, which are often the initial symptoms. These findings provide valuable insights into the molecular landscape of Alzheimer's disease, shedding light on its underlying mechanisms. The study also uncovers novel miRNAs associated with Alzheimer's pathogenesis that were not previously reported.

Keywords Alzheimer's disease, mRNAs, miRNA targets, neurodegeneration, therapeutic targets

Introduction

AD, or Alzheimer's disease, is a neurodegenerative disorder that causes progressive cognitive decline, memory loss, and neuronal dysfunction. It is characterized by molecular and cellular changes, including the accumulation of beta-amyloid protein outside neurons and the presence of twisted strands of tau protein within neurons. These changes result in synaptic loss, disrupted neuronal signaling, inflammation, and damage to brain tissue. AD is the leading cause of dementia globally, accounting for approximately 60% to 80% of cases [1].

Oxidative stress and chronic inflammation have been implicated in AD pathogenesis. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and antioxidant defenses, resulting in cellular damage and neuronal dysfunction. Chronic inflammation involving microglia, immune cells in the brain, contributes to the progression of AD. Thus, investigating genes associated with oxidative stress and inflammation is essential for understanding AD pathology and identifying potential therapeutic targets. The buildup of amyloid plaques, neurofibrillary tangles, oxidative stress, and inflammation collectively result in neuronal dysfunction and eventual cell death. This process leads to synaptic loss, disrupted neuronal signaling, and impaired cellular processes, all of which contribute to the cognitive decline observed in Alzheimer's disease [2].

MicroRNAs (miRNAs) are short RNA molecules, typically around 22 nucleotides in length, that play a critical role in the regulation of gene expression. They function as post-transcriptional regulators, exerting their effects after the synthesis of RNA. The miRNAs bind to specific messenger RNA (mRNA) molecules, primarily in the 3' untranslated region (UTR), resulting in the inhibition of mRNA translation or degradation [3]. This mechanism allows miRNAs to finely adjust gene expression by preventing the production of specific proteins. Dysregulation of miRNAs can have significant implications for cellular processes and contribute to the development of various diseases. Altered expression of miRNAs can disrupt normal gene expression patterns, leading to aberrant cellular functions and potentially contributing to the pathology of diseases [4].

Therefore, miRNAs have emerged as potential biomarkers for Alzheimer's disease. Changes in the expression levels of specific miRNAs in the brain or peripheral fluids, such as blood or cerebrospinal fluid, may serve as indicators of disease progression or as diagnostic markers. Manipulating the expression or function of miRNAs holds promise as a therapeutic strategy [5]. By targeting specific miRNAs, it may be possible to restore normal gene expression patterns and mitigate the cellular dysfunctions associated with various diseases, including Alzheimer's disease [6].

Understanding the molecular mechanisms and identifying inflammatory biomarkers and therapeutic targets are crucial for developing effective interventions in AD. Investigating the role of dysregulated miRNAs in AD pathogenesis shows promise in unraveling disease mechanisms and identifying novel therapeutic strategies. Additionally, the discovery of miRNAs as potential biomarkers offers valuable tools for monitoring disease progression and facilitating early diagnosis [7].

Given the potential of miRNAs to disrupt crucial genes and their functions, thereby affecting normal brain function and neuronal activity through stress, inflammation, and related mechanisms, it is imperative to investigate the role of miRNAs and their interaction with mRNAs in patients with AD [8]. Analyzing differentially expressed miRNAs and their target mRNAs in AD patients can shed light on which mRNAs are potentially targeted by upregulated miRNAs, leading to the downregulation of key genes involved in normal brain function. Such

investigations are essential for understanding the underlying molecular mechanisms and identifying potential therapeutic targets for AD [9].

The ultimate goal of this research is to improve interventions for AD and enhance the quality of life for affected individuals. To achieve this, it is crucial to understand the fundamental molecular mechanisms underlying AD, including the identification of inflammatory biomarkers that contribute to neuronal dysfunction and inflammation. Additionally, developing potential therapeutic interventions relies on elucidating therapeutic targets [10].

Our research aims to identify differentially expressed miRNAs and mRNAs in AD patients compared to healthy controls. Additionally, we investigate the dysregulation of mRNA targets caused by the overexpression of these miRNAs. Through the integration of miRNA and mRNA sequencing data, we conduct miRNA-mRNA correlation analysis to identify inflammatory biomarkers associated with brain inflammation in AD. Therefore, this study provides insights into novel miRNAs that can be targeted for therapeutic interventions.

Materials and Methods

This study focused on mRNA and miRNA sequencing analysis to identify therapeutic biomarkers for Alzheimer's disease. The datasets were obtained from Homo sapiens brain tissues of patients and normal individuals, excluding cell-line studies. The collected RNA-seq data underwent preprocessing, mapping, and post-processing steps. Differential expression analysis was performed to identify differentially expressed mRNAs and miRNAs. To understand the impact of upregulated miRNAs on gene expression, target mRNAs were identified using the miRWalk database, Functional and pathway enrichment analysis of the gene targets of differentially expressed miRNAs was performed using GeneCodis4 (https://genecodis.genyo.es/), exploring gene ontology terms (BP, MF, CC) and KEGG pathways. Network analysis was performed to identify the most interactive miRNAs with the mRNAs [11].

Messenger- and Micro-RNA Sequencing Data collection

To gather the datasets, we applied selection criteria that required them to originate from Homo sapiens and not be derived from cell-line studies. The datasets were retrieved from National Institute of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/). We specifically collected miRNA (ID: *PRJNA201039*) and mRNA (ID: *PRJNA727602*) sequencing data of samples that originated from brain tissues of patients and normal individuals.

RNA-seq preprocessing, mapping and post-processing

The initial set of reads obtained from the sequencer contains various contaminants, including low-quality reads, adapters, and primer remnants. To address this, the raw reads underwent preprocessing before alignment. The FastQC tool was utilized to assess the quality of the raw reads based on factors such as per base sequence quality, GC content, per base N content, sequence length distribution, sequence duplication levels, overrepresented sequences, and adapter content (https://github.com/csf-ngs/fastqc). To eliminate poor quality reads that could introduce PCR artifacts and hinder downstream analysis, the FastP tool was employed for trimming (with parameters like -i for raw FASTQ sample, -o for output, and -w for multi-core processing) to generate reliable data for further analysis [12]. Subsequently, the quality of the trimmed reads was reassessed using the FastQC tool. Furthermore, the cleaned reads were aligned to the reference human genome (GRCh38) using the Hisat2 aligner [13]. The duplicates, which may arise from PCR amplification during sample preparation and could lead to false gene expression estimates, were removed from the aligned reads using the Sambamba command-line module named markdup, incorporating the -r flag [14].

Read quantification and DEA analysis

Gene expression abundances were estimated using the StringTie tool [15]. StringTie performed a three-step process to obtain count reads. Initially, it assembled the alignments into partial and full-length transcripts, which contained millions of short read sequences, generating multiple isoforms. Next, the transcripts were merged to create a consistent set of transcripts across all samples. Finally, gene quantification was performed using the merged

transcripts as a reference, and the -eB option was utilized to generate expression counts in the Ballgown table format. Differentially expressed genes (DEGs) between Alzheimer's patients and normal individuals were analyzed using the Ballgown 2.30.0 package in R 4.2.2. Biologically and statistically significant genes were identified by analyzing the FPKM normalized expression data with Ballgown, using logFC values and p-values, respectively. A threshold of P-value < 0.05 and logFC values between 1 and -1 were applied to identify upregulated and downregulated genes, respectively. Additionally, a Volcano plot was used to visualize the significant DEGs.

miRNA-seq Data preprocessing and mapping

The raw trimmed reads are pre-processed similar to the steps mentioned for mRNA-seq data previously. The trimmed reads were then mapped to the reference genome (Homo sapiens GRCh38) using the miRDeep2 (v0.0.7) package's perl script called mapper.pl [16]. This script required a configuration file containing the accession numbers of all samples, the reference genome, and the trimmed reads. Execution of the script involved specifying parameters such as the number of CPU cores, input file format (fastq), retaining temporary files, converting raw reads to FASTA format, collapsing reads, mapping to the genome, and outputting progress reports. This process resulted in the generation of read counts, their sources, and genomic locations.

Novel miRNAs and Quantification

We used miRDeep2.pl, a perl script from the miRDeep2 package, to identify known and novel miRNAs. The script utilized collapsed reads, reference genome information, and miRNA sequences (hairpin and mature) obtained from the miRBase database. The collapsed reads were generated during the mapping process. Furthermore, we quantified the expression of both novel and known miRNAs using miRDeep2's 'quantifier.pl' script, applying specific parameters.

Differential expression analysis of miRNA

Raw counts obtained from the miRDeep2.pl quantification script were provided to the DESeq R/Bioconductor package for differential expression analysis (DEA). Only miRNA genes with a read count greater than 1 were included in the analysis. Differential expression was determined based on criteria such as a log fold change (logFC) greater than 2 and a p-value less than 0.05. MiRNAs meeting these thresholds were considered upregulated if the logFC was greater than 1 and downregulated if the logFC was less than -1.

Target identification and functional enrichment analysis

To identify the target mRNAs of upregulated miRNAs and understand their potential impact on gene expression, we utilized the miRWalk database [17]. The miRWalk database combines experimentally validated miRNA-target interactions with machine learning techniques to predict these interactions. The predicted miRNA-mRNA interactions were further refined using the filter function of the dplyr package (v1.0.10) in R (v4.2.2). In the miRWalk filtering process, specific criteria were applied, including an energy score of <= -30, a binding probability

of 1, and an AU content < 0.6. AU-rich elements refer to specific nucleotide sequences around the predicted binding sites (Li et al., 2021). Moreover, to identify gene targets of differentially expressed (DE) miRNAs and conduct functional and pathway enrichment analysis, we employed GeneCodis4. In this analysis, Homo sapiens was selected as the organism, miRNAs were chosen as the input type, and gene ontology (GO) terms were used to explore biological processes (BP), molecular functions (MF), cellular components (CC), and KEGG pathways.

Interaction between miRNA-mRNA and Hub Genes Identification

A network analysis was conducted using the cytohubba module of Cytoscape to identify miRNAs with the highest number of interactions with target genes, which could potentially disrupt the expression of these target mRNA genes [18]. Subsequently, the functional enrichment of the target genes associated with these highly interactive miRNAs was assessed using GeneCodis4.

Results

Identification of differentially expressed mRNAs

Differential gene expression analysis of 40 Alzheimer's samples (n=18 disease; n=22 normal) revealed a total of 2973 dysregulated (upregulated and downregulated) genes in AD patients. It was observed that a total of 1453 genes were overexpressed with a fold change (FC) value > 1 and P-value < 0.05, while 1520 genes were underexpressed, showing FC value < -1 and P-value < 0.05 in AD. The biologically and statistically significant dysregulated genes are shown in **Figure 1(a)**. Whereas, the top 10 upregulated and downregulated genes with respect to logFC values are shown in **Table 1** and **Table 2**, respectively. The top 10 significantly upregulated genes included STOML3, DEFA3, ENSG00000286679, HLA-DQB2, LINC03003, ENSG00000227713, DLK1, GZMK, FOXB1 and KRT16 and the top 10 downregulated genes included TMED8, ZBTB8A, FCER2, FSTL4, TTBK2, OR2L13, STYK1, SPECC1L-ADORA2A, CADPS2 and SLC6A7.

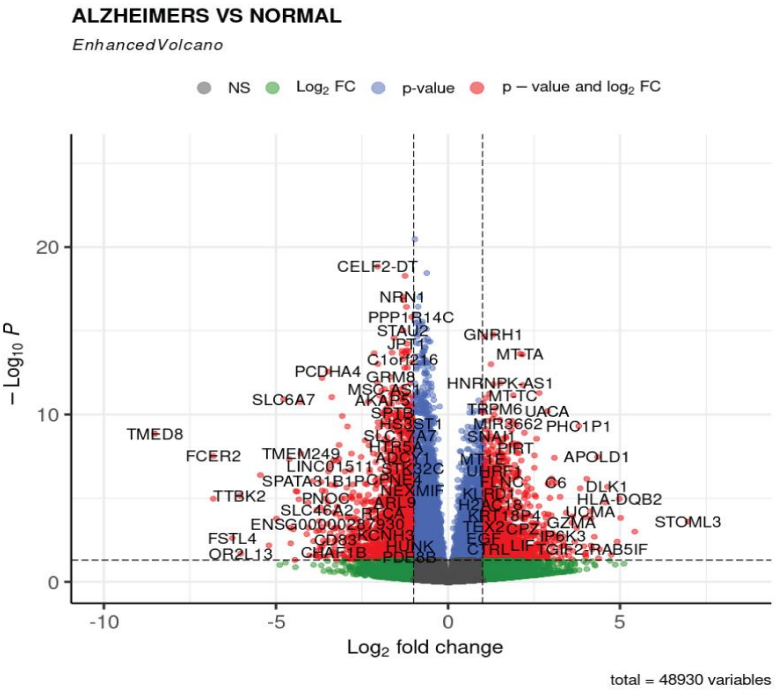


Fig. 1 (a) Enhanced volcano plot representing differentially expressed genes in AD. Red dots represent upregulated and downregulated genes. Biologically significant genes are shown on the x-axis w.r.t Log2FC ± 1, and statistically significant genes are shown on the y-axis w.r.t Log10 P-value < 0.05. **(b)** Enhanced volcano plot representing differentially expressed miRNAs in AD. Red dots represent upregulated and downregulated miRNAs. Biologically

significant miRNAs are shown on the x-axis w.r.t Log2FC ± 1, and statistically significant miRNAs are shown on the y-axis w.r.t Log10 P-value < 0.05

Table 1 Top 10 upregulated genes in AD.

Genes	P-value	logFC
STOML3	0.000250211216912787	6.97528076931273
DEFA3	0.000976918214962452	5.42613330801508
ENSG00000286679	0.000150795451621881	5.01847966659682
HLA-DQB2	1.07994912502603E-05	4.98238900639921
LINC03003	0.00390854262316584	4.91685180967915
ENSG00000227713	0.0245663666907518	4.7438430549598
DLK1	2.09335050401297E-06	4.63388626180104
GZMK	1.78972476802595E-05	4.56342371219177
FOXB1	0.0112465175548983	4.40483967741706
KRT16	0.0394371592272343	4.3718138104176

Table 2 Top 10 downregulated genes in AD.

Genes	P-value	logFC
TMED8	1.40337957447335E-09	-8.52202976812845
ZBTB8A	1.06254563853546E-05	-6.82221097770297
FCER2	3.09315866544678E-08	-6.82212359759237
FSTL4	0.00240566145281569	-6.27084471893716
TTBK2	7.17050723903973E-06	-6.04457022883382
OR2L13	0.0208472169257941	-6.02708295581613

STYK1	4.15894943440721E-07	-5.45210271981265
SPECC1L-ADORA2A	0.00657981554214427	-5.20332280983478
CADPS2	0.00016612249273122	-4.9847098458308
SLC6A7	1.24311023571547E-11	-4.77685606931429

GO term analysis of dysregulated genes

GO term and KEGG pathway analyses were performed using GeneCodis4 to analyze the dysregulated genes. GO term analysis identified the Biological processes (BP), Molecular Functions (MF), and Cellular Components (CC) of the dysregulated genes which were affected by AD. The BP analysis of upregulated genes showed that the overexpressed genes were enriched in the inflammatory response, immune response, blood vessel development, T cell activation, transmembrane receptor protein tyrosine kinase signaling pathway, positive regulation of cell population proliferation, chronic inflammatory response, peripheral nervous system development, forebrain dorsal/ventral pattern formation, regulation of cell death, programmed cell death involved in cell development and regulation of neuron death as shown in **Supplementary Information Figure S1**. Whereas the downregulated genes were enriched in chemical synaptic transmission, nervous system development, regulation of membrane potential, nervous system process, synaptic vesicle exocytosis, neuropeptide signaling pathway, synapse organisation, signal transduction, regulation of postsynaptic membrane potential, learning, neuron differentiation, brain development, neuron maturation, neuron development, memory, cognition, dendrite development, long term memory, oxygen transport, cellular response to increased oxygen levels and reactive oxygen species as shown in **Supplementary Information Figure S2**.

The MF analysis revealed that the upregulated genes were enriched in molecular functions like sequence-specific DNA binding, signaling receptor binding, kinase activity, interleukin-8 receptor binding, heat shock protein binding, complement component C4b binding, nerve growth factor receptor activity, interleukin-8 binding, interleukin-2 binding, interleukin-15 receptor activity, transcription factor binding, signaling adaptor activity, oxygen sensor activity, MHC class I receptor activity, chemokine receptor binding, oxidoreductase activity, monooxygenase activity, cytoskeletal motor activity, tumor necrosis factor-activated receptor activity, MAP kinase tyrosine/serine/threonine phosphatase activity, protein dimerization activity, hydrolase activity, unfolded protein binding, interleukin-1 receptor binding, peroxidase activity, protein kinase binding, MHC class I protein binding, antioxidant activity, tumor necrosis factor receptor binding, tau protein binding, scaffold protein binding and neurotransmitter receptor activity as shown in **Supplementary Information Figure S3**. Whereas, the downregulated genes were involved tau protein binding, tau-protein kinase activity, amyloid-beta binding, ion channel activity, voltage-gated ion channel activity, neurotransmitter receptor activity, extracellular ligand-gated ion channel activity, voltage-gated potassium channel activity, calmodulin binding, transmitter-gated ion channel activity involved in

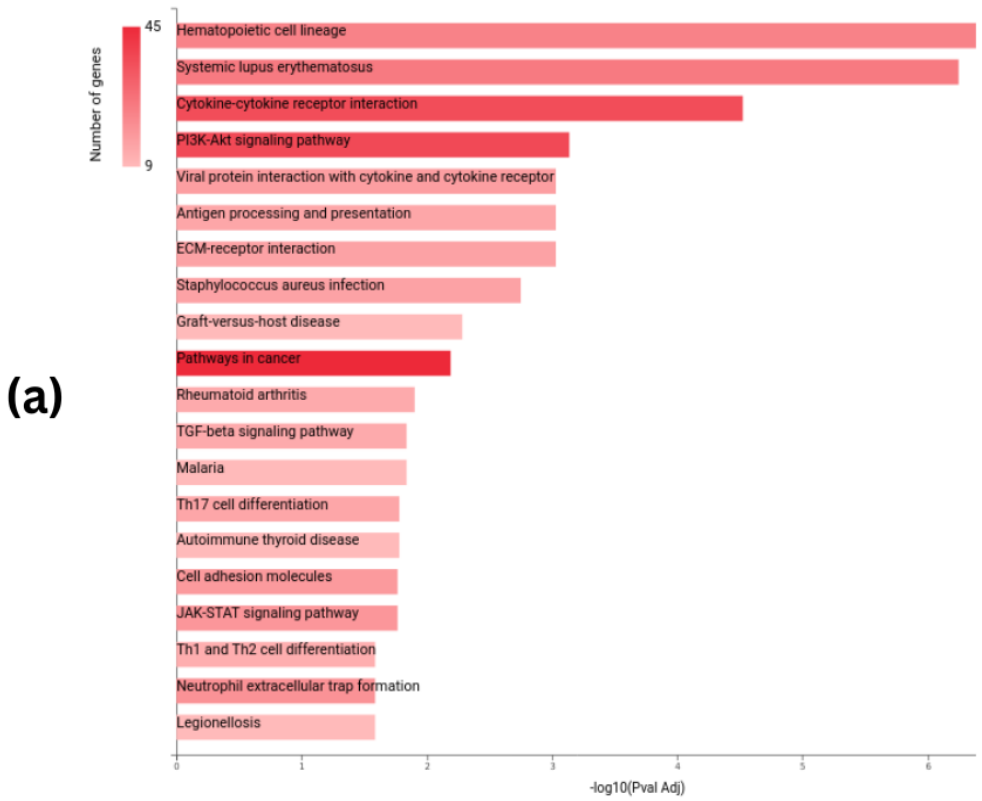
regulation of postsynaptic membrane potential, lysozyme activity, tumor necrosis factor-activated receptor activity, MAP-kinase scaffold activity, MAP kinase tyrosine/serine/threonine phosphatase activity, signaling adaptor activity, dioxygenase activity, growth factor activity, MAP kinase activity, protein kinase binding, protein kinase inhibitor activity, enzyme activator activity, cytokine activity, microtubule motor activity, Hsp90 protein binding, GTP-dependent protein binding, G-protein beta/gamma-subunit complex binding, cytokine binding, oxidoreductase activity, exonuclease activity and tumor necrosis factor receptor binding as shown in **Supplementary Information Figure S4**.

The CC analysis of upregulated genes showed that they were majorly located in the extracellular region, plasma membrane, collagen-containing extracellular matrix, extracellular space, cell surface, external side of the plasma membrane, nucleosome, clathrin-coated endocytic vesicle membrane, basement membrane, an integral component of membrane, blood microparticle, extracellular exosome, an integral component of the plasma membrane, extracellular matrix, integrator complex, chromatin, collagen trimer, collagen type IV trimer, IgG immunoglobulin complex and basal plasma membrane as shown in **Supplementary Information Figure S5**. In contrast, the downregulated genes were localized mostly in the synapse, cell junction, glutamatergic synapse, plasma membrane, postsynaptic membrane, neuron projection, axon, dendrite, an integral component of the plasma membrane, postsynaptic density, integral component of membrane, perikaryon, synaptic vesicle membrane, neuronal cell body, axon terminus, dendrite membrane, GABA-ergic synapse, postsynapse and an integral component of presynaptic membrane **Supplementary Information Figure S6**.

KEGG pathway analysis of dysregulated genes

The biological pathways interrupted due to the dysregulation of genes were identified through GeneCodis4. The overexpressed genes like WNT8B, NOX4, NDUFA4L2, LPL, FZD5, IL6, CSF1, FZD10, TRAF2, CR1L, IFNG, THBS4, ACKR1, IL6, VCAM1, CR1, CXCL8, THBS1 were involved in upregulating biological pathways such as hematopoietic cell lineage, cytokine-cytokine receptor interaction, TGF-beta signaling pathway, complement and coagulation cascades, MAPK signaling pathway, phagosome, IL-17 signaling pathway, necroptosis, TNF signaling pathway, p53 signaling pathway, chemokine signaling pathway, RAS signaling pathway, apoptosis, longevity regulating pathway, alzheimer disease, axon guidance, synaptic vesicle cycle, RNA degradation, parkinson disease, inflammatory mediator regulation of trp channels, RAP1 signaling pathway, chemical carcinogenesis reactive oxygen species, phosphatidylinositol signaling system, glioma, oxidative phosphorylation, neurotrophin signaling pathway and pathways of neurodegeneration multiple diseases as shown in **Figure 2(a)**. In contrast, underexpressed genes like ADCY1, ACTL6B, ADRB3, NDUFAF4, HTR5A, PDYN, NTSR1, GABRA1, CHRND, MAS1, CHRNA6, GABBR2, SSTR3, P2RX5, GPR83, CHRNA2, HTR1A, NPY2R, ADRA2A, AVPR2, GRM1, CHRNA3, GABRG2, UTS2B, ADORA2A, NPBWR1, KISS1R, GRM5 and CHRNA1 were involved in downregulating the pathways that included neuroactive ligand-receptor interaction, calcium signaling pathway, glutamatergic synapse, nicotine addiction, oxytocin signaling pathway, cAMP signaling pathway, synaptic vesicle cycle, MAPK signaling pathway, RAP1 signaling pathway, glucagon signaling pathway, HIF-1 signaling pathway, B cell receptor signaling pathway, neurotrophin signaling pathway, EGFR tyrosine kinase inhibitor resistance, histidine metabolism, metabolic

pathways, RIG-I-like receptor signaling pathway, thermogenesis, mismatch repair, autophagy, circadian rhythm and chemical carcinogenesis reactive oxygen species **Figure 2(b)**.


(a)
Number of genes
45
9
Hematopoietic cell lineage
Systemic lupus erythematosus
Cytokine-cytokine receptor interaction
PI3K-Akt signaling pathway
Viral protein interaction with cytokine and cytokine receptor
Antigen processing and presentation
ECM-receptor interaction
Staphylococcus aureus infection
Graft-versus-host disease
Pathways in cancer
Rheumatoid arthritis
TGF-beta signaling pathway
Malaria
Th17 cell differentiation
Autoimmune thyroid disease
Cell adhesion molecules
JAK-STAT signaling pathway
Th1 and Th2 cell differentiation
Neutrophil extracellular trap formation
Legionellosis
0
1
2
3
4
5
6
-log10(Pval Adj)

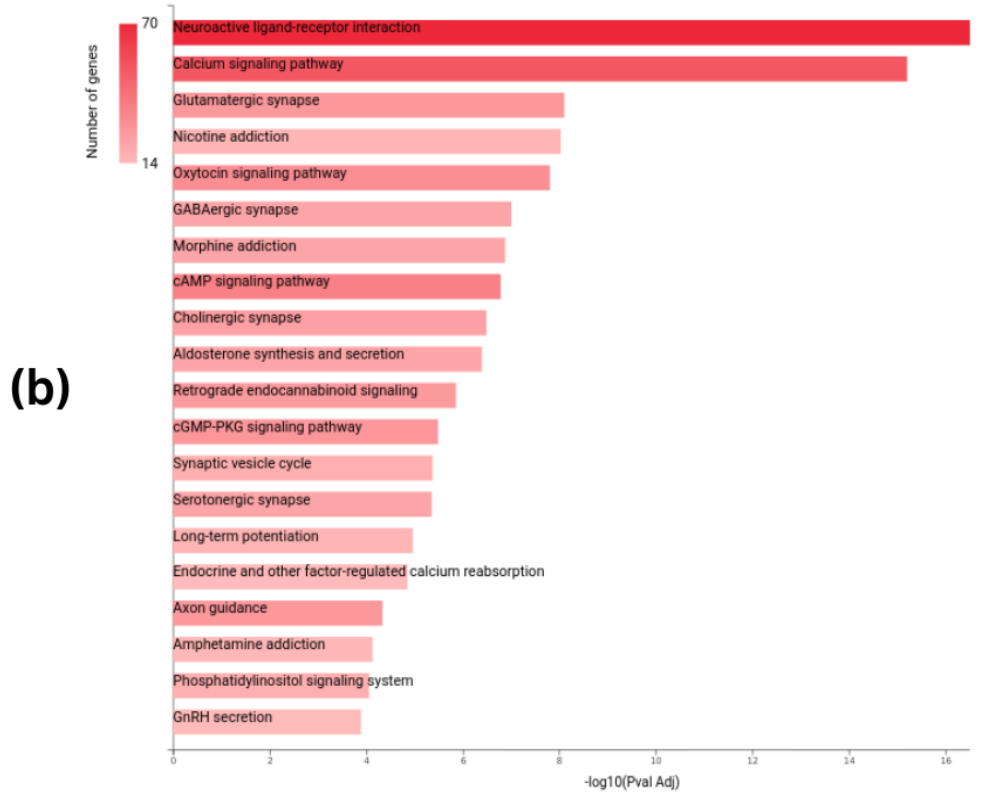

(b)
Number of genes
70
14
Neuroactive ligand-receptor interaction
Calcium signaling pathway
Glutamatergic synapse
Nicotine addiction
Oxytocin signaling pathway
GABAergic synapse
Morphine addiction
cAMP signaling pathway
Cholinergic synapse
Aldosterone synthesis and secretion
Retrograde endocannabinoid signaling
cGMP-PKG signaling pathway
Synaptic vesicle cycle
Serotonergic synapse
Long-term potentiation
Endocrine and other factor-regulated calcium reabsorption
Axon guidance
Amphetamine addiction
Phosphatidylinositol signaling system
GnRH secretion
0
2
4
6
8
10
12
14
16
-log10(Pval Adj)

Fig. 2 (a) KEGG pathway analysis of upregulated genes. Barchart plot of top 20 pathways in AD vs. Normal. The x-axis is the –log10(Pval Adj), while the gene number for each pathway is represented on the y-axis. It represents cytokine-cytokine receptor interaction, P13K-Akt signaling pathway as significant pathways. **(b)** KEGG pathways of downregulated genes. Barchart plot of top 20 pathways in AD vs. Normal. The x-axis is the –log10(Pval Adj), while the gene number for each pathway is represented on the y-axis. It represents neuroactive ligand-receptor interaction, calcium signaling pathway, and cAMP signaling pathway as significant pathways.

Identification of differentially expressed miRNAs

To Identify the mRNAs that are dysregulated by miRNAs, differential gene expression analysis of 70 Alzheimer's samples (n=48 disease; n=22 normal) was performed, which revealed a total of 58 dysregulated (upregulated and downregulated) miRNAs in Alzheimer's disease (AD) patients. It was observed that a total of 21 miRNAs were overexpressed with a fold change (FC) value > 1 and P-value < 0.05, while 37 miRNAs were underexpressed, showing FC value < -1 and P-value < 0.05 in AD. The biologically and statistically significant dysregulated miRNAs are shown in **Figure 1(b)**. Whereas, the top 10 upregulated and downregulated miRNAs with respect to logFC and P-value are shown in **Table 3** and **Table 4**, respectively. The top 10 significantly upregulated miRNAs are hsa-miR-1468-5p, hsa-miR-26b-3p, hsa-miR-4781-3p, hsa-let-7d-3p, hsa-miR-3127-3p, hsa-miR-6505-3p, hsa-miR-6762-3p, hsa-miR-5006-3p, hsa-miR-4661-5p and hsa-miR-561-5p and the top 10 downregulated miRNAs are hsa-miR-3200-3p, hsa-let-7f-5p, hsa-miR-148a-3p, hsa-miR-15a-5p, hsa-miR-17-3p, hsa-miR-144-5p, hsa-let-7g-5p, hsa-miR-660-5p, hsa-miR-144-3p and hsa-miR-199a-3p

Table 3 Top 10 upregulated miRNAs in AD

miRNA	P-value	logFC
hsa-miR-1468-5p	2.86903707148824E-08	1.37521741547912
hsa-miR-26b-3p	5.57825470811302E-08	1.06245716019891
hsa-miR-4781-3p	5.81444160995306E-08	1.04541352203666
hsa-let-7d-3p	5.07044705151362E-05	1.0568278344522
hsa-miR-3127-3p	7.53808424822284E-05	1.31741274516372
hsa-miR-6505-3p	0.000705436371462794	1.54603793453981
hsa-miR-6762-3p	0.00178536280645917	1.21359416351977
hsa-miR-5006-3p	0.0023937375603115	1.05746776037572
hsa-miR-4661-5p	0.00615059897706512	1.21104000197341

hsa-miR-561-5p	0.00728570335869978	2.06921400973115

Table 4 Top 10 down regulated miRNAs in AD

miRNA	P-value	logFC
hsa-miR-3200-3p	3.10235758675113E-10	-1.66979450865524
hsa-let-7f-5p	1.24344249402478E-09	-1.04204970862786
hsa-miR-148a-3p	8.26182068364948E-09	-1.60874245092525
hsa-miR-15a-5p	2.28972446698426E-08	-1.37265786687261
hsa-miR-17-3p	5.69872387183754E-08	-1.48894497751568
hsa-miR-144-5p	6.90236195844793E-08	-1.77003843591524
hsa-let-7g-5p	3.40832067377588E-07	-1.05818946420444
hsa-miR-660-5p	6.41796276603568E-07	-1.48330166025788
hsa-miR-144-3p	1.61527348206846E-06	-2.26743242362227
hsa-miR-199a-3p	6.06054363908788E-06	-1.39167512565558

miRNA-mRNA Expression Correlation Analysis

To identify the targets of 21 upregulated miRNAs involved in the downregulation of mRNA, the miRWalk database was used. Each miRNA was searched individually for a set of target genes, and the targets were retrieved. The target genes were then filtered based on binding point = 1, binding energy > -30, and AU < 0.6, and a filtered file was generated for each miRNA, and then it was compared with a list of significantly down regulated mRNAs identified in this study (Figure 1(a)) to retrieve common mRNAs using the Venny tool. The number of common genes between the filtered target genes and the significantly down regulated mRNAs is shown in Table 5.

Table 5 shows the list of upregulated miRNAs, their target and filtered genes, and common downregulated mRNA targets

miRNAs	Target genes	Filtered genes	Common mRNA targets

hsa-let-7d-3p	11,982	7	1
hsa-miR-23c	7,924	1	1
hsa-miR-26b-3p	13,206	24	0
hsa-miR-103a-2-5p	15292	59	3
hsa-miR-561-5p	2182	0	0
hsa-miR-758-3p	12,960	13	2
hsa-miR-1233-3p	15,436	441	28
hsa-miR-1468-5p	14,082	38	3
hsa-miR-1908-3p	14,434	2057	130
hsa-miR-3127-3p	16,499	1257	78
hsa-miR-3180-5p	13,857	800	59
hsa-miR-4661-5p	14,523	26	3
hsa-miR-4772-3p	12,228	9	0
hsa-miR-4781-3p	8,183	2	0
has-miR-5006-3p	15,220	104	9
hsa-miR-5701	1,620	0	0
hsa-miR-6505-3p	11,936	5	0
hsa-miR-6754-3p	16410	1206	77
hsa-miR-6762-3p	17,308	1720	117
hsa-miR-6866-3p	13,026	10	1
hsa-miR-7155-3p	11,421	11	1

Interaction between miRNA-mRNA and Hub Genes Identification

To identify miRNAs that have the most interactions with target genes that may have the potential to dysregulate the target mRNA genes, a network analysis was performed through the cytohubba module of Cytoscape. Based on the network analysis results, the top 5 hub miRNAs and their mRNA genes were identified that are hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p, and hsa-miR-3180-5p, presented in (Figure 3(a), Table 6). It provided valuable information about the miRNAs' functional relationships and potential interactions. These hub miRNAs target multiple genes, play a crucial role in the network, and are likely to have significant functional and regulatory implications.

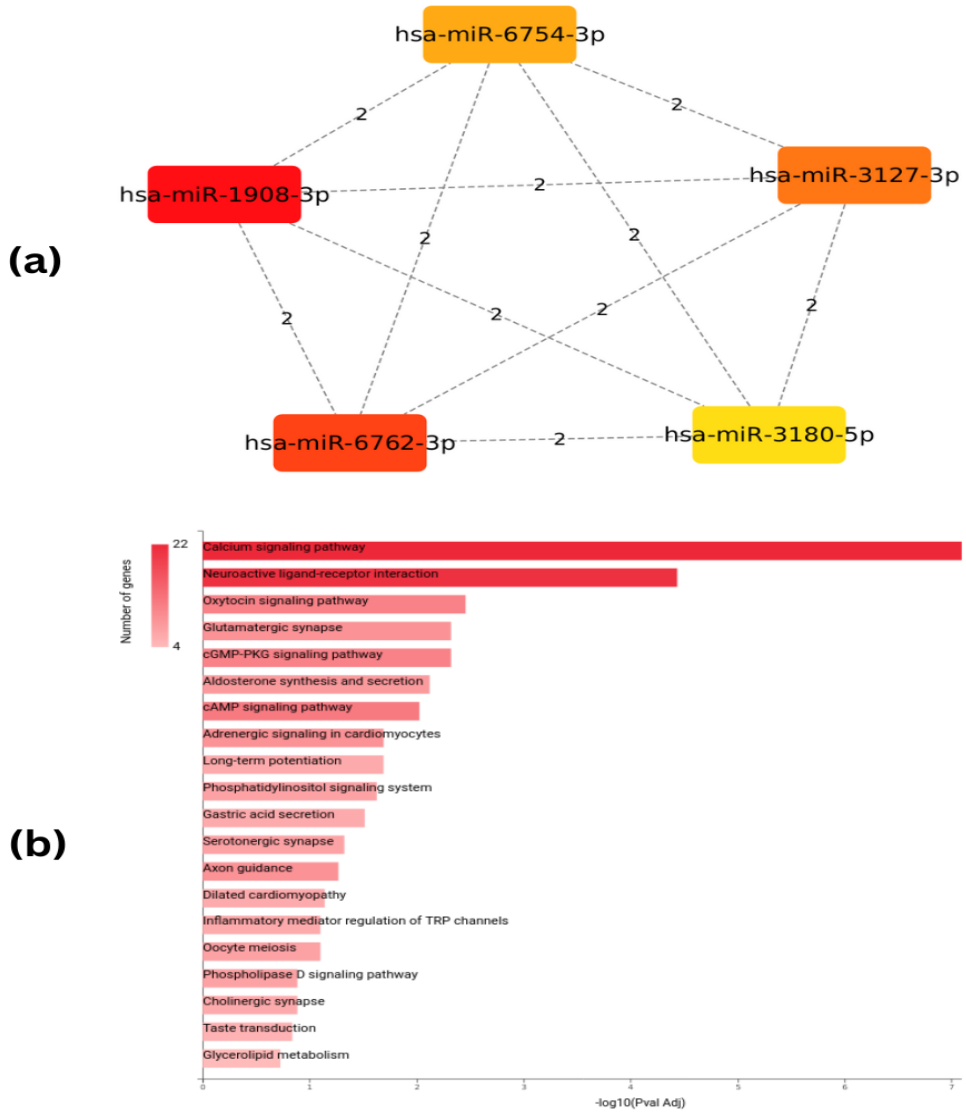


Fig. 3 (a) The top 5 upregulated hub miRNAs with the most number of interactions with other mRNAs were identified through miRNA-mRNA interaction network analysis based on miRNA-seq data. The darker color represents more interactions within the same network. **(b)** KEGG pathway analysis of miRNA-mRNA target genes. Barchart plot of

top 20 pathways in AD vs. Normal. The x-axis is the –log10(Pval Adj), while the gene number for each pathway is represented on the y-axis. It represents the calcium signaling pathway, neuroactive ligand-receptor interaction, and cAMP signaling as significant pathways.

Table 6 Enlists top 5 miRNAs in the miRNA-mRNA network ranked by Degree method. The rank tells about the position, while the score represents the number of mRNA genes targeted by the miRNA.

Rank	Name	Score
1	hsa-miR-1908-3p	130
2	hsa-miR-6762-3p	117
3	hsa-miR-3127-3p	78
4	hsa-miR-6754-3p	77
5	hsa-miR-3180-5p	59

GO term analysis of the miRNA-mRNA targets

To identify the BP and MF of the top 5 miRNA targets, GO term analysis was performed using GeneCodis4. The BP analysis showed that they were enriched in chemical synaptic transmission, regulation of synaptic plasticity, nervous system development, signal transduction, regulation of synaptic vesicle cycle, neuromuscular synaptic transmission, synaptic vesicle priming, regulation of transmembrane ion transport, axon guidance, learning, regulation of postsynaptic membrane potential, regulation of synaptic transmission, axon choice point recognition, synaptic vesicle endocytosis, postsynapse organization, synaptic vesicle maturation, dendrite development, neurofilament bundle assembly, negative regulation of axonogenesis, neurotransmitter secretion, dendritic spine development, protein localisation to the synapse, synaptic vesicle membrane organization, long-term memory, regulation of long-term neuronal synaptic plasticity and regulation of neuron migration as shown in **Table 7** and **Supplementary Information Figure S7**. The MF analysis revealed that the miRNA-mRNA interactions were enriched in molecular functions like voltage-gated ion channel activity, ligand-gated ion channel activity, EH domain binding, adenylate cyclase inhibiting G protein-coupled glutamate receptor activity, neurotransmitter receptor activity, kinase activity, structural constituent of the presynaptic active zone, glutamate receptor activity, somatostatin receptor activity, NAD+ kinase activity, calcium channel activity, G protein-coupled serotonin receptor activity, transmembrane-ephrin receptor activity and potassium channel activity as shown in **Table 8** and **Supplementary Information Figure S8**.

Table 7 lists significant biological processes dysregulated by miRNA-mRNA interactions

Description	P-value	Top genes
chemical synaptic transmission	1.48E-09	HTR3B, NTSR1, FGF12, CHRND, SV2B
regulation of synaptic plasticity	5.04E-07	ERC2, YWHAG, PPP3CB, JPH4, SYNGAP1
nervous system development	2.31E-06	SRRM4, TRNP1, FGF12, EFNB3, RET
regulation of synaptic vesicle cycle	1.16E-05	DNAJC5, PRKAR1B, BSN, RAB3B
neuromuscular synaptic transmission	4.51E-05	RIMBP2, ERC2, RIMBP3C, EGR3, STXBP1
synaptic vesicle priming	8.62E-05	ERC2, SYNJ1, UNC13A, STXBP1
axon guidance	0.000119	NRXN3, EFNB3, RET, LRTM2, GAP43
learning	0.000154	NTSR1, NRXN3, SYNJ1, SLC12A5, PPP3CB
regulation of postsynaptic membrane potential	0.000225	CHRND, GRM1, GRIN3A, KCNA1, ADRB1
axon choice point recognition	0.000317	EFNB3, GAP43
synaptic vesicle endocytosis	0.000689	SYNJ1, PIP5K1C, SYP, AMPH, PACSIN1
postsynapse organization	0.000833	SPTBN2, CNKSR2, SHANK1
synaptic vesicle maturation	0.000833	SYP, UNC13A, STXBP1
dendrite development	0.000965	GRIP1, PAK3, SYNGAP1, GRIN3A, LAMC2

neurofilament bundle assembly	0.00094	NEFH, NEFM
negative regulation of axonogenesis	0.001096	EFNB3, SYNGAP1, THY1
neurotransmitter secretion	0.001449	SYN3, UNC13A, STXBP1, GRM4
dendritic spine development	0.001407	SLC12A5, PAK3, CAMK2A
long-term memory	0.003359	ADCY1, RASGRF1, SHANK1, NPAS4
regulation of neuron migration	0.004403	CAMK2A, NTNG1, SCRT2

Table 8 lists significant molecular functions dysregulated by miRNA-mRNA interactions

Description	P-value	Top genes
voltage-gated ion channel activity	0.00014	KCNH1, KCNH5, CACNA1E, CACNB2, KCNA2,
calcium ion binding	7.53E-05	CALM3, DGKB, FBN3, RET, TCHH
calmodulin binding	9.94E-05	KCNH1, GAP43, KCNH5, ADCY1, PPP3CB
ligand-gated ion channel activity	0.000445	HTR3B, CHRND, CNGB1, GRIN3A, ANXA6
EH domain binding	0.000345	SYNJ1, EPN3

adenylate cyclase inhibiting G protein-coupled glutamate receptor activity	0.000494	GRM1, GRM7, GRM4
neurotransmitter receptor activity	0.000899	HTR3B, CHRND, HTR1A, HTR4, HTR6
kinase activity	0.000736	CALM3, BUB1B, HUNK, DCLK3, STK32C
diacylglycerol kinase activity	0.000943	DGKB, DGKZ, AGK
voltage-gated potassium channel activity	0.001304	KCNH1, KCNK9, KCNH5, KCNA2, LRRC38
structural constituent of presynaptic active zone	0.003327	ERC2, BSN
glutamate receptor activity	0.002988	GRM1, GRM7, GRM4
somatostatin receptor activity	0.003327	SSTR3, SSTR2
structural constituent of cytoskeleton	0.002803	PPL, SPTB, SPTBN2, ADD2, NEFH
ion channel activity	0.002809	HTR3B, KCNH1, CHRND, KCNH5
NAD+ kinase activity	0.003578	DGKB, DGKZ, AGK
calcium channel activity	0.00223	CACNA1E, CACNB2, GRIN3A, CACNG8, CACNB3

G protein-coupled serotonin receptor activity	0.003517	HTR1A, HTR4, HTR6, CHRM2
transmembrane-ephrin receptor activity	0.003578	EFNB3, EPHA10, EPHA6
potassium channel activity	0.004784	KCNH1, KCNK9, KCNH5, KCNA2, KCNK12

KEGG pathway analysis of miRNA-mRNA targets

The biological pathways interrupted due to the dysregulation of the top 5 miRNA-mRNA targets were identified through GeneCodis4. The miRNAs hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p, and hsa-miR-3180-5p were enriched in pathways such as neuroactive ligand-receptor interaction, cAMP signaling pathway, axon guidance, Inflammatory mediator regulation of TRP channels, gap junction, MAPK signaling pathway, Ras signaling pathway, glioma, synaptic vesicle cycle, long-term depression, glycosaminoglycan degradation, Rap1 signaling pathway, neurotrophin signaling pathway, PI3K-Akt signaling pathway, pathways in cancer, autophagy, pathways of neurodegeneration, insulin signaling pathway, pertussis, Parkinson's disease, mRNA surveillance pathway, apoptosis and Alzheimer's disease (**Figure 3(b)**, **Table 9**).

Table 9 lists significant KEGG pathways dysregulated by miRNA-mRNA interactions

Description	P-value	Top genes
Calcium signaling pathway	5.44857999692531E-11	CALM3, FGF9, FGF22, NTSR1, RET
Neuroactive ligand-receptor interaction	3.78539085677113E-07	NTSR1, CHRND, MAS1, SSTR3, HTR1A
Oxytocin signaling pathway	5.48456863355718E-05	CALM3, ADCY1, PPP3CB, CACNB2, CAMK2A
Glutamatergic synapse	0.000124547465268 81	ADCY1, PPP3CB, GRM1, GRIN3A, GRM7

cGMP-PKG signaling pathway	0.000114298625194497	CALM3, ADCY1, PPP3CB, PRKCE, CNGB1
Aldosterone synthesis and secretion	0.000237291283343143	CALM3, KCNK9, ADCY1, PRKCE, CAMK2A
cAMP signaling pathway	0.000343764979022131	CALM3, ADCY1, HTR1A, CNGB1, CAMK2A
Adrenergic signaling in cardiomyocytes	0.000955875187539909	CALM3, ADCY1, CACNB2, CAMK2A, CACNG8
Long-term potentiation	0.000894573241593521	CALM3, ADCY1, PPP3CB, GRM1, CAMK2A, ITPR1
Phosphatidylinositol signaling system	0.00122449186064002	CALM3, DGKB, SYNJ1, PIP5K1C, PLCD4, DGKZ
Gastric acid secretion	0.00173160537837671	CALM3, ADCY1, CAMK2A, SSTR2, CCKBR, ITPR1
Serotonergic synapse	0.00294629798031653	HTR3B, TPH2, HTR1A, HTR4, KCNJ6, HTR6, ITPR1
Axon guidance	0.00361807481834624	SRGAP3, EFNB3, PPP3CB, PAK3, SEMA3E, CAMK2A
Dilated cardiomyopathy	0.00530626811087242	ADCY1, CACNB2, CACNG8, CACNB3, SLC8A1

Discussion

AD is a degenerative disease of neurons that negatively hampers the brain's functioning and impacts its physical structure [19]. Hence, leading to cognitive impairment, dementia, and memory loss. The molecular and cellular changes resulting in Alzheimer's are the augmentation of beta-amyloid protein outside the neurons, while the twisted strands of tau protein inside the neurons are reported to be the hallmarks of AD [20]. Consequently, it causes synaptic loss, disrupted neuronal signaling, death of neurons, inflammation, atrophy, and damage to brain tissue [21]. According to the latest report, Alzheimer's is the most frequent cause of dementia, with approximately 60% to 80% of cases reported globally [22]. Therefore, understanding the fundamental molecular mechanisms involved in Alzheimer's is necessary to identify inflammatory biomarkers that cause inflammation and dysfunction of neurons and to develop potential therapeutic interventions. Thus, to elucidate potential therapeutic targets, RNA sequencing is a gold-standard quantitative tool for differential expression analysis that identifies the expression level of miRNA and mRNA with greater accuracy and higher efficiency [23].

To identify the miRNAs involved in the dysregulation of mRNAs, differential expression analysis (DEA) was performed on miRNA and mRNA datasets. The DEA of the RNA-seq dataset identified a total of 2973 dysregulated mRNA genes in AD patients. Among these, 1453 genes were overexpressed, while 1520 genes were underexpressed. Among the top 10 downregulated genes included CALM3, CAMK2A, CAMK1D, CALM3, FGF9, FGF22, RET, ADCY1, CALM3, PPP3CB, ITPR1 that were involved in cellular signaling, neuronal development, synaptic plasticity, and organogenesis [24]. Whereas the DEA of miRNA dataset identified a total of 58 dysregulated miRNAs in AD patients. It was observed that 21 miRNAs were overexpressed, while 37 miRNAs were underexpressed. The top 10 significantly upregulated miRNAs are hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p, hsa-miR-3180-5p, hsa-miR-103a-2-5p, hsa-miR-1233-3p, hsa-miR-1468-5p, hsa-miR-4661-5p and hsa-miR-5006-3p that may play a role in the progression of cancer, inflammation, neuronal development, cell proliferation, immune response, insulin signaling, inflammatory responses, autoimmune diseases, and immune regulation. The dysregulation of these mRNAs and miRNAs contributes to neuronal dysfunction, leading to AD progression [25].

Identification of miRNA-mRNA correlation, network, and hub genes analysis revealed the top 5 upregulated hub miRNAs involved in downregulating several targeted mRNAs. Moreover, functional enrichment analysis of the miRNA-mRNA targets was performed. It indicated that these miRNAs were mostly involved in the dysregulation of biological pathways and processes such as chemical synaptic transmission, regulation of synaptic plasticity, nervous system development, signal transduction, neuromuscular synaptic transmission, axon guidance, learning, dendrite development, neurofilament bundle assembly, negative regulation of axonogenesis, neurotransmitter secretion, dendritic spine development, long-term memory, regulation of long-term neuronal synaptic plasticity and regulation of neuron migration. Dysfunctioning of synapses and impairment in the transmission of information between neurons play a role in AD [26]. Dysregulation of pathways such as synaptic plasticity, signal transduction and nervous system development leads to the decline of cognitive ability [27]. Deformity in neuromuscular synaptic transmission, axon guidance and dendrite development negatively impact the connectivity of neurons [28]. Dysregulation of all these

pathways, along with other processes like learning and memory disorders, neurofilament bundle assembly, and regulation of neuron migration, lead to the development of AD [29].

Genes such as NTSR1, FGF12, CHRND, SV2B, SLC12A5 and CACNA1E are involved in biological processes such as chemical synaptic transmission. Due to the dysfunction of these genes, synaptic loss occurs, which leads to neurodegeneration and dementia. In patients with AD, healthy synapses are lost and act as an early sign of neuronal dysfunction and progression of AD [30].

Regulation of synaptic plasticity and long-term neuronal synaptic plasticity processes are disrupted due to the dysfunctioning of genes such as YWHAG, PPP3CB, JPH4, SYNGAP1, RASGRF1, LZTS1, SYP and SYNGR1. Due to the dysregulation of these genes, impairment of synaptic plasticity occurs. Synaptic plasticity is the mechanism through which neurons modify the structure or composition of synapses according to neural functions [31]. Abnormalities in synaptic activities are activated by depositing β-amyloid (Aβ) oligomers that bind to presynaptic and postsynaptic neurotransmitters [32]. These abnormalities lead to a gradual decrease in cognitive function and the onset of AD [33].

Similarly, genes such as PAK3, SYNGAP1, GRIN3A, LAMC2 and CAMK2A play a role in dendrite and dendritic spine development. Dendritic spines are physical structures where synapses take place. Dysregulation of these genes leads to changes in the mechanisms, such as loss of dendritic spines that are directly linked with loss of synaptic activities, and synaptic failure is the direct cause of the decline in memory and cognitive abilities in AD [34,35].

Likewise, genes like TRNP1, FGF12, EFNB3, RET, GAP43 and PCSK2 are involved in nervous system development. Dysregulation of these genes leads to inflammation of neurons in AD, and neuron death occurs throughout the brain, which causes the breakdown of connections between the network of neurons, and the brain region starts to shrink, and a significant volume of the brain is lost, known as brain atrophy [36].

Moreover, genes like RASGRF1, SHANK1 and NPAS4 are involved in regulating long-term memory processes, but due to the dysregulation of these genes, the area of the brain which is responsible for creating and retrieving memories is damaged and causes dementia or memory loss and is one of the first symptoms reported among AD patients [37,38].

Furthermore, this study also revealed the top 5 upregulated hub miRNAs i.e., hsa-miR-1908-3p, hsa-miR-6762-3p, hsa-miR-3127-3p, hsa-miR-6754-3p and hsa-miR-3180-5p that were inhibiting the role of mRNAs and thus, were involved in the progression of AD. The miRNA, hsa-miR-1908-3p, is reported to be upregulated in breast cancer and reproductive system diseases but is not reported in the literature in terms of AD. However, the miR-1908-5p is found to be upregulated in cancers and other diseases like AD, glioma, bipolar disorder and unipolar disorder. Furthermore, hsa-miR-6762-3p miRNA was reported to be upregulated and was influencing the expression of genes associated with AD [39]. However, some studies were reported to show the role of miR-6762 in dementia and AD [40,41]. Thus, this miRNA can be proved as a potential biomarker to help diagnose AD and predict diagnosis. Likely, hsa-miR-3127-3p miRNA is reported in the expression analysis of AD [42]. Whereas miR-3127 was dysregulated in multiple tumor types and has an important role in tumorigenesis and cancer progression [43]. Some studies reported that miR-3127-5p was down-regulated in lung cancer tissues [44]. Furthermore, hsa-miR-6754-3p was dysregulated

in intracranial aneurysms [45], while miR-6754 was dysregulated in AD. Moreover, hsa-miR-3180-5p was reported in the expression profile of miRNA in AD and was dysregulated in a COVID-19 study [46], whereas miR-3180 was reported to be differentially expressed in an insulin-resistant human hepatocellular carcinoma cell line [47].

KEGG pathway enrichment analysis showed that DE miRNA-mRNA targets were significantly associated with neuroactive ligand-receptor interaction, cAMP signaling pathway, inflammatory mediator regulation of TRP channels, MAPK signaling pathway, Ras signaling pathway, synaptic vesicle cycle, neurotrophin signaling pathway, autophagy, pathways of neurodegeneration, Parkinson's disease, mRNA surveillance pathway, apoptosis and Alzheimer's disease pathways leading to abnormal cell growth, synaptic dysfunction, neurodegeneration and pathogenesis of AD [48].

This study found that NTSR1, CHRND, MAS1, SSTR3, HTR1A, and GRM1 miRNA-mRNA targets were enriched in the neuroactive ligand-receptor interaction pathway. This pathway plays a significant role in maintaining the function of neurons by regulating transcription factors and gene expression. The dysregulation of the genes involved in the neuroactive ligand-receptor interaction pathway can reduce memory function [49].

It has been reported that genes CALM3, ADCY1, HTR1A, CNGB1 and CAMK2A are involved in the cAMP signaling pathway. Dysregulation of these genes causes disruption in the pathway and is associated with neuropathology in AD. The cAMP response element binding protein (CREB) pathway is involved in synaptic function and cognition. It enhances cognitive processes like memory and learning and is required for a healthy connection between neurons. In neurodegenerative diseases like Alzheimer’s, there is an overproduction of another protein called GSK3β which inhibits the activity of CREB. This disruption causes a loss of connection between neurons and hence causes impairment in cognitive abilities in AD [50].

Likewise, genes such as CALM3, ADCY1, PRKCE, CAMK2A and BDKRB2 are involved in the inflammatory mediator regulation of TRP channels. The dysregulation of these genes causes neurotoxicity in AD that is associated with the production of inflammatory moderators that are linked with cellular pathways such as TRP channels [51].

Moreover, genes like FGF9, FGF22, PPP3CB, CACNA1E and CACNB2 play a role in the mitogen-activated protein kinase (MAPK) signaling pathway. The regulation of neuronal apoptosis, β- and γ-secretase activity, and phosphorylation of amyloid precursor protein and tau protein is caused due to the activation of MAPK cascades that cause AD progression [52].

Furthermore, genes like CALM3, FGF9, FGF22, RASAL1, and PAK3 play a role in the Ras signaling pathway that utilizes Amyloid Precursor Protein and Amyloid Beta protein for activation of Ras-ERK cascade and glycogen synthase kinase 3 (GSK-3) signaling that helps in neurodegeneration in AD [53].

Moreover, UNC13A, STXBP1 and SLC6A7 genes play a role in the synaptic vesicle cycle (SVC). SVC is a prime site for the production of amyloid β and toxicity and is crucial for signaling, allowing entrance to various pathogens and toxins that have deleterious effects in AD [54].

Additionally, CALM3, PPP3CB, SPTBN2, GRM1 and CAMK2A genes are involved in pathways of neurodegeneration. Dysregulation of these genes causes aggregation of toxic proteins, such as alpha-synuclein, beta-

amyloid plaques, Tau tangles, altered neurotransmission of dopamine, acetylcholine and TDP-43 that play role in neurodegenerative diseases such as AD [55].

Lastly, CALM3, PPP3CB, and ITPR1 play a role in Alzheimer's disease pathway. The dysregulation of these genes causes imbalance and damage of neurons accumulating over years that finally lead to neuron death in areas of the brain that are responsible for learning, memory and cognition by apoptosis [56].

Thus, this study provides key insights into the upregulated miRNA and its effect on the targeted mRNA genes. Moreover, a novel set of 2 miRNAs (hsa-miR-1908-3p and hsa-miR-6754-3p) have been identified that were not reported previously and are involved in the progression and development of AD.

Conclusion

In conclusion, this study elucidated the dysregulated molecular landscape of AD, highlighting the role of dysregulated miRNAs and mRNAs in disease progression. The identified miRNAs and genes were associated with key biological pathways involved in synaptic function, neurodevelopment, inflammation, and neurodegeneration. The findings support the importance of understanding the fundamental molecular mechanisms of AD to identify potential therapeutic targets and biomarkers for diagnosis and treatment. The novel miRNAs, hsa-miR-1908-3p and hsa-miR-6754-3p, identified in this study expand the knowledge of miRNA involvement in AD. Further research on these miRNAs and their specific targets may provide valuable insights into AD pathogenesis and offer new avenues for intervention and management of the disease.

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