

Genetic insights and molecular pathways in Alzheimer's disease: Unveiling the complexity of neurodegeneration

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by the accumulation of amyloid-beta plaques and tau tangles in the brain. We conducted a comprehensive Differential Gene Expression (DGE) analysis using RNA sequencing and microarray datasets from the Gene Expression Omnibus (GEO) database to elucidate the molecular mechanisms underlying AD. Forty-three datasets, encompassing 902 samples from various biological sources, were analysed. The study identified 157 frequently upregulated and 177 down-regulated genes associated with AD. Upregulated genes include *DDX3Y*, *H6PD* and *KIF1B*, while down-regulated genes include *CREM*, *CD44*, and *HES4*. Functional enrichment analysis using Enrichr revealed significantly upregulated pathways, including the PI3K-Akt signalling and Wnt signalling pathways. The downregulated pathways include the immune system and TNF signalling pathway. Network analysis with STRING identified key interactive genes, including *MYC*, *HSP90AB1* and *CENPA* among the upregulated genes, and *ENO1*, *RPLP0*, and *RPS3A* among down-regulated genes. Additionally, the research focused on identifying transcription factors and miRNAs associated with AD, revealing critical regulatory elements influencing disease progression. These findings provide insights into the dysregulated pathways, key genes, and regulatory mechanisms involved in AD, offering potential targets for therapeutic intervention.

1. Introduction

Dementia refers to a diverse group of neurodegenerative diseases. The primary symptoms include a significant decline in cognitive function, which disrupts the ability to engage in daily tasks. Among the dementia, Alzheimer's Disease (AD) stands out as the prevailing type, making up 80 % of all Dementia instances [1]. According to different estimations, the number of patients with AD will increase from 26.6 million worldwide in 2006 to an alarming rate of 107 million by 2050 [2]. The biological definition of AD is the existence of plaques that contain β -amyloid and tangles containing tau in the brain [3]. As the disease progresses, the entorhinal area, hippocampus, amygdala and associative parts of the neocortex are affected by macroscopic atrophy [4].

Although much work has been done examining AD's pathological and molecular mechanisms using different techniques such as animal models, expression profiling, genome-wide association studies, neuro-imaging methods, system biology framework, etc., the actual cause of AD still needs to be well understood [5]. Like many other complicated

disorders, AD is influenced by different factors such as lifestyle, environmental and hereditary factors [6]. However, when comparing all these factors, genetics plays a significant role in AD development.

A rare kind of AD called Early-Onset Familial Alzheimer's Disease (FAD) is caused by the mutations of three different genes such as APP, PSEN1, and PSEN2. These genes code for amyloid precursor protein, presenilin-1 and presenilin-2, respectively, which cause symptoms of the disease that appear in people usually in their 30s, 40s, or 50s [7]. The most common type of Alzheimer's is late-onset Alzheimer's Disease (LOAD), which usually develops after 65 years of age and is impacted by both environmental and genetic factors. The most significant genetic risk factor for LOAD is the APOE ϵ 4 allele. The risk of getting this AD is increased by inheriting one copy of APOE ϵ 4 and much more by inheriting two copies of APOE ϵ 4 [8]. While genetic factors are recognised as an important cause in the development of AD, our understanding of their contribution remains incomplete.

To bridge this gap, our research focuses on understanding the differential gene expression, signalling pathway analysis, miRNA regulation, protein-protein interactions (PPI) and distant regulatory elements

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Table 1

Description of the AD datasets used in the study.

SI NO:	TITLE	GSE NO:	COUNTRY	SAMPLE NO:	SOURCE
1	Modeling early-onset sporadic Alzheimer's disease using iPSC-derived neurons	GSE231341	USA	24	Skin
2	Music compensates for altered gene expression in age-related cognitive disorders	GSE239282	Spain	60	Capillary blood
3	SPLICER: A Highly Efficient Base Editing Toolbox That Enables in vivo Exon Skipping for Targeting Alzheimer's Disease [RNA-seq]	GSE246587	USA	6	Kidney
4	Long RNA profiles of human brain extracellular vesicles in Alzheimer's disease	GSE197505	China	18	Brain frontal cortex
5	Human Microglial State Dynamics in Alzheimer's Disease Progression [RNA-seq]	GSE227221	USA	12	IPSCs
6	MEG3 activates necroptosis in human neuron xenografts modeling Alzheimer's disease	GSE195455	Belgium	12	H9-Stem cell derived neurons
7	Choroid plexus mis-splicing and altered cerebrospinal fluid composition in myotonic dystrophy type 1 [human]	GSE228458	USA	13	Choroid plexus lateral
8	Identification of genes associated with Periodontal pathogens induced endothelial dysfunction using RNA-seq data	GSE222136	South Korea	9	Brain
9	Functional characterisation of Alzheimer's disease genetic variants in microglia	GSE206478	USA	8	WTC11 iPSC-derived microglia
10	APOE Expression and Secretion are Modulated by Copper-Dependent and -Independent Mitochondrial Dysfunction	GSE201889	USA	12	HAP1
11	Up-regulated c-JUN induces aberrant transposable element mobilisation, cGAS-STING activation and cell death in Alzheimer's disease	GSE213610	Italy	61	HpNPCs
12	Recapitulation of pathophysiological features of AD in SARS-CoV-2 infected subjects	GSE236562	USA	32	Brain
13	Transcriptomic profiling of sporadic Alzheimer's disease patients	GSE203206	USA	47	Occipital Lobe
14	BisDemethoxyCurcumin (BDC) loaded H-Ferritin-Nanocages Mediate Regulation of inflammation in Alzheimer's Disease Patients	GSE203408	Italy	23	Blood
15	Microarray analysis of the astrocyte transcriptome in the ageing brain: relationship to Alzheimer's pathology and ApoE genotype	GSE29652	United Kingdom	18	JS1-18 temporal cortex
16	Differential Neuropathology, Genetics, and Transcriptomics in two kindred cases with Alzheimer's Disease and Lewy Body Dementia	GSE193438	Italy	48	Basal ganglia
17	Transcriptomes in Peripheral Blood Mononuclear Cells of Dementia and Alzheimer Patients	GSE18309	Taiwan	9	peripheral blood mononuclear cells (AD and MCI patients)
18	Alzheimer disease diagnostic prediction using peripheral BMC gene expression	GSE4229	Canada	40	Peripheral blood
19	Expression of mRNAs Regulating Synaptic Function and Neuroplasticity in Incipient AD	GSE12685	USA	14	Frontal cortex synaptoneurosome
20	Expression of mRNAs Regulating Synaptic Function and Neuroplasticity in Incipient AD	GSE180793	USA	14	iPSC derived neurons
21	The Alzheimer's gene SORL1 is a key regulator of endosomal recycling in human neurons	GSE95673	USA	11	hiPSC derived neurons
22	Endotype reversal as a novel strategy for screening drugs targeting familial Alzheimer's disease	GSE4226	Canada	28	Peripheral blood
23	Alzheimer Disease peripheral blood mononuclear cell expression	GSE184942	China	10	Hippocampus
24	Transcriptomic analysis of the chippocampus in Alzheimer's disease	GSE164012	China	9	Prostate stromal cell
25	Transcriptomic profiling of human senescent cells upon treatment with the senolytic agent procyanidin C1	GSE182761	USA	9	hiPSC derived neurons
26	Computational Repurposing of Bumetanide for Preventing or Treating Alzheimer's Disease [AT2123B iPSC Bumex RNA-seq]	GSE159333	Japan	9	iPS microglia
27	Expression data of iPS microglia treated with TREM2 agonist antibody	GSE163150	USA	24	Neuroblastoma SHSY-5Y cells
28	Transcriptome analyses reveal tau isoform-driven changes in transposable element and gene expression	GSE153110	Germany	8	Brain organoids
29	A microRNA signature for early detection and RNA therapy in Alzheimer's disease (3-microRNA mimic)	GSE160224	China	9	iPSC derived neurons
30	Efficient manipulation of gene dosage in human iPSCs using CRISPR/Cas9 nickases	GSE155567	Germany	24	THP1 macrophages
31	Deletion of the Alzheimer's Disease-associated CD33 results in increased phagocytosis, oxidative burst and inflammation	GSE161199	Italy	23	Blood
32	Alzheimer's, Parkinson's disease and Amyotrophic Lateral Sclerosis gene expression patterns divergence reveals different grade of RNA metabolism involvement	GSE85238	Germany	8	Differentiated THP-1
33	Expression data from THP-1 cells treated with GSK3 β inhibitors	GSE159541	USA	90	Temporal and inferior parietal cortex
34	Small RNA sequencing of brain-derived extracellular vesicles from post-mortem brain tissue of controls and Alzheimer's disease patients with different APOE genotypes	GSE157652	USA	24	Differentiated in vitro from iPSCs
35	Transcriptomic and functional deficits in human TREM2-/- microglia impair response to Alzheimer's pathology in vivo [RNA-seq]	GSE143951	Germany	7	iPSC derived neuronal cultures
36	iPSC-derived neuronal cultures expressing the Alzheimer's disease associated rare TREM2 R47H variant enables the construction of an A β -induced gene regulatory network	GSE108038	USA	18	3D Cell culture
37	Interleukin-4 restores neurogenic plasticity of the primary human neural stem cells through suppression of Kynurenic acid production upon Amyloid-beta42 toxicity	GSE78117	USA	15	2D and 3D cell culture
38	A novel hydrogel-based instructive biohybrid 3D culture system for modeling human neural stem cell plasticity, neurogenesis, neurodevelopment, and neurodegeneration	GSE107006	USA	9	CD34 Positive hemopoietic stem cell
	Antibodies That Convert Bone Marrow Into Trafficking Microglia-Like Cells Reduce Brain Amyloid				

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Table 1 (continued)

<u>SI NO:</u>	<u>TITLE</u>	<u>GSE NO:</u>	<u>COUNTRY</u>	<u>SAMPLE NO:</u>	<u>SOURCE</u>
39	Identification of downstream genes regulated by YAP1 through knockdown and overexpression of YAP1 in U251 cell with a stably expression of mutant APP	GSE100891	China	11	U251 cell with a stably expression of mutant APP 128(M671L)
40	RNAseq in Alzheimer's Disease patients	GSE53697	Switzerland	17	Brain
41	Transcriptomics profiling of Alzheimer's disease reveal novel molecular targets	GSE67333	USA	8	LOAD Hippocampi
42	Gene expression profile of sporadic and PSEN1 early-onset Alzheimer's Disease	GSE39420	Spain	21	Frozen brain tissue: posterior cingulate at thalamus level
43	Microarray analyses of laser-captured hippocampus reveal distinct gray and white matter signatures associated with incipient Alzheimer's disease	GSE28146	USA	30	laser capture CA1 tissue gray matter from formalin fixed, paraffin embedded specimens

interactions involved in Alzheimer's disease. We use publicly available gene expression datas from the GEO database to find altered gene expression linked to AD. Moreover, using Enrichr analysis, we try to elucidate the key signalling pathways involved in disease pathogenesis. We also try to investigate dysregulated microRNA expression profiles and protein-protein interaction networks in Alzheimer's disease.

The research into Alzheimer's disease is vital due to its prevalence and predicted increase in cases, making it a serious public health concern. Even with the progress made in comprehending the disease's genetic and molecular foundations, there is still an urgent need to clarify the intricacies of this condition. By examining multiple facets, such as protein interactions, microRNA regulation, gene expression, and signalling pathways, we try to identify critical mechanisms underlying the pathophysiology of AD. This knowledge is essential for creating efficient therapies, including targeted therapy and interventions to lessen the effects of AD on patients and society as a whole.

2. Materials and methods

2.1. Collection of relevant data sets of Alzheimer's patients from the GEO database

The expression array datasets pertaining to Alzheimer's disease were retrieved from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>) database of the National Centre for Biotechnology Information (NCBI) using the keyword "Alzheimer's disease". The selection process was limited to primary experimental investigations examining genetic variations between Alzheimer's patients and healthy controls. A set of inclusion and exclusion criteria were used to screen the datasets [9]. The Inclusion criteria include;

- Datasets which include controls and patients
- Datasets having expressional arrays
- Datasets outlining the diagnostic criteria
- Datasets studied in *Homo sapiens*
- Datasets containing at least two samples including control and treated
- Drug-treated datasets

The excluded data set include:

- Methylation studies
- Datasets having no diagnostic criteria
- Datasets from other species
- Datasets having no details about controls
- Mutation studies

Technical replicates were assessed to ensure consistency, and any discrepancies were investigated, with outliers being excluded from the analysis.

2.2. Analysis of gene expression in normal individuals and Alzheimer's patients was performed using GEO2R

The selected datasets were investigated using the GEO2R tool, accessible at <http://www.ncbi.nlm.nih.gov/geo/geo2r/> provided by the Gene Expression Omnibus. Following the GEO2R analysis, p-values were determined using the 't' test. Additionally, fold changes for each gene were computed using the GEO2R. Fold change is represented by logFC values, indicating the magnitude of gene expression alteration. Based on the p-value, the differentially expressed genes (DEGs) were chosen and divided into upregulating genes and downregulating genes [10].

2.3. Identification of functional gene enrichment and Gene ontology analysis using Enrichr analysis

Gene ontology and pathway enrichment analyses were utilised to elucidate the pertinent biological concepts and signalling pathways associated with prevalent DEGs of AD. The two categories of gene ontology (GO) and functional processes include biological processes and molecular functions. Selected upregulated and downregulated genes are used to find common pathways in AD. The study used KEGG, Wiki-Pathways, Reactome, Bioplane, Elsevier pathway, MSigDB, HumanCyc, Panther, NCI-Nature, and BioCarta databases as sources of pathway annotations. The selection of pathways is based on the p-value with a p-value of < 0.05, which was considered significantly enriched [11]. The further selection of enriched pathways is based on the combined score.

2.4. Compilation of the human protein-protein interaction network using STRING analysis

STRING database (<http://string-db.org>), was used for the retrieval of interacting Genes to construct the PPI network. STRING is a database that comprises information on known and predicted protein-protein interactions. Ambiguous terms were clarified by establishing the confidence cutoff and setting the maximum additional interactors to import the network. A PPI network utilising the STRING database is constructed to identify AD-associated hub proteins and key genes. The protein-protein interaction network from STRING consists of nodes (representing the genes) and edges (representing the interconnection between genes). Following the STRING analysis, the most interactive genes were chosen based on the number of interactions [12].

Table 2

List of frequently upregulated and downregulated genes with their frequency of occurrence.

Si No:	Up-regulated genes	Frequency of occurrence	Down-Regulated Genes	Frequency of occurrence
1.	DDX3Y	8	CREM	8
2.	H6PD	6	CD44	5
3.	KIF1B	6	HES4	5
4.	TOP2A	5	LBH	5
5.	RERE	5	PADI2	5
6.	LOC100129534	5	LOC107984910	5
7.	PRKCZ	5	GPR157	5
8.	CLSTN1	5	SPSB1	4
9.	PER3	5	NAMPT	4
10.	LINC01786	5	BCL2A1	4
11.	FHAD1	5	PFKP	4
12.	SYNPR	5	ITGB3	4
13.	PKIB	5	GNB1	4
14.	KIAA0101	5	RER1	4
15.	S1PR1	4	CPLANE2	4
16.	MEIS2	4	HSPB7	4
17.	LOC100132287	4	RPL22	4
18.	LOC100288069	4	PGD	4
19.	LOC112268260	4	SLC2A5	4
20.	TMEM201	4	ISG15	4
21.	C1orf159	4	NQO1	4
22.	LOC107985733	4	C3	4
23.	KAZN	4	MT1F	4
24.	LINC01409	4	CRLF1	4
25.	SLC35E2B	4	BMP2K	4
26.	INTS11	4	RPS13	4
27.	LOC105376794	4	RPS3A	4
28.	MXRA8	4	RPS16	4
29.	FBXO42	4	GBAP1	4
30.	CAMTA1	4	TGIF1	4
31.	CDK11B	4	LINC00115	3
32.	TAS1R3	4	MEG3	3
33.	LOC107984847	4	PPIA	3
34.	NPHP4	4	CD99	3
35.	AGRN	4	ID2	3
36.	UBR4	4	STOM	3
37.	DISP3	4	C1orf21	3
38.	SMC4	4	LGR6	3
39.	PDCD4	4	CCL4	3
40.	NEK2	4	MSC-AS1	3
41.	CENPA	4	PARVG	3
42.	CDKN3	4	HSPA1A	3
43.	BIRC5	4	PCGF5	3
44.	KDM5D	4	DAPP1	3
45.	UBE2C	4	TNFRSF10A	3
46.	MYC	3	GSAP	3
47.	TCF7	3	FGR	3
48.	TRABD2A	3	PLK3	3
49.	ITGA6	3	MTF1	3
50.	FOXP1	3	NRIP3	3
51.	HSP90AB1	3	GOS2	3
52.	IPCEF1	3	CSF1	3
53.	LEF1	3	HS3ST3B1	3
54.	HK2	3	TFAP2A	3
55.	MST1P2	3	TRAF1	3
56.	TRNK	3	WNT5A	3
57.	MEGF6	3	OLR1	3
58.	SLC6A6	3	TREM1	3
59.	RUNX3	3	CCL8	3
60.	DHRS3	3	CXCL3	3
61.	EOMES	3	ADORA2A	3
62.	PLPP3	3	AQP9	3
63.	TUBB	3	CXCL5	3
64.	SH2D5	3	NF2	3
65.	NPR3	3	ATP2B1	3
66.	SKI	3	GNB4	3
67.	FNDC10	3	CDKN1A	3
68.	SCNN1D	3	VCAM1	3
69.	PLEKHG5	3	ITIH5	3
70.	NPPA-AS1	3	IER3	3
71.	EPHA2	3	KCNE4	3
72.	UBE4B	3	IL1R2	3

Table 2 (continued)

Si No:	Up-regulated genes	Frequency of occurrence	Down-Regulated Genes	Frequency of occurrence
73.	LOC107984841	3	SERPINE1	3
74.	ATAD3B	3	LOC112268219	3
75.	ACAP3	3	ICMT	3
76.	MFAP2	3	SDHB	3
77.	ZBTB17	3	LZIC	3
78.	PLEKHM2	3	ANO7L1	3
79.	ACOT7	3	MASP2	3
80.	NOC2L	3	SPEN-AS1	3
81.	EXOSC10-AS1	3	CENPS-CORT	3
82.	CDK11A	3	TARDBP	3
83.	PEX10	3	UQCRHL	3
84.	SZRD1	3	VWA1	3
85.	MRPL20-AS1	3	CENPS	3
86.	SPEN	3	ESPN	3
87.	MTOR	3	FBXO6	3
88.	TP73-AS1	3	MMEL1	3
89.	NBPF1	3	GPR153	3
90.	MIR34AHG	3	MIIP	3
91.	MIR12136	3	BARD1	3
92.	MORN1	3	MT2A	3
93.	EFHD2	3	BLZF1	3
94.	PIK3CD	3	TOR1B	3
95.	AGTRAP	3	SP140	3
96.	WASH7P	3	TRIM5	3
97.	LOC105378948	3	APOBEC3G	3
98.	PANK4	3	IFT3	3
99.	RNF207	3	MYO10	3
100.	MIIP	3	FBXO2	3
101.	MRPL20	3	PANX2	3
102.	ENO1	3	BTG2	3
103.	DNAJC16	3	GLIS3	3
104.	SLC25A33	3	CADM1	3
105.	LOC107984913	3	TMEM54	3
106.	CCNL2	3	PLPP3	3
107.	ARHGEF10L	3	NWD1	3
108.	SLC45A1	3	CFI	3
109.	UBIAD1	3	TAS1R3	3
110.	LOC112268218	3	VAT1	3
111.	EPHA2-AS1	3	EFNB1	3
112.	LOC100133331	3	ITGB5	3
113.	KCNJ3	3	EPHB4	3
114.	TAC1	3	WWTR1	3
115.	SERTM1	3	MAFF	3
116.	CALB1	3	NAA50	3
117.	BEX3	3	LIMS1	3
118.	ENC1	3	ABHD2	3
119.	CHD5	3	ABCA1	3
120.	ACTG1	3	SEMA3C	3
121.	DCAF12	3	CD109	3
122.	XRCC6	3	CD163	3
123.	TMEM51	3	CNR1	3
124.	TERF2IP	3	LINC00689	3
125.	MON1A	3	PRTG	3
126.	MGP	3	SH3BGRL3	3
127.	SERPINF1	3	MST1L	3
128.	GPR12	3	CYBB	3
129.	ZBTB48	3	PRDX1	3
130.	ISG15	3	EIF2S2	3
131.	TPRG1L	3	RPLP0	3
132.	DFFB	3	NCK1	3
133.	WRAP73	3	TUBB6	3
134.	NADK	3	RPL23AP7	3
135.	RPL22	3	ATF3	3
136.	APOC1	3	ATF5	3
137.	RELN	3	DHRS7	3
138.	TNFRSF21	3	ZNF3	3
139.	LBH	3	MLLT11	3
140.	AXL	3	CYFIP2	3
141.	ESPN	3	RPL6	3
142.	AKR7A3	3	RPL24	3
143.	CROCCP2	3	ATP13A2	3
144.	PMEP1A	3	ARID1A	3
145.	RGS16	3	EIF3CL	3
146.	CDC20	3	CAMK2B	3
147.	DPYSL2	3	GABBR2	3

(continued on next page)

Table 2 (continued)

Si No:	Up-regulated genes	Frequency of occurrence	Down-Regulated Genes	Frequency of occurrence
148.	<i>MTURN</i>	3	<i>ENC1</i>	3
149.	<i>NUSAP1</i>	3	<i>GSN</i>	3
150.	<i>ZNF558</i>	3	<i>FAM20C</i>	3
151.	<i>USP9Y</i>	3	<i>DRAXIN</i>	3
152.	<i>CKS1B</i>	3	<i>ABCC3</i>	3
153.	<i>MCM10</i>	3	<i>RHCE</i>	3
154.	<i>MAD2L1</i>	3	<i>AURKAIP1</i>	3
155.	<i>SLC8A1</i>	3	<i>SSU72</i>	3
156.	<i>SEPT5-GP1BB</i>	3	<i>MTOR-AS1</i>	3
157.	<i>DDX39B</i>	3	<i>MAD2L2</i>	3
158.	-	-	<i>LOC107984868</i>	3
159.	-	-	<i>ECE1</i>	3
160.	-	-	<i>VAMP3</i>	3
161.	-	-	<i>SAMD11</i>	3
162.	-	-	<i>S100A10</i>	3
163.	-	-	<i>VIM</i>	3
164.	-	-	<i>GIMAP8</i>	3
165.	-	-	<i>SIPA1L2</i>	3
166.	-	-	<i>NPL</i>	3
167.	-	-	<i>CTSB</i>	3
168.	-	-	<i>CLBL</i>	3
169.	-	-	<i>OPTN</i>	3
170.	-	-	<i>STRIP2</i>	3
171.	-	-	<i>GSTA4</i>	3
172.	-	-	<i>KLF5</i>	3
173.	-	-	<i>MMP7</i>	3
174.	-	-	<i>LGMN</i>	3
175.	-	-	<i>FAM198B</i>	3
176.	-	-	<i>LG12</i>	3
177.	-	-	<i>ENO1</i>	3

2.5. Identification of distance regulatory elements in Alzheimer's patients using DiRE analysis

DiRE (<https://dire.dcode.org/>) is used to identify the distance regulatory elements and transcription factors that play a significant role in modulating gene expression associated with the AD. Transcription factors that exhibited a notable degree of enrichment in binding sites which is found in regulatory regions linked to disease were recognised as the possible candidates involved in the pathophysiology of AD [13].

2.6. Identification of miRNA linked to AD and construction of miRNA network with miRNet

The miRNA database identifies the list of miRNAs corresponding to target genes (<https://www.mirnet.ca/miRNet/upload/GeneUploadView.xhtml>). The upregulated and downregulated genes were submitted to the database. A table of miRNAs, hits and their p-value, and a graphic depiction of the relationships between hub genes and miRNAs were produced using the miRNet database [14].

3. Results

3.1. Retrieval of AD dataset from the GEO database

RNA sequencing (RNAseq) and microarray expression datasets were utilised for Differential Gene Expression (DGE) analysis. A total of 43 datasets relevant to Alzheimer's disease were retrieved from the database, encompassing 902 samples. The primary source of the biological material of the samples consists of the brain, peripheral blood, and iPSC-derived neurons. A comprehensive overview of the microarray datasets

is provided in Table 1.

3.2. Identification and screening of differentially expressed genes using GEO2R analysis

The GEO2R tool was utilised to identify the differentially expressed genes. According to the p-value DEGs were selected. According to the p-value, DEGs were selected. The DEGs were categorised into two groups: up-regulated genes and down-regulated genes. DEGs were identified with a t-test, and statistically significant DEGs were defined by the GEO2R online tool with a $|\log FC| > 2$ and adjusted P value < 0.05 . The DEGs with a $\log FC < 2$ were considered down-regulated genes, while those with a $\log FC > 2$ were considered up-regulated. From the 3516 genes 157 frequently up-regulated genes were selected. Likewise, from the 4466 genes, 177 Down-regulated frequently repeated genes were selected (Table 2).

3.3. Identification of frequently upregulated and down-regulated pathways in AD using Enrichr analysis

Functional enrichment and gene ontology analysis of 157 upregulated and 177 down-regulated genes was performed with Enrichr analysis. The pathways with a p-value < 0.05 were considered significantly enriched. The upregulated pathways include Type II diabetes mellitus, PI3K-Akt signalling pathway, Neuroblastoma, Wnt signalling pathway, Hippo signalling pathway, ion channel inactivation, inactivation of APC/C via direct inhibition of the APC/C complex, TGF-beta signalling, Myc Targets, apoptosis and presenilin pathway. The down-regulated pathways include the immune system, cytoplasmic ribosomal proteins, TNF signalling pathway, translation, ephrin receptor B forward pathway, heparan sulfate biosynthesis, beta-arrestins in GPCR desensitisation, p53 Pathway and signalling pathway from G-Protein. The significant upregulated and downregulated AD pathways are shown in Fig. 1 A and B.

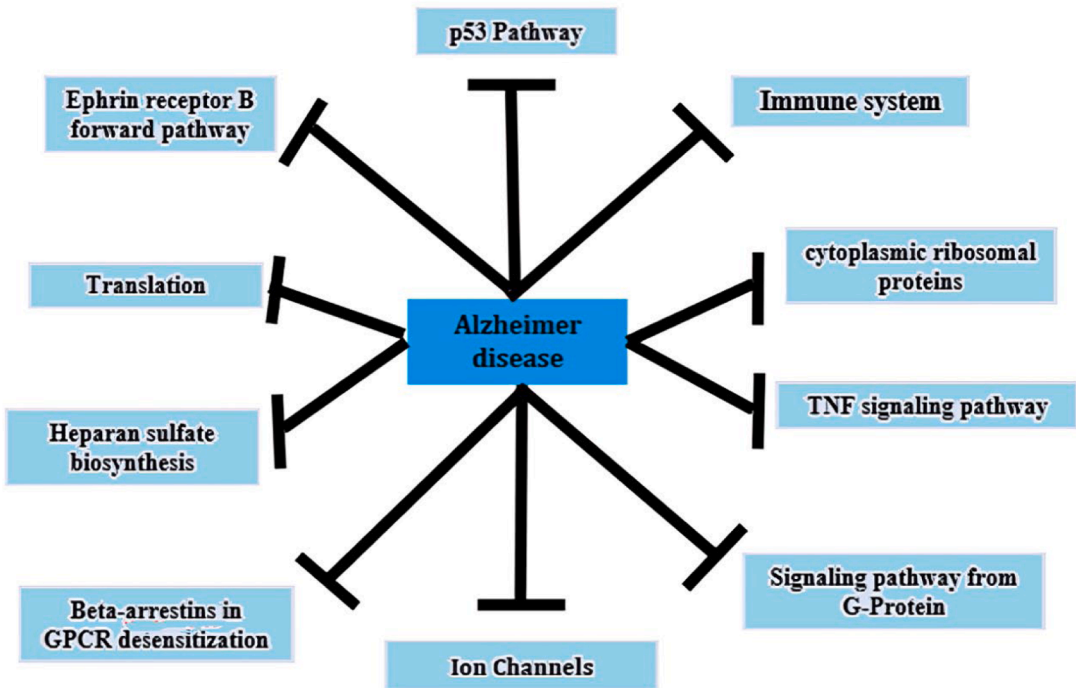
3.4. Construction of interaction network between the differentially expressed genes

Network construction of 157 up-regulated and 177 down-regulated genes was performed using STRING analysis, with a confidence level set to 0.700. In the network, nodes (circles) represent genes, while edges (lines) indicate gene-gene interactions (Fig. 2 A and Fig. 2 B). STRING analysis identifies the most interactive genes in AD. Eight key genes show high interaction from the 157 up-regulated genes, including MYC, HSP90AB1, CENPA, MAD2L1, TUBB, UBE2C, BIRC5, and NEK2 (Table 3). Similarly, four genes show high interaction from the 177 down-regulated genes, including ENO1, CCL4, ISG15, and CD44 (Table 4). The combined score of genes and connected nodes is represented in Supplementary Table 1 and 2.

3.5. Identification of top transcription factors in using DiRE analysis

Transcription factors associated with up-regulated and down-regulated genes were identified using DiRE analysis. The chromosome distribution of up-regulated and down-regulated genes and the regulatory element are shown in Fig. 3. From up-regulated and down-regulated genes, the top 10 transcription factors were selected (Fig. 4). The top 10 transcription factors in up-regulated genes are SMAD, OCT, MYOGENIN, ZIC2, NERF, STAT6, PEA3, T3R, HSF2 and top 10 transcription factors in down-regulated genes are RFX1, LBP1, MEF2, NFE2, STAT6, PAX2,

A



B

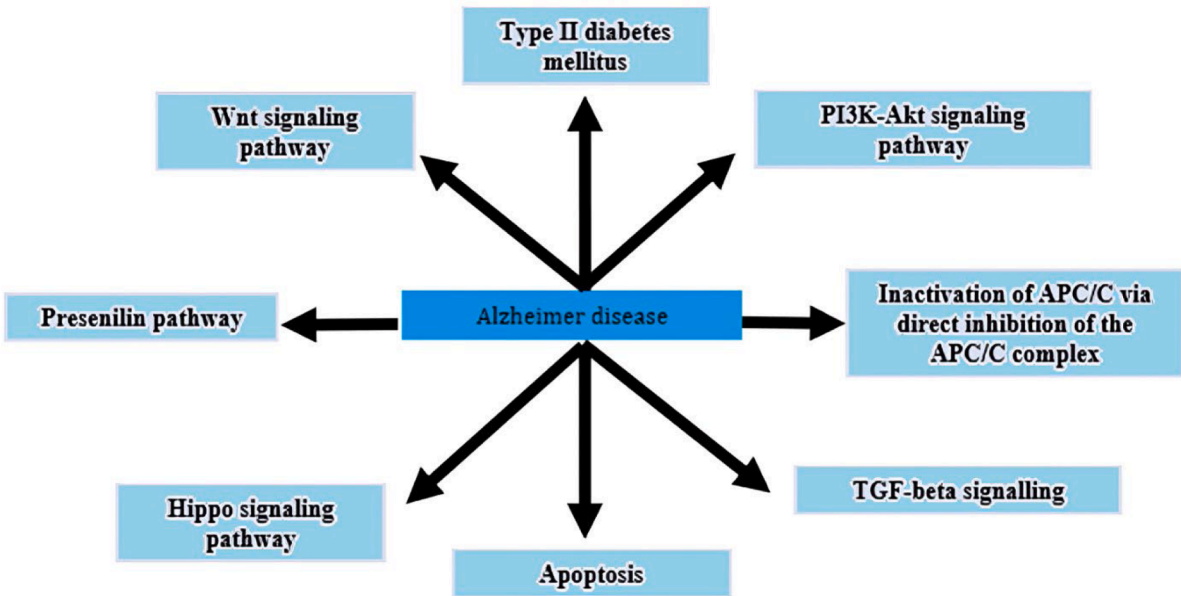


Fig. 1. A. Significant down- regulated pathways in Alzheimer diseases. The up-regulated pathways include includes Type II diabetes mellitus, PI3K-Akt signaling pathway, Neuroblastoma, Wnt signalling pathway, Hippo signalling pathway, ion channel inactivation, inactivation of APC/C via direct inhibition of the APC/C complex, TGF-beta signalling, Myc Targets, apoptosis and presenilin pathway. The selection of pathways is based on the p-value with a p-value of < 0.05, which was considered significantly enriched

Fig. 1 B: Significant up-regulated pathways in Alzheimer diseases. The down-regulated pathways include immune system, cytoplasmic ribosomal proteins, TNF signaling pathway, translation, ephrin receptor B forward pathway, heparan sulfate biosynthesis, beta-arrestins in GPCR desensitisation, p53 Pathway and signaling pathway from G-Protein. The selection of pathways is based on the p-value with a p-value of < 0.05, which was considered significantly enriched.

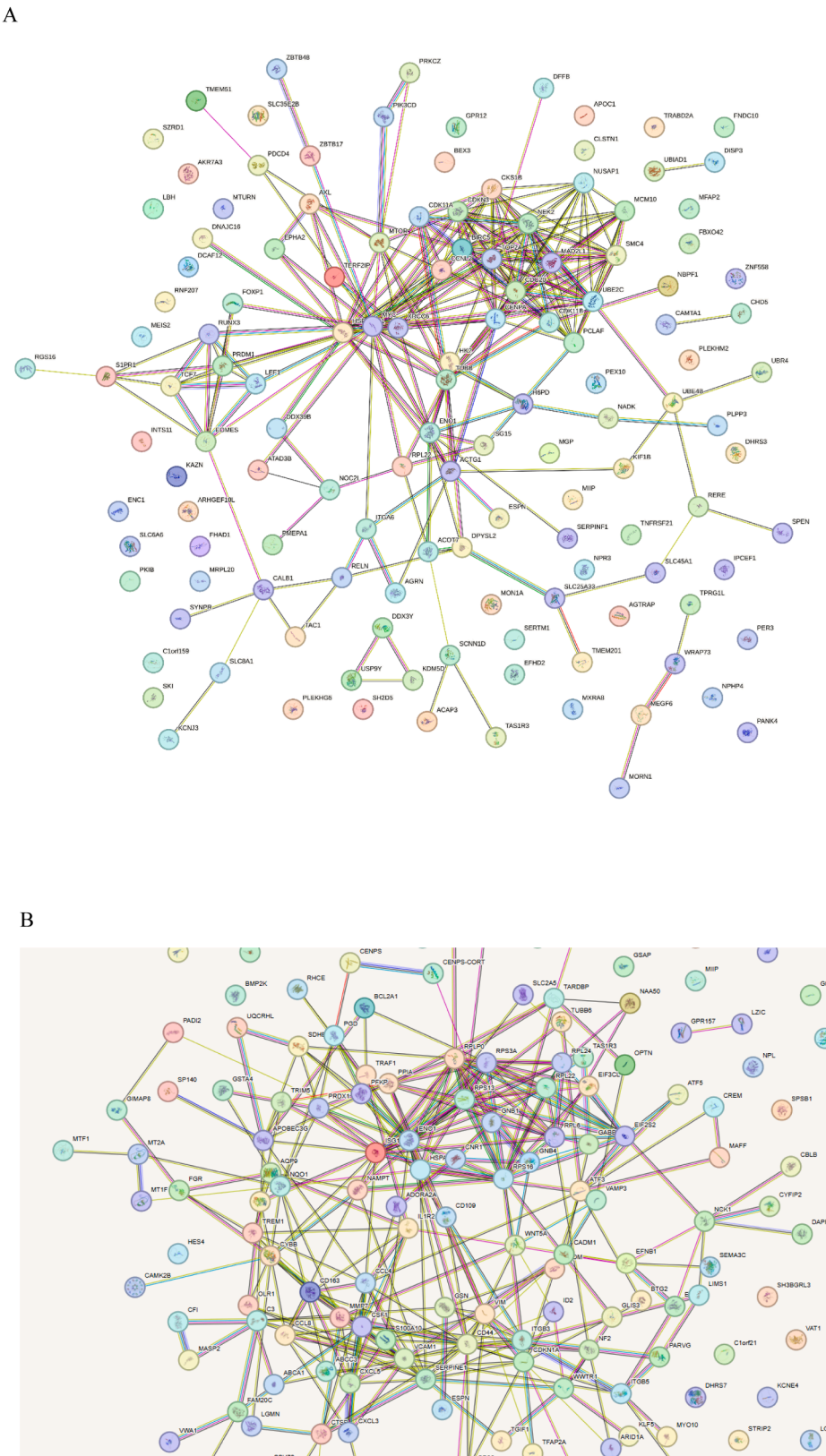


Fig. 2. A: STRING analysis which shows the interaction between the up-regulated genes. From the 157 up-regulated genes, 8 key genes show high interaction which include *MYC*, *HSP90AB1*, *CENPA*, *MAD2L1*, *TUBB*, *UBE2C*, *BIRC5*, and *NEK2*.
Fig. 2 B. STRING analysis which shows the interaction between the down-regulated genes. From the 177 down-regulated genes, 4 genes show high interaction which include *ENO1*, *CCL4*, *ISG15*, and *CD44*.

Table 3

The number of interactions among the up-regulated genes in STRING analysis.

SI No:	Up-regulated genes	No: of genes interacted nodes
1	<i>MYC</i>	29
2	<i>HSP90AB1</i>	15
3	<i>CENPA</i>	15
4	<i>MAD2L1</i>	14
5	<i>TUBB</i>	13
6	<i>UBE2C</i>	14
7	<i>BIRC5</i>	15
8	<i>NEK2</i>	15

Table 4

The number of interactions among the down-regulated genes in STRING analysis.

SI No:	Down-regulated genes	No: of genes interacted-nodes
1	<i>ENO1</i>	19
2	<i>CCL4</i>	11
3	<i>CD44</i>	15
4	<i>ISG15</i>	9

LMO2COM, *NFKAPPAB*, *CEBPB*, *TEF1* (Table 5).

3.6. Identification of miRNA related to AD and construction of miRNA network

To explore the correlation of candidate target genes with potential miRNAs, miRNet was employed to construct and analyse the miRNA-hub gene network (Fig. 5 A and B). Through miRNet analysis, the top 9 up-regulated miRNAs and their related genes and 10 down-regulated miRNAs and their related genes were selected based on the degree and betweenness (Tables 6 and 7).

4. Discussion

In this study, 43 datasets related to AD were retrieved from the NCBI Gene Expression Omnibus (GEO), following specific inclusion and exclusion criteria. The primary sources of samples included brain tissue, peripheral blood, and iPSC-derived neurons. Differentially expressed genes (DEGs) were identified using the GEO2R tool, with selection based on p-value significance. The DEGs were then categorised into two groups: upregulated and down-regulated genes. Out of 3516 genes, 157 were frequently upregulated, while 177 out of 4466 down-regulated genes were frequently observed. Various studies on AD have identified significant up-regulation and down-regulation in the expression levels of genes such as *NDNF*, *ROR2*, *CD36*, *HADH*, *CPLX1*, *TSPAN6*, *VCAM1*, *SFRP1*, and *ERBB2*. These findings highlight the potential of these genes as biomarkers for Alzheimer's disease. Additionally, *CD36*, *HADH*, *SFRP1*, and *ERBB2* were identified as potential therapeutic targets [15].

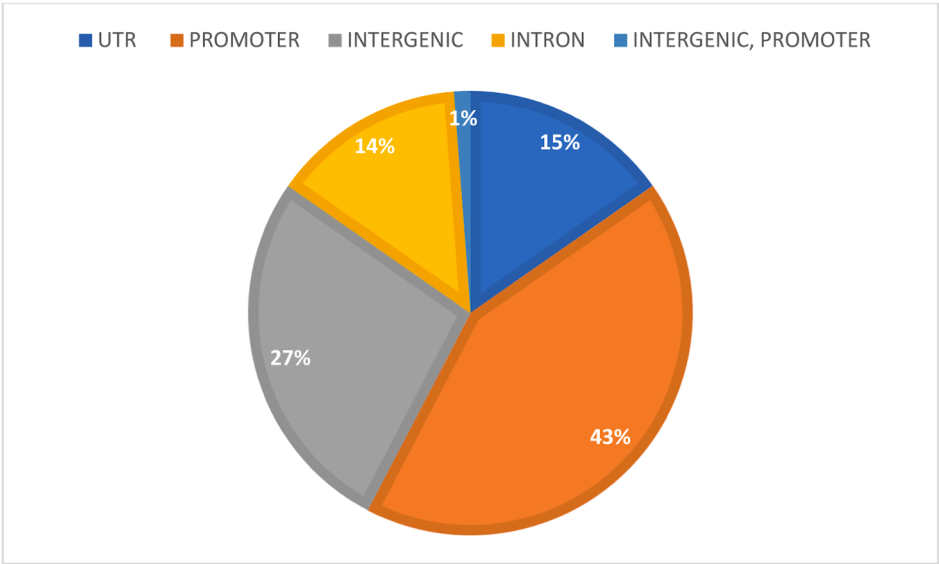
Upon enrichment analysis, several pathways were found to be upregulated in AD. These include Type II diabetes mellitus, PI3K-Akt signalling, Neuroblastoma, Wnt signalling, Hippo signalling, ion channel inactivation, APC/C inactivation, TGF-beta signalling, Myc Targets, apoptosis, and the presenilin pathway. Type 2 diabetes mellitus and Alzheimer's disease are strongly correlated, with each increasing the risk of the other due to shared risk factors like obesity and insulin resistance. Lifestyle changes and certain diabetes medications may help slow cognitive decline associated with Alzheimer's disease [16]. Increased A β 42/A β 40 ratio in AD upregulates CRABP1 in neuroblastoma cells, impairing neurogenesis and linking Alzheimer's pathology with

neuroblastoma mechanisms [17]. The PI3K-Akt pathway, involved in growth, survival, and metabolism, links amyloid- β , neurofibrillary tangles, and brain atrophy, making it a potential target for therapy [18]. Aberrant WNT signalling disrupts neurogenesis, synaptic formation, and memory processes in AD [19]. Likewise, the Hippo signalling pathway may influence Alzheimer's pathogenesis by affecting microglial activation and neuroinflammation [20]. The ion channel dysfunction in AD exacerbates symptoms by disrupting neuronal signalling and cell stability [21]. In AD, the disruption of the APC/C complex due to A β oligomers leads to neurodegenerative processes [22]. Impairment of TGF- β 1 signalling contributes to A β accumulation, neurodegeneration and tau pathology, suggesting potential therapeutic approaches [23]. While N-myc and c-myc proteins do not affect neuronal death directly, their upregulation in astrocytes may contribute to neurodegenerative disorders [24]. Apoptosis is critical in AD, driving neuronal loss and cognitive decline through β -amyloid accumulation and tau hyperphosphorylation [25]. The presenilin pathway's upregulation accelerates β -amyloid plaque formation, exacerbating neuronal damage and cognitive decline [26].

In AD, several pathways are down-regulated, contributing to the disease's progression and neurodegeneration. The immune system pathways are impaired, reducing the brain's ability to manage inflammation and infections, which can lead to increased accumulation of amyloid-beta plaques and tau tangles [27]. Cytoplasmic ribosomal proteins are also down-regulated, affecting protein synthesis crucial for neuronal health and function, which further exacerbates neurodegeneration [28]. The TNF signalling pathway down-regulation disrupts inflammatory responses and neuronal survival, worsening neuroinflammation and neuronal loss [29]. Moreover, the translation processes are impaired, leading to the reduced synthesis of essential proteins for neuronal function and synaptic plasticity, which contributes to cognitive decline [30]. The ephrin receptor B forward pathway, involved in cell-cell communication and synaptic plasticity, is down-regulated, disrupting neuronal connectivity and memory [31]. Heparan sulfate biosynthesis is affected, impairing the production of glycosaminoglycans, which is critical for cell signalling and extracellular matrix integrity and impacts neuronal growth and survival [32]. Beta-arrestins, which play a role in desensitising G-protein-coupled receptors (GPCRs), are down-regulated, altering GPCR signalling and affecting neurotransmitter release and neuronal communication. The p53 pathway, crucial for cell cycle regulation, apoptosis, and DNA repair, is down-regulated, impairing the cell's ability to manage stress and repair damage, leading to increased neuronal apoptosis [33]. Lastly, signalling pathways from G-proteins, involved in various cellular processes, including neurotransmission, are down-regulated, disrupting neuronal signalling and exacerbating cognitive deficits [34].

In the STRING analysis, eight upregulated genes were selected from a pool of 157 genes, including *MYC*, *HSP90AB1*, *CENPA*, *MAD2L1*, *TUBB*, *UBE2C*, *BIRC5*, and *NEK2*. These genes are associated with various cellular processes, such as cell cycle regulation, stress response, and mitosis, which are relevant to AD pathology. Conversely, four down-regulated genes were chosen from 177 genes: *ENO1*, *CCL4*, *ISG15* and *CD44*. These genes involve glycolysis, ribosomal function, immune responses and cell adhesion. Their down-regulation may contribute to impaired cellular metabolism, reduced protein synthesis, and compromised immune response in AD. *MYC* is the most important gene in upregulated genes because it interacts with 29 other genes. In Alzheimer's disease, *MYC* is upregulated, contributing to neurodegeneration by promoting abnormal neuronal proliferation, affecting synaptic plasticity, and exacerbating amyloid-beta and tau pathology [35]. Understanding the intricate control of *MYC* in the brain holds

A



B

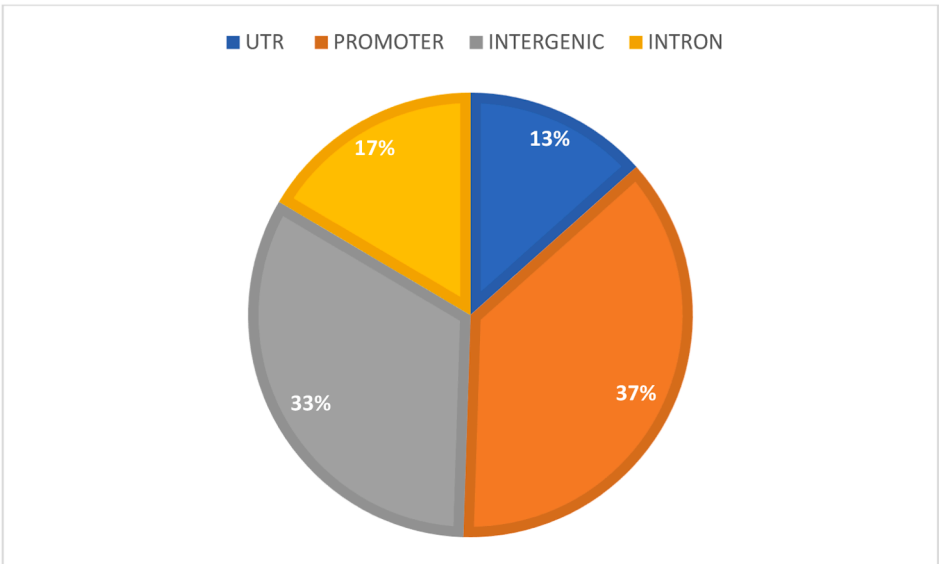


Fig. 3. Distribution of regulatory elements in up-regulated and down-regulated genes. The chromosome distribution of up-regulated and down-regulated genes and the regulatory element.

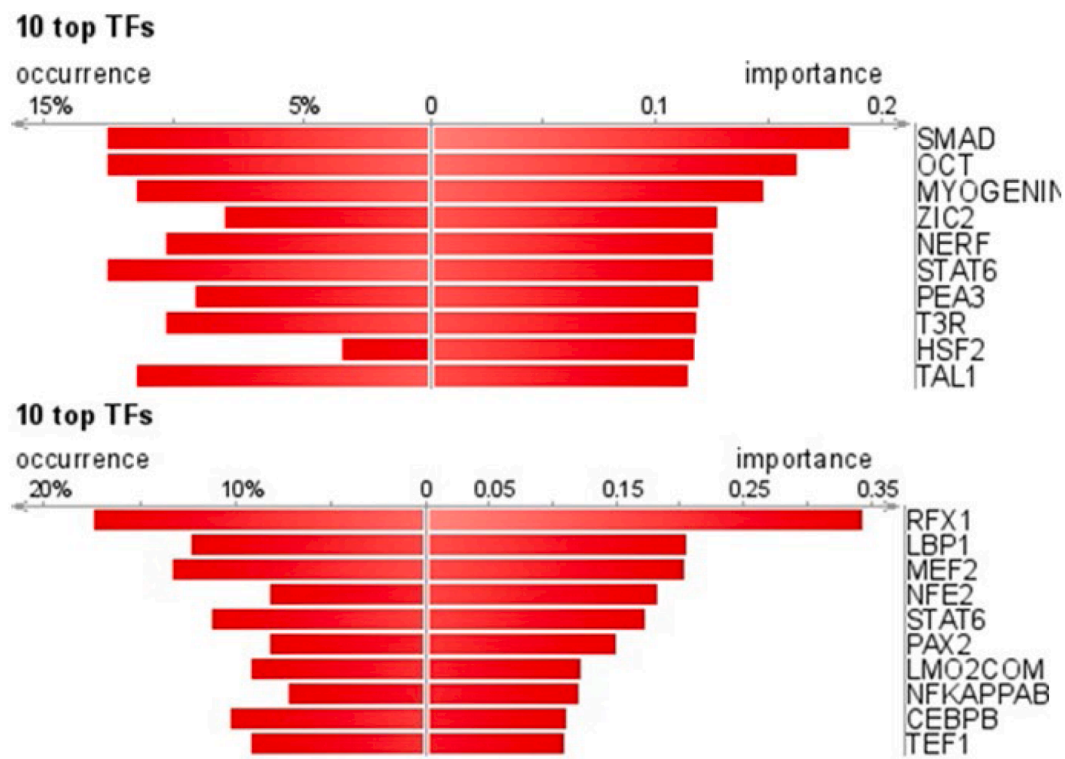


Fig. 4. Top 10 transcription factors in up-regulated and down-regulated genes: The top 10 transcription factors in up-regulated genes are *SMAD*, *OCT*, *MYOGENIN*, *ZIC2*, *NERF*, *STAT6*, *PEA3*, *T3R*, *HSF2* and top 10 transcription factors in down-regulated genes are *RFX1*, *LBPI*, *MEF2*, *NFE2*, *STAT6*, *PAX2*, *LMO2COM*, *NFKAPPAB*, *CEBPB*, *TEF1*.

Table 5
Major classes of transcription factor with occurrence.

SI No	Transcription factor of up-regulated genes	Occurrence	Transcription factor of down-regulated genes	Occurrence
1	<i>SMAD</i>	12.50 %	<i>RFX1</i>	17.35 %
2	<i>OCT</i>	12.50 %	<i>LBP1</i>	12.24 %
3	<i>MYOGENIN</i>	11.36 %	<i>MEF2</i>	13.27 %
4	<i>ZIC2</i>	7.95 %	<i>NFE2</i>	8.16 %
5	<i>NERF</i>	10.23 %	<i>STAT6</i>	11.22 %
6	<i>STAT6</i>	12.50 %	<i>PAX2</i>	8.16 %
7	<i>PEA3</i>	9.09 %	<i>LMO2COM</i>	9.18 %
8	<i>T3R</i>	10.23 %	<i>NFKAPPAB</i>	7.14 %
9	<i>HSF2</i>	3.41 %	<i>CEBPB</i>	10.20 %
10	<i>TAL1</i>	11.36 %	<i>TEF1</i>	9.18 %

therapeutic promise and sheds light on the development of neurodegenerative diseases. *ENO1* is the most important gene in down-regulated genes because it interacts with 19 other genes and has 57 interactions between them. *ENO1*, also known as 2-phospho-D-glycerate hydrolase, is a glycolytic enzyme that is expressed in most tissues and is responsible for the conversion of 2-phosphoglyceric acid to phosphoenolpyruvic acid in the glycolytic pathway. A study using Redux proteomics reported dysregulation of *ENO1* in cases of mild cognitive impairment (MCI), which is associated with modified hippocampus proteins and malfunction, indicating that the inactivation of *ENO1* leads to the development of AD [36].

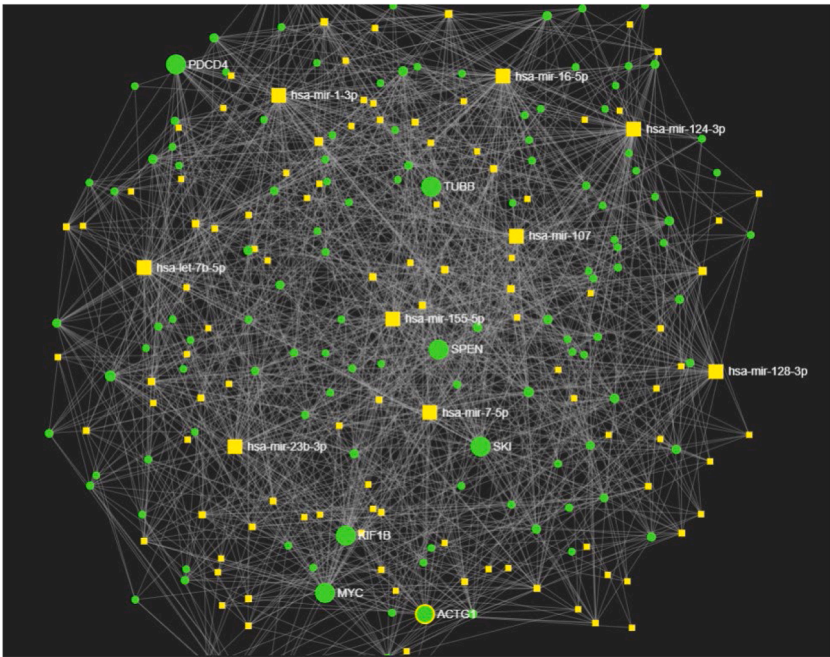
In the DiRE analysis, the top 10 transcription factors associated with upregulated genes are SMAD, OCT, STAT6, MYOGENIN, ZIC2, NERF, PEA3, T3R, and HSF2, while those for down-regulated genes are RFX1, LBP1, MEF2, NFE2, STAT6, PAX2, LMO2COM, NFKAPPAB, CEBPB, and TEF1. SMAD proteins, downstream of TGF-β signalling, regulate gene expression related to neurodegeneration [37]. In AD STAT3, are implicated in neuronal responses to stress and inflammation. Dysregulation of

the JAK/STAT signalling pathway, including impaired STAT3 activation, can contribute to neurodegenerative processes and cognitive decline [38]. In this study, the gene *CYBB*, located in the intergenic region of chromosome X, is identified. Increasing evidence suggests that women have a higher risk of developing AD compared to men [39]. Enhancing the function of transcription factors could potentially mitigate neuroinflammation, support neuronal health, and improve cognitive functions in AD.

Moreover, microRNAs play a crucial role in AD by regulating gene expression related to neuroinflammation, amyloid-beta metabolism, tau phosphorylation, and neuronal survival. Dysregulation of specific miRNAs can contribute to AD pathology by influencing these processes, thereby affecting cognitive function and disease progression [40]. In the present study, we have identified top 9 upregulated miRNAs (hsa-mir-16-5p, hsa-mir-124-3p, hsa-mir-1-3p, hsa-let-7b-5p, hsa-mir-155-5p, hsa-mir-23b-3p, hsa-mir-107, hsa-mir-7-5p, hsa-mir-128-3p) and their related genes (*MYC*, *SKI*, *SPEN*, *TUBB*, *PDCD4*, *KIF1*, *BACTG1*), as well as the six down-regulated miRNAs (hsa-mir-1-3p, hsa-mir-16-5p, hsa-mir-124-3p, hsa-mir-155-5p, hsa-let-7b-5p, hsa-mir-107) and their related genes *BTG2*, *CDKN1A*, *ARID1A*, *NAA50*, *SERPINE1* in Alzheimer diseases based on degree and betweenness through miRNet analysis. Targeting microRNAs (miRNAs) offers a promising approach to AD therapy by modulating gene expression in disease pathology.

In conclusion, this work highlights the critical role of gene regulation, transcription factors, and microRNAs in Alzheimer's disease pathogenesis. This research underscores the complexity of AD and its underlying mechanisms by identifying key upregulated and down-regulated genes and understanding the impact of specific transcription factors and miRNAs. The findings provide valuable insights into how these molecular factors contribute to neurodegeneration and cognitive decline, offering potential targets for novel therapeutic strategies. The importance of this work lies in its potential to inform the development of

A



B

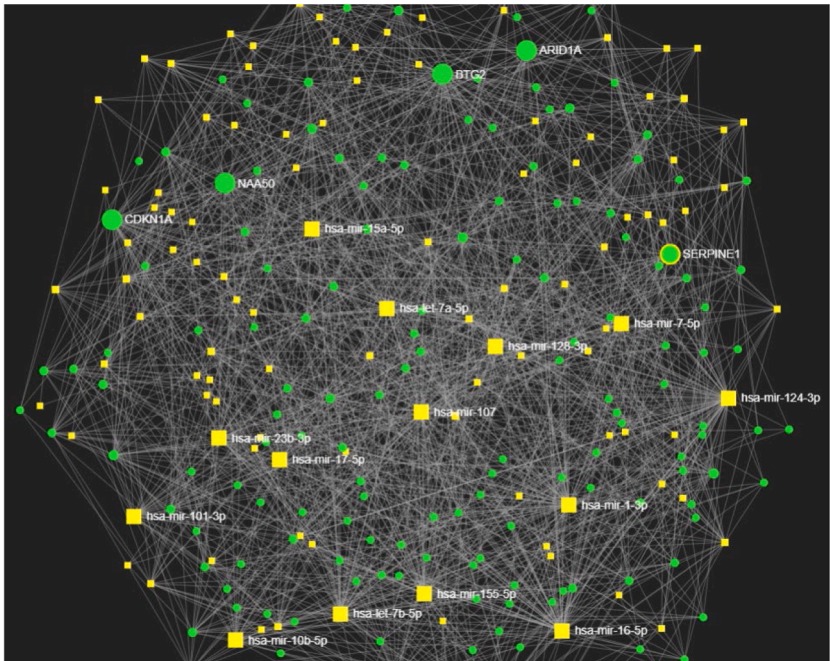


Fig. 5. A. Network of the up-regulated miRNAs. Through miRNet analysis, top up-regulated miRNAs and its related genes were selected based on the degree and betweenness.
Fig. 5 B. Network of the down-regulated miRNAs. Through miRNet analysis, top down-regulated miRNAs and its related genes were selected based on the degree and betweenness.

Table 6
List of Up-regulated miRNA and genes with degree and betweenness.

SI No	Up-regulated DE-miRNA	Degree	Betweenness	Genes	Degree	Betweenness
1	hsa-mir-16-5p	72	2639.158	MYC	40	1658.54
2	hsa-mir-124-3p	70	3017.502	SKI	35	1119.742
3	hsa-mir-1-3p	62	2204.085	SPEN	34	861.4532
4	hsa-let-7b-5p	50	1214.178	TUBB	31	767.2746
5	hsa-mir-155-5p	50	1396.3	PDCD4	31	1448.381

Table 7
List of Up-regulated DE-miRNA and genes with degree and betweenness.

SI No	Down-regulated DE-miRNA	Degree	Betweenness	Genes	Degree	Betweenness
1	hsa-mir-1-3p	92	4632.562	<i>BTG2</i>	52	2384.242
2	hsa-mir-16-5p	87	4209.87	<i>CDKN1A</i>	49	2523.344
3	hsa-mir-124-3p	82	3605.823	<i>ARID1A</i>	36	1056.943
4	hsa-mir-155-5p	66	2165.744	<i>NAA50</i>	30	643.3914
5	hsa-let-7b-5p	62	1789.082	<i>SERPINE1</i>	30	897.0549

targeted treatments that could modulate gene expression and improve clinical outcomes for AD patients, ultimately advancing our approach to combating this devastating disease.

CRediT authorship contribution statement

Chandana Yesudas: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Neethu P:** Formal analysis. **III-akkiam Devaraj:** Writing – review & editing, Writing – original draft, Supervision.

Declaration of competing interest

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.

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Supplementary materials

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