Exploring the Molecular Pathology of Alzheimer's Disease and Type 2 Diabetes Mellitus: Network Biology of Overlap Transcriptomic and Epigenetic Analysis Factors

Yadav Ravina[1], Mani Ruchi Jakhmola[1], Sharma Arun K.[2], Ahmad Saif [3] and Katare Deepshikha Pande[1*]
1. Proteomics and Translational Research Lab, Centre for Medical Biotechnology, Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, 201301, INDIA
2. Department of Pharmacology, Amity Institute of Pharmacy, Amity University, Gurugram, Haryana, 122413, INDIA
3. Department of Translational Neuroscience, Barrow Neurological Institute 350 West Thomas Road Phoenix, Arizona, 85013, USA
*dpkatare@amity.edu

Abstract

Type 2 diabetes mellitus (T2DM) has been globally recognized to trigger the risk of Alzheimer's Disease (AD) [commonly known as Type 3 diabetes (T3DM)] which has towering prevalence across the world. This study delineates the molecular crosstalk of these comorbidities using bioinformatics and network biology. Stringent analysis of Affymetrix microarray data from datasets GSE26168 and GSE63063 has provided substantial molecular correlations of T2DM to AD. Estimating common differentially expressed genes, regulating transcriptional factors, involving signaling kinase and coinciding biological processes of these insidious pathologies open new windows for therapeutic targets and diagnostic biomarkers. Numerous pivotal genes including FCER1G, FGR, ALOX5AP, NCF2 and CSF3R, have been studied to regulate insulin signalling pathways and interestingly noticed differential expression patterns in AD.

A bunch of DEG genes from this series are also involved in neuroinflammation and synaptic dysfunction concurring the molecular interplay between T2DM and AD. The results of the network analysis further revealed complex interrelations between the dysregulated genes, central hubs and potential regulatory nodes critical to the pathophysiology of T3DM comorbidity. Overall, these findings unveil the molecular underpinnings of T3DM interplay and provide a roadmap towards developing targeted therapeutic strategies for debilitating neurodegenerative disorders.

Keywords: Neurodegenerative Comorbidities, Molecular Crosstalk, Bioinformatics Analysis, Network Biology.

Introduction

Alzheimer's disease (AD) is the most prevalent progressive cognitive disorder and is predominantly associated with aging[8]. Approximately 50 million people worldwide suffer from AD or other dementias and this number is expected to increase to 152 million by 2050[25]. Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder that has seen a significant increase over the past two decades[10]. Despite its high prevalence, T2DM has been extensively studied for its potential role as a primary etiological factor in various neurological disorders, including AD[40]. Due to its significant association with neurodegenerative conditions, T2DM is sometimes referred to as 'Type 3 diabetes' (T3DM), highlighting its impact on the central nervous system[24]. Recent statistics revealed that T2DM could exacerbate a 50-60% risk of instigation of AD. Besides, a high prevalence of T2DM has been observed in AD patients, where around 80% have impaired glucose tolerance or manifest diabetes[36].

Ample evidence suggests that insulin resistance is the main culprit instigating cognitive impairment in the brain by altered glucose metabolism. Insulin resistance in AD patients is due to altered sensitivity of brain insulin receptors, which affects the degradation and expression of Tau and beta-amyloid (Aβ) precursor proteins[9]. Accumulation of amyloid fibrils in the brain causes cognitive impairment, while amyloid plaque deposition in pancreatic β-cells disrupts glucose homeostasis in T2DM individuals[32].

Moreover, chronic hyperglycemia may provoke the formation of AGEs, oxidative stress and inflammation which are further involved in AD progression[29,30]. While numerous theories are proposed for understanding the signaling interplay between these allied pathologies, there is no key regulator where promising biomarkers have been revealed. Indeed, the identification of promising targets can play a strategic role in restricting the health hazards of these irreversible comorbidities[14,35].

The present study is designed to investigate the role of genetic and epigenetic factors associated with these diseases. The genes related to T2DM and AD were comprehensively collected and scrutinized for their functional characteristics. This comprehensive investigation involved the exploration of key genes, transcriptional factors (TFs) and protein kinases (PKs) associated with both conditions which were predicted using DEGs by bioinformatic tools. This integrated approach comprises of OMIC data and computational analysis to elucidate the underlying molecular mechanisms for the pursued comorbidity. Shared biomarkers and pathways in T2DM-induced AD may

provide insights into the development of putative preventive drugs and therapies.

Material and Methods

Affymetrix Microarray Data: A wide search strategy was implemented to gather datasets pertaining to the molecular linkages between type 2 Diabetes Mellitus (T2DM) and Alzheimer's disease (AD). Utilizing the gene expression omnibus (GEO) repository hosted by the National Center for Biotechnology Information (NCBI), susceptibility datasets were systematically obtained. For T2DM, the search query employed included ("diabetes mellitus, type 2"[MeSH Terms] or type 2 diabetes mellitus [All Fields]) and "Homo sapiens"[porgn] and ("gse"[Filter] and "Expression profiling by array"[Filter] and "attribute name tissue"[Filter]).

For AD, the search strategy comprised of ("alzheimer disease"[MeSH Terms] or alzheimers[All Fields]) and "Homo sapiens"[porgn] and ("gse"[Filter] and "Expression profiling by array"[Filter] and "attribute name tissue"[Filter]). The datasets GSE26168[18] and GSE63063[33] were specifically selected for analysis, ensuring meticulous downloading to preserve data integrity and reproducibility.

Assessment of DEGs: The DEGs were extracted using the platform and series matrix file of the GEO dataset through the R programming language with an appropriate annotation package. In addition, the limma package was used within the R framework[26,31].

Venn diagram to visualize the overlap of DEGs: Employing the `VennDiagram` package within the R programming environment, we executed a comparative analysis of differentially expressed genes (DEGs) across two distinct datasets[5]. Initially, DEG lists were procured and segregated into discrete vectors delineating upregulated and downregulated gene sets. Subsequently, the `Venn diagram function was invoked, delineating the input sets corresponding to the upregulated and downregulated DEGs from the respective datasets. Parameter optimization was conducted to refine the Venn diagram's aesthetics, including color schemes, opacity levels and annotation dimensions, ensuring optimal legibility and visual fidelity.

Gene Enrichment Analysis: We utilized the enrichGO and enrichKEGG functions in the ClusterProfiler package to conduct gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analyses on the identified DEGs[38]. Subsequently, we employed the "ggplot2" package to visualize the results, aiming to evaluate the enriched biological pathways and functions associated with the DEGs.

Specifically, for the GO analyses, we investigated the enriched categories including cellular components (CCs), molecular functions (MFs) and biological processes (BPs). This comprehensive approach allowed us to gain insights into the underlying biological mechanisms, providing valuable information for further exploration and interpretation.

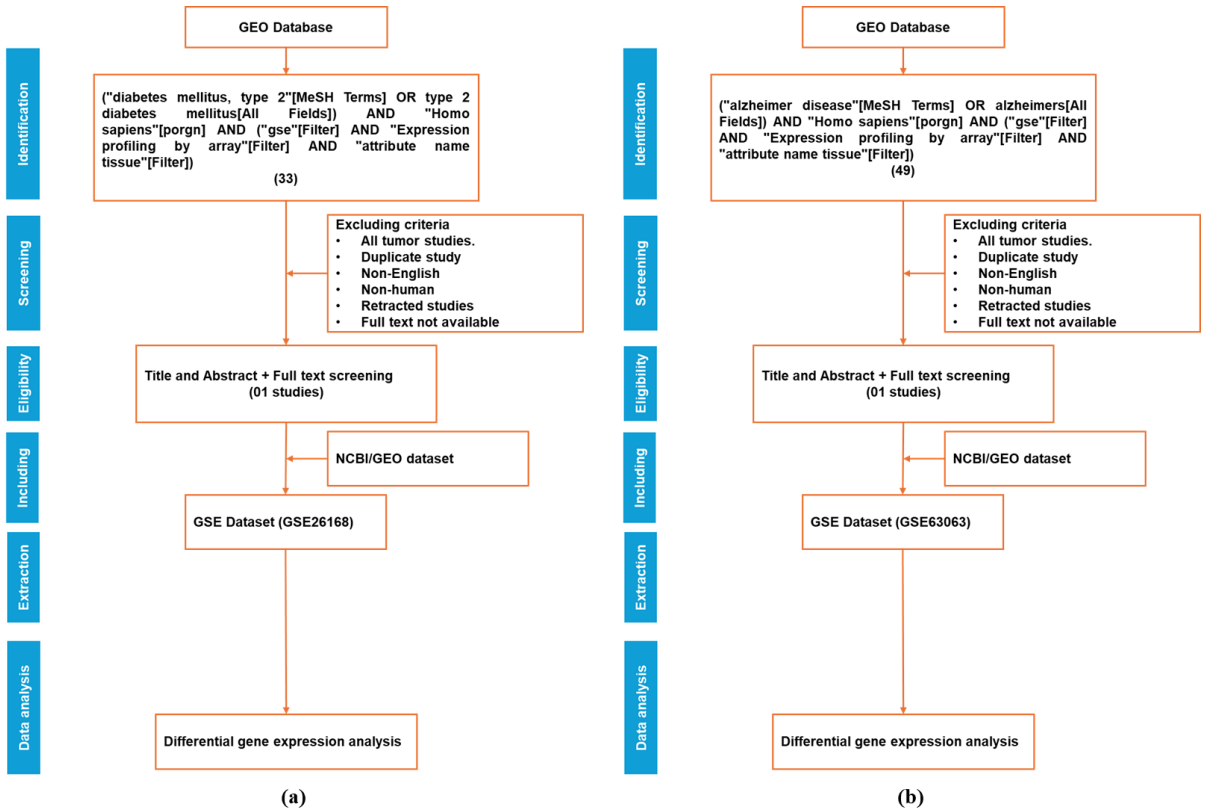


Figure 1: Systematic search strategy for identifying T2DM and AD datasets in the GEO repository.

PPI Network Analysis, MCODE and Hub Genes Identification: We have carried out a Protein-Protein Interaction (PPI) network analysis to understand the interactions between common DEGs between the two datasets GSE26168 and GSE63063 at a protein level. The PPI network was constructed from publicly available databases such as cytoscape, which depicted functional relationships and potential molecular mechanisms for the pathogenesis of T3DM[12,34]. Afterward, the Molecular Complex Detection (MCODE) analysis revealed densely connected regions in the PPI network, representing protein complexes or functional modules related to T3DM. In addition, we identified the hub's highly connected nodes ranked by topological properties such as degree centrality, betweenness centrality and closeness centrality. These hub genes act as critical regulators in the molecular network and provide insight towards potential therapeutic targets to be studied further.

Integrative Analysis of TF Enrichment, PPI and Kinase Activity: Systematically, transcription factors were identified through Chromatin Immunoprecipitation sequencing datasets. Following this, a PPI network was constructed from transcription factors with putative functional regulatory importance using the Genes2Networks computational platform. Putative protein kinases were predicted for their possible involvement in mRNA expression changes using kinase enrichment analysis. Finally, the X2K (eXpression2Kinases) algorithm integrates enriched transcription factors and the predicted kinases[2,6,13].

Discovery of Promising Therapeutic Candidates: Therapeutic targets shared between Alzheimer's disease and type 2 diabetes mellitus have been identified through analysis of common differentially expressed genes using the drug design database. This global resource is widely utilized for identifying associations between drugs and gene targets. It serves as a valuable tool for analysing novel genes and establishing connections between therapeutic agents and their molecular targets. This tool GSEA software enabled the correlation of transcriptional activation patterns with potential drug candidates for research and pharmacological applications. By linking pharmacological signatures to DEGs, this approach may uncover drugs with inhibitory effects on both T2DM and AD. These insights hold promise for advancing therapeutic strategies targeting the shared molecular pathways of these complex diseases.

Statistical analysis: Statistical analysis was performed using RStudio (https://www.R-project.org). The data was tested using the Wilcoxon rank sum test when it was not normal and used the T-test when it was normal. A ‘p’ value of ≤0.05 was set as a significance threshold for screening DEGs.

Results

Differential gene expression analysis: A comprehensive analysis of Differentially Expressed Genes (DEGs) was conducted using the R programming language and the limma package within the R framework. DEGs were extracted from the gene expression omnibus (GEO) dataset using platform and series matrix files, carefully considering appropriate annotation packages. The selection criteria for DEGs were established based on a log2 fold change (log2FC) magnitude ≤ -0.1 or ≥ 0.1, accompanied by a statistically significant p-value cutoff of ≤ 0.05. This rigorous approach identified 2973 DEGs (1323 up-regulated and 1650 down-regulated) in T2DM and 577 DEGs (374 up-regulated and 203 down-regulated) in AD.

In addition to the analysis, a volcano plot was generated to visualize the distribution of DEGs based on their fold change and statistical significance (p-value). The volcano plot reveals distinct clusters of DEGs, with those meeting both the fold change and p-value criteria prominently displayed (Figure 2). This analysis provides valuable insights into the molecular mechanisms implicated in the studied phenomenon and forms the basis for further investigation.

Venn Diagram Analysis: Venn diagram analysis revealed a notable convergence of 119 common DEGs (77 upregulated and 42 downregulated DEGs) between the two datasets, GSE26168 and GSE63063 (Figure 3). The cohort of upregulated DEGs within this shared domain likely constitutes a cadre of pivotal genes central to the organism’s adaptive response to the experimental stimuli. Conversely, the downregulated DEGs may indicate a concerted suppression of specific cellular functions or pathways, which are uniformly affected across the datasets. Integrating these findings could yield a more comprehensive understanding of the molecular underpinnings governing the observed phenotypic outcomes.

Gene Enrichment Analysis: We employed enrichGO and enrichKEGG functions from the ClusterProfiler package, followed by visualization with ggplot2, to conduct gene enrichment analysis aimed at elucidating the biological pathways and functions associated with 119 commonly identified DEGs. Our findings revealed enriched categories across Cellular Components (CCs), Molecular Functions (MFs) and Biological Processes (BPs) in gene ontology (GO) analysis (Table 1).

Notably, enriched CCs included the secretory granule lumen, cytosolic ribosome, specific granule, tertiary granule lumen, tertiary granule membrane, ficolin−1−rich granule membrane, focal adhesion, cell−substrate junction, tertiary granule and secretory granule membrane, indicative of roles in cellular organization and signaling. Enriched MFs primarily involved oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor, phosphatidylinositol−3−phosphate binding, superoxide−generating NAD(P)H oxidase activity, complement receptor activity, IgG binding, superoxide−generating NADPH oxidase activator activity, immune receptor activity, carbohydrate binding,

double−stranded RNA binding and pattern recognition receptor activity.

Enriched BPs encompassed SRP−dependent cotranslational protein targeting to membrane, myeloid leukocyte migration, interleukin−8 production, positive regulation of interleukin−8 production, regulation of interleukin−8 production, regulation of response to biotic stimulus, astrocyte differentiation, positive regulation of response to biotic stimulus, neutrophil activation involved in immune response and neutrophil degranulation, highlighting the diverse functional roles of DEGs in cellular homeostasis and environmental adaptation.

Moreover, KEGG analysis identified enriched pathways such as the biosynthesis of amino acids, platelet activation, ribosome, lipid and atherosclerosis, ferroptosis, osteoclast differentiation, endocytosis, pentose phosphate pathway, neutrophil extracellular trap formation and Coronavirus disease − COVID−19, underscoring potential involvement of DEGs in critical signaling cascades and regulatory networks (Table 2). Visualization of results facilitated the interpretation and exploration of enriched biological pathways and functions associated with DEGs, offering valuable insights into the molecular mechanisms driving observed gene expression changes in our study, thus laying the foundation for further investigation and interpretation.

Network Dynamics in T3DM Based on Hub Gene Centrality and MCODE Clustering: The PPI network analysis was meticulously conducted by querying publicly available databases, notably STRING which aggregates protein interaction data from various sources, including experimental results, computational prediction methods and public text collections. The resultant network was visualized using the cytoscape software (Figure 4).

Enhancing the analysis, we applied the MCODE algorithm, a cytoscape plugin designed to detect densely connected regions within large PPI networks. These regions indicate protein complexes or functional modules that play pivotal roles in cellular processes. Our MCODE analysis, parameterized with a node score cutoff of 0.2 and a k-core threshold of 2, identified seven clusters (Table 3). The first cluster displayed a high degree of interconnectivity and biological significance, suggesting its involvement in crucial pathways integral to disease progression (Figure 5).

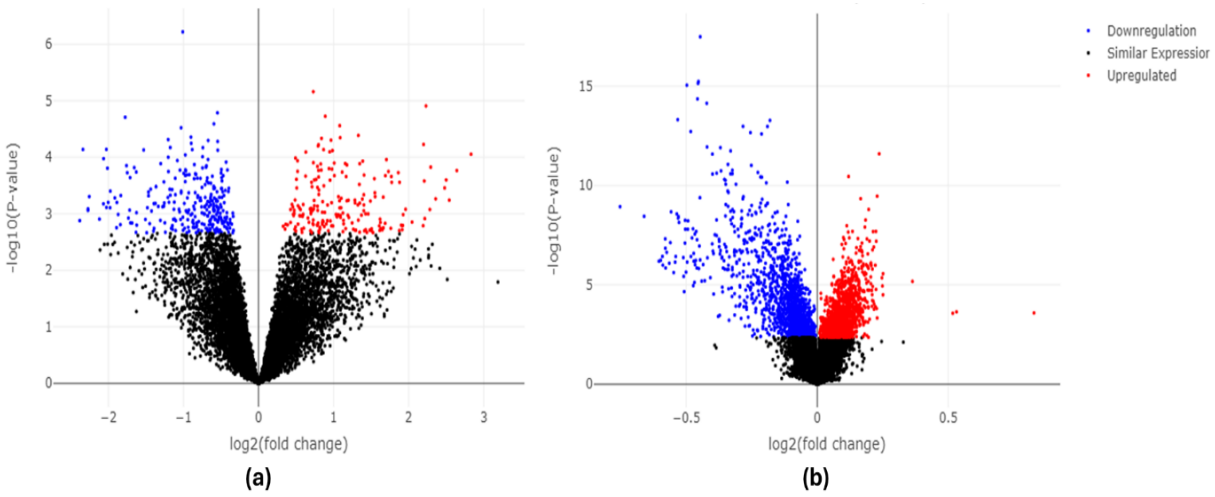


Figure 2: Volcano plot (a) DEGs in GSE26168 (b) DEGs in GSE63063.

Figure 3: Venn diagram (a) Common down-regulated DEGs in GSE26168 and GSE63063 (b) Common up-regulated DEGs in GSE26168 and GSE63063.

Table 1
Gene Ontology (Cellular Components (CCs), Molecular Functions (MFs), Biological Processes (BPs) of 119 common DEGs in GSE26168 and GSE63063

	ID	Description	Gene Ratio	Bg Ratio	p value	p. adjust	q value	gene ID	Count
CCs	GO:0030667	Secretory granule membrane	16/112	306/19559	2.145755E-11	4.93522E-09	3.72682E-09	FCGR2A/SIRPA/SIGLEC5/CA4/CXCR1/SELL/MGAM/CLEC12A/CD14/FPR1/ADGRG3/ADGRE5/MME/CD68/PLAUR/FPR2	16
	GO:0070820	Tertiary granule	10/112	164/19559	3.540611E-08	4.0717E-06	3.07474E-06	SIRPA/SIGLEC5/PGLYRP1/MGAM/CLEC12A/FPR1/TIMP2/FPR2/MMP9/ALDOA	10
	GO:0030055	Cell-substrate junction	13/112	423/19559	9.20168E-07	7.05462E-05	5.32729E-05	RPS29/RPL10A/ANXA1/RPS10/RPL27/ACTN1/PCBP2/VIM/HSPA1B/ADGRE5/MME/PLAUR/FERMT3	13
	GO:0005925	Focal adhesion	12/112	415/19559	4.63545E-06	0.000266538	0.000201276	RPS29/RPL10A/ANXA1/RPS10/RPL27/ACTN1/PCBP2/VIM/HSPA1B/ADGRE5/MME/PLAUR	12
	GO:0101003	Ficolin-1-rich granule membrane	5/112	61/19559	2.593266E-05	0.001192902	0.000900818	SIRPA/SIGLEC5/MGAM/FPR1/FPR2	5
	GO:0070821	Tertiary granule membrane	5/112	73/19559	6.199944E-05	0.002376642	0.001794718	SIRPA/SIGLEC5/MGAM/CLEC12A/FPR2	5
	GO:1904724	Tertiary granule lumen	4/112	55/19559	0.000277467	0.009116779	0.006884525	PGLYRP1/TIMP2/MMP9/ALDOA	4
	GO:0042581	Specific granule	6/112	160/19559	0.000319522	0.009186263	0.006936995	PGLYRP1/CLEC12A/ADGRG3/TIMP2/PLAUR/FPR2	6
	GO:0022626	Cytosolic ribosome	5/112	110/19559	0.000427178	0.009899829	0.007475843	RPS29/RPL10A/RPS10/RPL27/RSL24D1	5
	GO:0034774	Secretory granule lumen	8/112	322/19559	0.000533878	0.009899829	0.007475843	CYB5R3/ACTN1/SERPINA1/PGLYRP1/TIMP2/FERMT3/GRN/ALDOA	8
MFs	GO:0038187	Pattern recognition receptor activity	4/111	23/18352	1.027114E-05	0.003297113	0.003135474	LY96/TLR8/PGLYRP1/CD14	4
	GO:0003725	Double-stranded RNA binding	5/111	76/18352	9.705801E-05	0.01557783	0.014814136	LSM14A/ACTN1/VIM/TLR8/ADAR	5
	GO:0030246	Carbohydrate binding	7/111	267/18352	0.001197174	0.101474351	0.096499629	CLEC4A/TALDO1/SIGLEC5/SELL/MGAM/CLEC12A/ALDOA	7
	GO:0140375	Immune receptor activity	5/111	135/18352	0.001373988	0.101474351	0.096499629	CXCR1/IL17RA/FPR1/CSF3R/FPR2	5
	GO:0016176	Superoxide-generating NADPH oxidase activator activity	2/111	10/18352	0.001580597	0.101474351	0.096499629	NCF4/NCF1C	2
	GO:0019864	IgG binding	2/111	11/18352	0.001924219	0.102945721	0.097898866	FCGRT/FCGR2A	2

	GO:0004875	Complement receptor activity	2/111	12/18352	0.002299957	0.10546944	0.100298861	FPR1/FPR2	2
	GO:0016175	Superoxide-generating NAD(P)H oxidase activity	2/111	13/18352	0.002707416	0.108635067	0.103309295	NCF4/NCF1C	2
	GO:0032266	Phosphatidylinositol-3-phosphate binding	3/111	48/18352	0.003055877	0.108992952	0.103649634	VPS36/NCF4/NCF1C	3
	GO:0050664	Oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor	2/111	19/18352	0.005796707	0.177438309	0.168739497	NCF4/NCF1C	2
BPs	GO:0043312	Neutrophil degranulation	24/109	487/18866	4.741644E-16	6.18165E-13	5.71653E-13	CYB5R3/ABR/FCGR2A/HSPA1B/SIRPA/SIGLEC5/SERPINA1/PGLYRP1/CXCR1/SELL/MGAM/CLEC12A/CD14/FPR1/ADGRG3/TIMP2/ADGRE5/MME/CD68/PLAUR/FPR2/MMP9/GRN/ALDOA	24
	GO:0002283	Neutrophil activation involved in immune response	24/109	490/18866	5.441559E-16	6.18165E-13	5.71653E-13	CYB5R3/ABR/FCGR2A/HSPA1B/SIRPA/SIGLEC5/SERPINA1/PGLYRP1/CXCR1/SELL/MGAM/CLEC12A/CD14/FPR1/ADGRG3/TIMP2/ADGRE5/MME/CD68/PLAUR/FPR2/MMP9/GRN/ALDOA	24
	GO:0002833	Positive regulation of response to biotic stimulus	8/109	251/18866	0.000104896	0.058995262	0.054556404	LY96/CLEC4A/LSM14A/MATR3/TLR8/FYN/FPR2/GRN	8
	GO:0048708	Astrocyte differentiation	5/109	83/18866	0.000119125	0.058995262	0.054556404	NOTCH1/VIM/STAT3/FPR2/GRN	5
	GO:0002831	Regulation of response to biotic stimulus	10/109	409/18866	0.000129831	0.058995262	0.054556404	LY96/CLEC4A/LSM14A/MATR3/PCBP2/TLR8/FYN/ADAR/FPR2/GRN	10
	GO:0032677	Regulation of interleukin-8 production	5/109	93/18866	0.000203706	0.069080126	0.063882474	ANXA1/HSPA1B/TLR8/STAT3/CD14	5
	GO:0032757	Positive regulation of interleukin-8 production	4/109	54/18866	0.000266942	0.069080126	0.063882474	HSPA1B/TLR8/STAT3/CD14	4
	GO:0032637	Interleukin-8 production	5/109	101/18866	0.000299329	0.069080126	0.063882474	ANXA1/HSPA1B/TLR8/STAT3/CD14	5
	GO:0097529	Myeloid leukocyte migration	7/109	222/18866	0.000306591	0.069080126	0.063882474	ANXA1/SIRPA/CXCR1/IL17RA/DYSF/CSF3R/FPR2	7
	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	5/109	105/18866	0.000358302	0.069080126	0.063882474	RPS29/SRP9/RPL10A/RPS10/RPL27	5

Table 2
KEGG analysis of 119 common DEGs in GSE26168 and GSE63063

ID	Description	Gene Ratio	Bg Ratio	p value	p. adjust	q value	gene ID	Count
hsa05171	Coronavirus disease - COVID-19	9/62	232/8223	5.34605E-05	0.007965618	0.007484474	RPS29/RPL10A/RPS10/RPL27/RSL24D1/FCGR2A/TLR8/STAT3/ADAR	9
hsa04613	Neutrophil extracellular trap formation	7/62	190/8223	0.000528727	0.039390181	0.037010908	VDAC3/FCGR2A/NCF4/PADI4/TLR8/FPR1/FPR2	7
hsa00030	Pentose phosphate pathway	3/62	30/8223	0.001433488	0.071196594	0.066896129	TALDO1/TKT/ALDOA	3
hsa04144	Endocytosis	7/62	251/8223	0.002666655	0.08016971	0.075327245	VPS36/CAPZA2/EHD1/IQSEC1/HSPA1B/RAB11FIP1/CXCR1	7
hsa04380	Osteoclast differentiation	5/62	128/8223	0.002690259	0.08016971	0.075327245	GAB2/FCGR2A/NCF4/SIRPA/FYN	5
hsa04216	Ferroptosis	3/62	41/8223	0.003548664	0.088125151	0.082802155	VDAC3/PCBP2/ACSL1	3
hsa05417	Lipid and atherosclerosis	6/62	215/8223	0.005410505	0.115166456	0.108210093	LY96/NCF4/HSPA1B/STAT3/CD14/MMP9	6
hsa03010	Ribosome	5/62	167/8223	0.008286685	0.154339512	0.145016991	RPS29/RPL10A/RPS10/RPL27/RSL24D1	5
hsa04611	Platelet activation	4/62	124/8223	0.014021524	0.232134125	0.218112601	FCGR2A/TBXAS1/FYN/FERMT3	4
hsa01230	Biosynthesis of amino acids	3/62	75/8223	0.018762433	0.279560253	0.262674063	TALDO1/TKT/ALDOA	3
hsa04936	Alcoholic liver disease	4/62	142/8223	0.021960273	0.297461876	0.27949438	LY96/ACADM/IL17RA/CD14	4
hsa05150	Staphylococcus aureus infection	3/62	96/8223	0.035541321	0.414236288	0.389215304	FCGR2A/FPR1/FPR2	3
hsa04640	Hematopoietic cell lineage	3/62	99/8223	0.038406482	0.414236288	0.389215304	CD14/CSF3R/MME	3
hsa00071	Fatty acid degradation	2/62	43/8223	0.041445526	0.414236288	0.389215304	ACADM/ACSL1	2
hsa04620	Toll-like receptor signaling pathway	3/62	104/8223	0.043436864	0.414236288	0.389215304	LY96/TLR8/CD14	3
hsa04066	HIF-1 signaling pathway	3/62	109/8223	0.048781711	0.414236288	0.389215304	MKNK2/STAT3/ALDOA	3

Furthermore, a Hub Genes analysis was performed to discern critical regulators within the PPI network. Hub genes were identified and prioritized based on their topological properties, quantified by metrics such as Degree, MCC, Closeness and MNC. These centrality measures were instrumental in pinpointing hub genes such as MNDA, FCER1G, ALOX5AP, NCF2, CSF3R, FPR1, FGR and HCK, which are hypothesized to be central drivers of molecular interactions in the pathogenesis of T3DM (Figure 6). Identifying these hub genes not only enhances our understanding of the diseases' molecular underpinnings but also underscores potential therapeutic targets warranting further exploration and validation.

Integrative Analysis to explore the Transcriptional Regulatory Landscape: Transcription Factor Enrichment Analysis (TFEA) identified 11 putatively enriched transcription factors (TFs) governing the expression of DEGs based on ChIP-seq data. Notably, TFs such as RUNX1, SPI1, POU5F1, RELA, NANOG, GATA2, GATA1, KLF4, TCF3, NFE2L2 and REST were among the top enriched regulators, suggesting their potential roles in orchestrating gene expression changes (Figure 7a) (Table 4). Subsequent construction of a PPI network connecting these enriched TFs using the Genes2Networks (G2N) method revealed a complex network of interactions, highlighting potential cooperative and regulatory relationships among the identified TFs (Figure 7b). Kinase enrichment analysis (KEA) predicted several protein kinases potentially regulating mRNA expression changes, with kinases such as HIPK2, ERK1, MAPK1, MAPK3, MAPK14 and CK2ALPHA (Figure 7c).

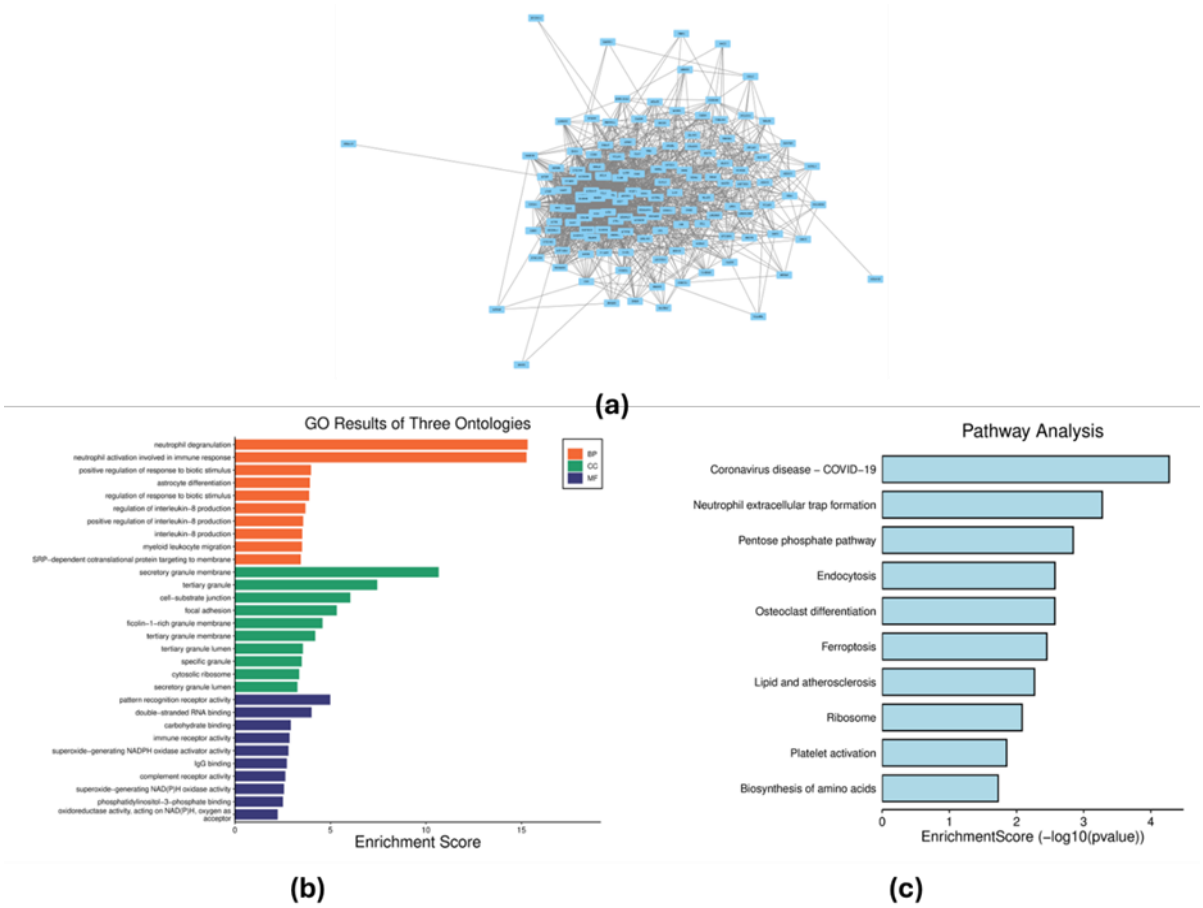


Figure 4: PPI networks.

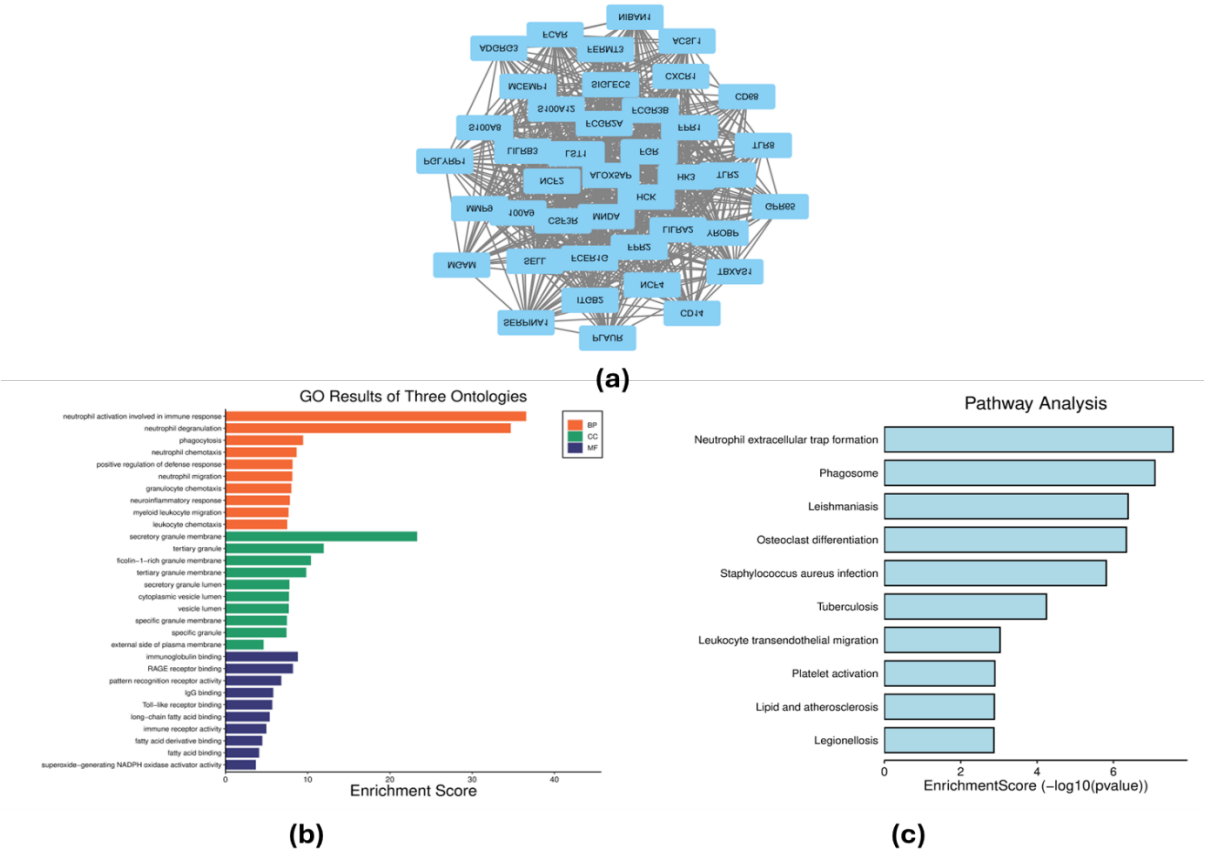
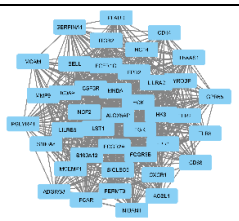
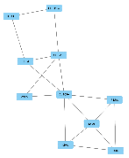
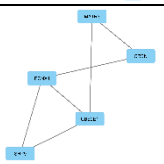
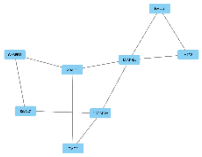


Figure 5: First MCODE cluster (Score 33.65) displayed a high degree of interconnectivity and biological significance.

Table 3
MCODE Clusters.

Cluster	Score	Node	Edge	Network
Cluster 1	33.65	41	673	
Cluster 2	4	7	12	
Cluster 3	3.556	10	16	
Cluster 4	3	5	6	
Cluster 5	2.857	8	10	
Cluster 6	2.667	4	4	
Cluster 7	2.5	5	5	

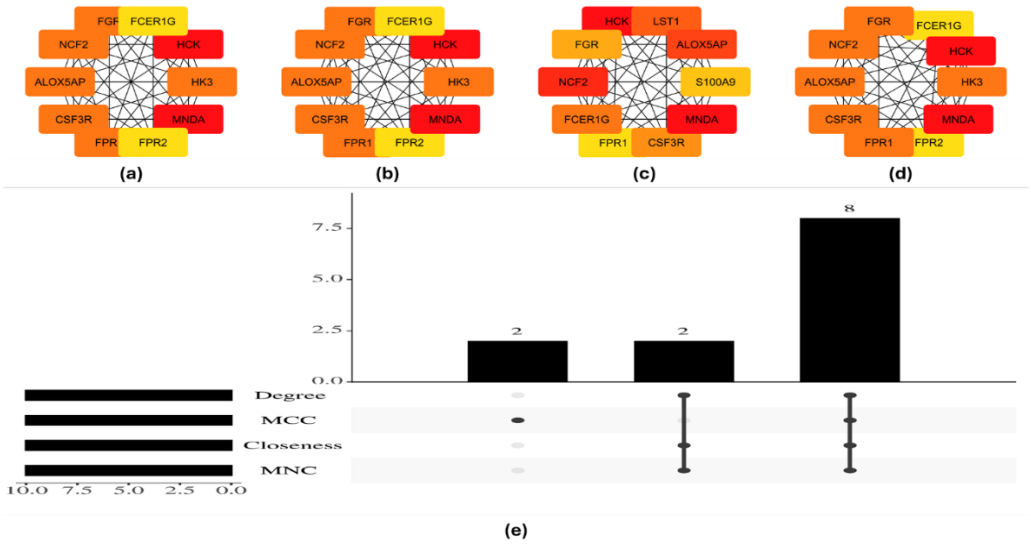


Figure 6: Hub genes (a) Degree (b) MCC (c) Closeness (d) MNC (e) UpSet plot.

Integration of enriched TFs and kinases via known PPIs using the X2K algorithm generated a comprehensive upstream pathway, unveiling intricate regulatory mechanisms underlying gene expression alterations (Figure 7d). This integrative analysis provides valuable insights into the regulatory landscape governing gene expression dynamics in the context of the studied biological system.

Understanding the structural properties of small molecules in relation to their receptor sensitivity necessitates a comprehensive examination of the protein-drug connection. The ten most important therapeutic drugs interacting with the DEGs have been determined based on their P value ($p < 0.01$) utilizing the EnrichR program from the DSigDB database.

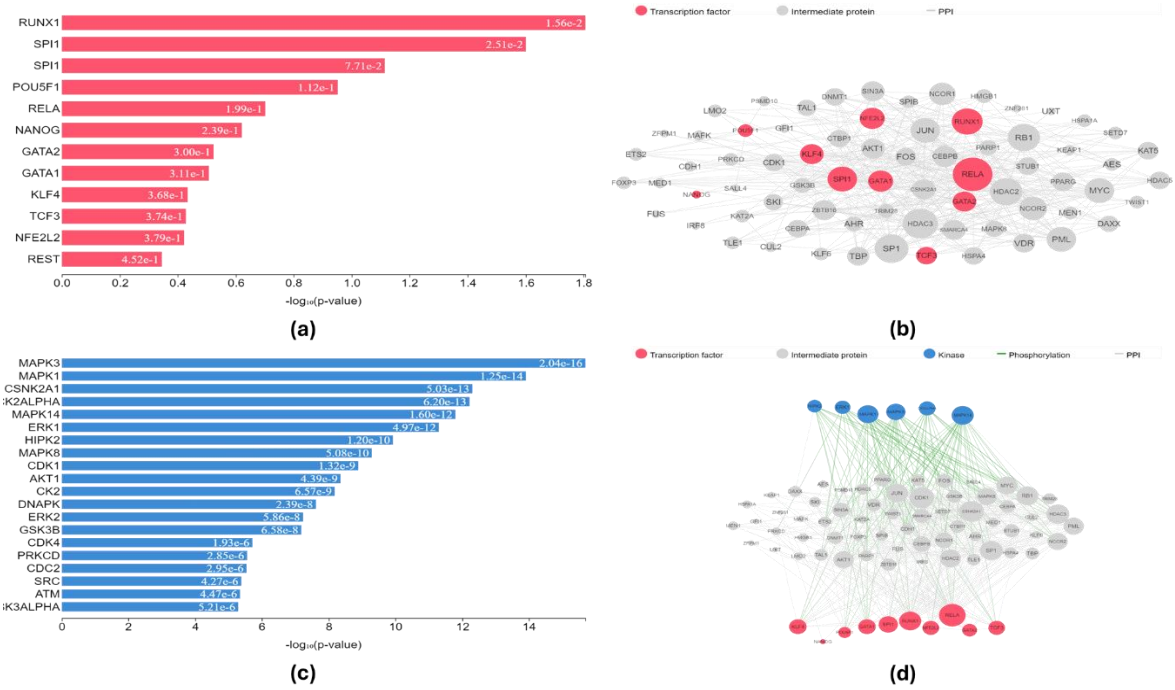


Figure 7: Transcriptional Regulatory Landscape (a) Transcription Factor Enrichment Analysis (TFEA) (b) Protein-Protein Interaction Expansion (c) Kinase Enrichment Analysis (KEA) (d) eXpression2Kinases Network.

Table 4
Enriched transcription factors (TFs) governing the expression of DEGs.

Rank	Transcription Factor	Hypergeometric p-value	Enriched Targets
1	RUNX1	0.01564	FCER1G; FGR; ALOX5AP
2	SPI1	0.02505	NCF2; MNDA; ALOX5AP
3	SPI1	0.07709	NCF2; ALOX5AP
4	POU5F1	0.112	HCK
5	RELA	0.1987	NCF2
6	NANOG	0.2391	HCK
7	GATA2	0.2999	MNDA
8	GATA1	0.3114	CSF3R
9	KLF4	0.3681	HCK
10	TCF3	0.3738	HCK
11	NFE2L2	0.3786	ALOX5AP
12	REST	0.4518	CSF3R

The predicted drugs were allopurinol CTD00005353, neostigmine bromide PC3 Down, indomethacin CTD 00006147, copper sulfate CTD00007279, methyl methanesulphonate CTD 00006304, hydrogen peroxide CTD00006118, phenethyl isothiocyanate CTD 00002443 and Diclofenac CTD 00005804.

Comprehensive intricate signaling between T2DM and AD based on genetic and epigenetic factors

HIPK2 Regulation: The intersection of T2DM and AD (T3DM) is particularly interesting due to the shared molecular pathways involving HIPK2. In individuals with T2DM, the dysregulation of HIPK2 may exacerbate AD pathology. The aberrant activity of HIPK2 in T2DM could further promote Aβ accumulation and tau hyperphosphorylation, thereby intensifying neuronal damage and cognitive decline associated with AD[16,27,28,39]. Moreover, the interplay between insulin resistance, a

defining characteristic of T3DM, suggests a bidirectional relationship where each condition may influence the progression of the other. Insulin resistance has been shown to affect brain metabolism and function, potentially contributing to the development and progression of AD[20,21] (Figure 8).

ERK1 Regulation: The interplay between T3DM is underscored by the observation that insulin resistance and the resultant hyperglycaemia may exert deleterious effects on brain function. Specifically, alterations in ERK1 activity associated with T2DM could compromise of synaptic integrity and disrupt cognitive functions. This suggests a mechanistic link whereby T2DM-associated metabolic disturbances might contribute to the cognitive deficit characteristic of AD[27,37]. Moreover, dysregulated ERK1/2 signaling has been identified in patients with AD, with potential implications for the multifaceted underlying pathologies of the disease including amyloid-β plaque formation, tau phosphorylation and neuroinflammation. The dysregulation of ERK1 in T2DM could thus have a cascading effect on these AD-related pathologies, potentially exacerbating the disease process[19] (Figure 8).

MAPK1, MAPK3 and MAPK14 Regulation: MAPK1, MAPK3 and MAPK14 are integral components of the Mitogen-Activated Protein Kinase (MAPK) family, a group of serine/threonine-specific protein kinases involved in a myriad of cellular processes. These kinases play pivotal roles in transducing extracellular signals to the cellular nucleus, thereby influencing gene expression and cellular responses. The dysregulation of MAPK1, MAPK3 and MAPK14 in T2DM could potentially influence the pathophysiology of AD[17].

Insulin resistance and chronic inflammation associated with T2DM may exacerbate synaptic dysfunction and neuroinflammation, thereby contributing to the cognitive decline observed in AD[4]. The interplay between these kinases in T3DM underscores the complex relationship between metabolic disorders and neurodegenerative diseases.

Understanding the molecular mechanisms by which MAPK1, MAPK3 and MAPK14 contribute to the pathogenesis of T3DM could provide insights into potential therapeutic targets for these conditions. Targeting the dysregulated MAPK signaling in T2DM may improve metabolic outcomes and mitigate the risk or progression of AD (Figure 8).

CK2ALPHA Regulation: CK2ALPHA is also a serine/threonine kinase that plays a crucial role in various cellular processes including glucose metabolism and insulin signaling[3,23]. The interplay between T3DM is particularly noteworthy in the context of CK2ALPHA activity. The metabolic disturbances associated with T2DM such as hyperglycaemia and insulin resistance, could influence the activity of CK2ALPHA[10,15]. This, in turn, may exacerbate tau pathology by promoting further tau phosphorylation. The presence of T2DM could thus potentiate the neurodegenerative processes in AD, potentially accelerating the onset and progression of cognitive decline[1,7]. Moreover, the dysregulation of CK2ALPHA in T2DM may have broader implications for neuronal health. Given the kinase's involvement in cell survival, apoptosis and response to oxidative stress, its dysfunction could contribute to the neuronal cell loss observed in AD (Figure 8).

Discussion

The current study aims to explore the molecular interplay between these comorbidities using bioinformatics tools and network biology. The comprehensive analysis of Affymetrix microarray data from datasets GSE26168 and GSE63063 revealed significant molecular linkages between T2DM and AD, commonly known as T3DM. After stringent preprocessing and quality control steps, differential gene expression analysis identified many genes dysregulated in both conditions. Notably, several key genes implicated in insulin signaling pathways, such as FCER1G, FGR, ALOX5AP, NCF2 and CSF3R, exhibited altered expression patterns across both datasets. Additionally, genes associated with neuroinflammation and synaptic dysfunction including FCER1G, FGR, ALOX5AP, NCF2, HCK, CSF3R, displayed differential expression profiles consistent with the pathogenesis of T3DM.

Furthermore, pathway enrichment analysis highlighted the involvement of shared biological processes including SRP−dependent cotranslational protein targeting to membrane, myeloid leukocyte migration, interleukin−8 production, positive regulation of interleukin−8 production, regulation of interleukin−8 production, regulation of response to biotic stimulus, astrocyte differentiation, positive regulation of response to biotic stimulus, neutrophil activation involved in immune response and neutrophil degranulation, underscoring the interconnectedness of these two complex diseases at the molecular level.

Network analysis unveiled intricate interactions among dysregulated genes, pinpointing central hubs and potential regulatory nodes driving the pathophysiology of T3DM comorbidity. Moreover, integrating clinical metadata enabled the identification of candidate biomarkers associated with disease progression and severity. Collectively, these findings provide novel insights into the molecular underpinnings of T3DM interplay, laying the groundwork for targeted therapeutic strategies and precision medicine approaches to mitigate the burden of these devastating neurodegenerative disorders.

Conclusion

Comprehensively, the current study investigating molecular overlaps between T2DM and AD explored several key genes MNDA, FCER1G, ALOX5AP, NCF2, CSF3R, FPR1, FGR and HCK that are commonly expressed in these pathology.

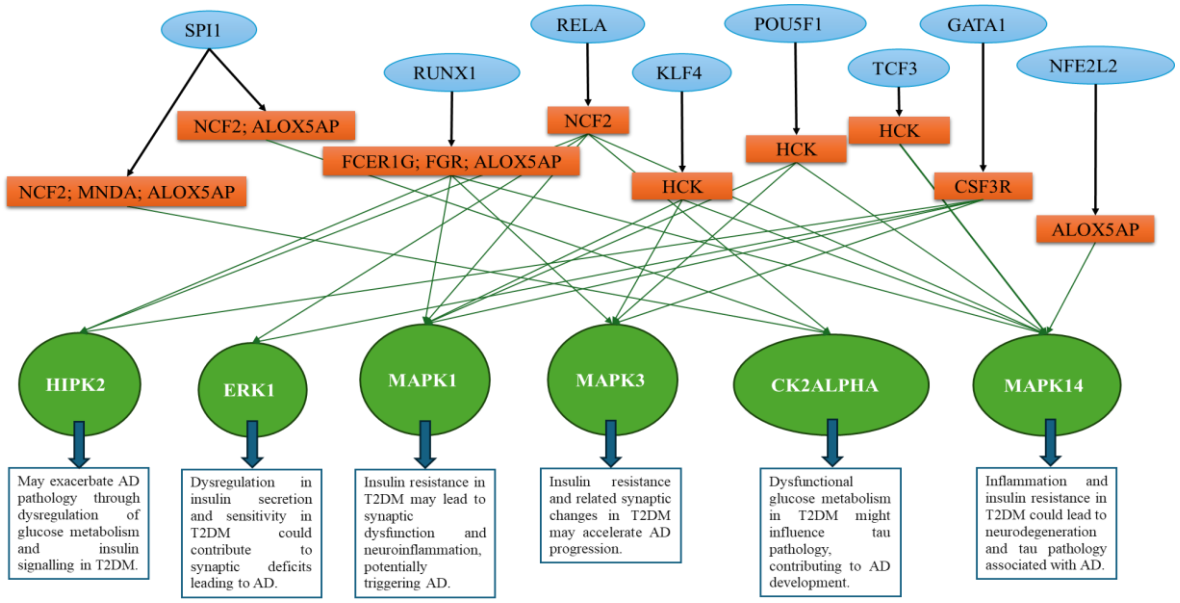


Figure 8: TF-Gene-Kinase Dynamics in T2DM and AD, or T3DM Pathogenesis.

These genes fall into critical biological pathways including insulin signaling, immune response and neuroinflammation, all of which are key pathophysiology factors between both maladies. Moreover, the central regulator of these investigated genes has been identified as RUNX1, SPI1 and RELA transcription factors that may orchestrate a complex network of interactions at the molecular level.

These interactions further include kinase pathways such as MAPK1, MAPK3, MAPK14 and CK2ALPHA, which are critically involved in cellular stress responses, inflammatory signaling, oxidative stress response and synaptic function. Interestingly, the implication of RUNX1 and SPI1 modulate the MAPK signaling pathways which play a fundamental role in cellular responses to stress and inflammation. This shared molecular framework suggests common pathophysiological landscape exacerbating T2DM and AD. The potential biomarkers and therapeutic targets in this research, comprising DEGs, transcription factors and kinases, offer exciting opportunities for the development of novel therapeutic agents that modulate the implicated pathways and, therefore, address the interlinked pathologies of T2DM and AD. The illumination of these molecular connections not only deepens our understanding of the diseases but also forms the foundation for future research to be directed at exploration and validation of these biomarkers and targets towards the development of novel treatments for these chronic conditions.

References

1. Abasi L.S., Elathram N., Movva M., Deep A., Corbett K.D. and Debelouchina G.T., Phosphorylation regulates tau's phase separation behavior and interactions with chromatin, *Communications Biology*, **7**, https://doi.org/10.1038/s42003-024-05920-4 **(2024)**

2. Berger S.I., Posner J.M. and Ma'ayan A., Genes2Networks: Connecting lists of gene symbols using mammalian protein interactions databases, *BMC Bioinformatics*, **8**, https://doi.org/10.1186/1471-2105-8-372/FIGURES/2 **(2007)**

3. Borgo C., D'Amore C., Sarno S., Salvi M. and Ruzzene M., Protein kinase CK2: a potential therapeutic target for diverse human diseases, *Signal Transduction and Targeted Therapy*, **6**, https://doi.org/10.1038/s41392-021-00567-7 **(2021)**

4. Chatterjee S. and Mudher A., Alzheimer's disease and type 2 diabetes: A critical assessment of the shared pathological traits, *Front Neurosci*., **12**, 359050, https://doi.org/10.3389/FNINS.2018.00383/BIBTEX **(2018)**

5. Chen H. and Boutros P.C., VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R, *BMC Bioinformatics*, **12**, https://doi.org/10.1186/1471-2105-12-35 **(2011)**

6. Clarke D.J.B., Kuleshov M.V., Schilder B.M., Torre D., Duffy M.E., Keenan A.B., Lachmann A., Feldmann A.S., Gundersen G.W., Silverstein M.C., Wang Z. and Ma'Ayan A., eXpression2Kinases (X2K) Web: linking expression signatures to upstream cell signaling networks, *Nucleic Acids Res*, **46**, W171–W179, https://doi.org/10.1093/NAR/GKY458 **(2018)**

7. Congdon E.E., Ji C., Tetlow A.M., Jiang Y. and Sigurdsson E.M., Tau-targeting therapies for Alzheimer disease: current status and future directions, *Nature Reviews Neurology*, **19**, 715–736, https://doi.org/10.1038/s41582-023-00883-2 **(2023)**

8. Conte F. and Paci P., Alzheimer's disease: insights from a network medicine perspective, *Scientific Reports*, **12**, https://doi.org/10.1038/s41598-022-20404-3 **(2022)**

9. Cui Y., Tang T.Y., Lu C.Q. and Ju S., Insulin Resistance and Cognitive Impairment: Evidence from Neuroimaging, *J Magn Reson Imaging*, **56**, 1621–1649, https://doi.org/10.1002/JMRI.28358 **(2022)**

10. Deepthi B.V. and Isac Sobana Raj C., Amalgamation, characterization and biological assay of

benzyltrimethylammonium chloride, *Res. J. Chem. Environ*., **28(2)**, 76-81 **(2024)**

11. DeFronzo R.A., Ferrannini E., Groop L., Henry R.R., Herman W.H., Holst J.J., Hu F.B., Kahn C.R., Raz I., Shulman G.I., Simonson D.C., Testa M.A. and Weiss R., Type 2 diabetes mellitus, *Nature Reviews Disease Primers*, **1**, https://doi.org/10.1038/nrdp.2015.19 **(2015a)**

12. Doncheva N.T., Morris J.H., Gorodkin J. and Jensen L.J., Cytoscape StringApp: Network Analysis and Visualization of Proteomics Data, *J Proteome Res*., **18**, 623–632, https://doi.org/10.1021/ACS.JPROTEOME.8B00702 **(2019)**

13. Gershman A., Sauria M.E.G., Guitart X., Vollger M.R., Hook P.W., Hoyt S.J., Jain M., Shumate A., Razaghi R., Koren S., Altemose N., Caldas G.V., Logsdon G.A., Rhie A., Eichler E.E., Schatz M.C., O'Neill R.J., Phillippy A.M., Miga K.H. and Timp W., Epigenetic patterns in a complete human genome, *Science*, https://doi.org/10.1126/SCIENCE.ABJ5089 **(2022)**

14. González P., Lozano P., Ros G. and Solano F., Hyperglycemia and Oxidative Stress: An Integral, Updated and Critical Overview of Their Metabolic Interconnections, *Int J Mol Sci*., **24**, https://doi.org/10.3390/IJMS24119352 **(2023)**

15. Hameed I., Masoodi S.R., Mir S.A., Nabi M., Ghazanfar K. and Ganai B.A., Type 2 diabetes mellitus: From a metabolic disorder to an inflammatory condition, *World J Diabetes*, **6**, 598, https://doi.org/10.4239/WJD.V6.I4.598 **(2015)**

16. Hampel H., Hardy J., Blennow K., Chen C., Perry G., Kim S.H., Villemagne V.L., Aisen P., Vendruscolo M., Iwatsubo T., Masters C.L., Cho M., Lannfelt L., Cummings J.L. and Vergallo A., The Amyloid-β Pathway in Alzheimer's Disease, *Molecular Psychiatry*, **26**, 5481–5503, https://doi.org/10.1038/s41380-021-01249-0 **(2021)**

17. Johnson L.N., Noble M.E.M. and Owen, D.J., Active and inactive protein kinases: Structural basis for regulation, *Cell*, **85**, 149–158, https://doi.org/10.1016/S0092-8674(00)81092-2 **(1996)**

18. Karolina D.S., Armugam A., Tavintharan S., Wong M.T.K., Lim S.C., Sum C.F. and Jeyaseelan K., MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus, *PLoS One*, https://doi.org/10.1371/JOURNAL.PONE.0022839 **(2011)**

19. Khezri M.R., Yousefi K., Esmaeili A. and Ghasemnejad-Berenji M., The Role of ERK1/2 Pathway in the Pathophysiology of Alzheimer's Disease: An Overview and Update on New Developments, *Cellular and Molecular Neurobiology*, **43**, 177–191, https://doi.org/10.1007/S10571-022-01191-X **(2022)**

20. Kullmann S., Heni M., Hallschmid M., Fritsche A., Preissl H. and Häring H.U., Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans, *Physiol Rev*., **96**, 1169–1209 **(2016)**

21. Laws S.M., Gaskin S., Woodfield A., Srikanth V., Bruce D., Fraser P.E., Porter T., Newsholme P., Wijesekara N., Burnham S., Doré V., Li Q.X., Maruff P., Masters C.L., Rainey-Smith S., Rowe C.C., Salvado O., Villemagne V.L., Martins R.N. and Verdile G., Insulin resistance is associated with reductions in specific cognitive domains and increases in CSF tau in cognitively normal adults, *Sci Rep.*, https://doi.org/10.1038/S41598-017-09577-4 **(2017)**

22. Leng F. and Edison P., Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here?, *Nature Reviews Neurology*, **17**, 157–172, https://doi.org/10.1038/s41582-020-00435-y **(2020)**

23. Lou D.Y., Dominguez I., Toselli P., Landesman-Bollag E., O'Brien C. and Seldin D.C., The alpha catalytic subunit of protein kinase CK2 is required for mouse embryonic development, *Mol Cell Biol*., **28**, 131–139, https://doi.org/10.1128/MCB.01119-07 **(2008)**

24. Mittal K., Mani R.J. and Katare D.P., Type 3 Diabetes: Cross Talk between Differentially Regulated Proteins of Type 2 Diabetes Mellitus and Alzheimer's Disease, *Scientific Reports*, **6**, https://doi.org/10.1038/srep25589 **(2016)**

25. Nichols E. et al, Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019, *Lancet Public Health*, **7**, e105–e125, https://doi.org/10.1016/S2468-2667(21)00249-8 **(2022)**

26. Ritchie M.E., Phipson B., Wu D., Hu Y., Law C.W., Shi W. and Smyth G.K., Limma powers differential expression analyses for RNA-sequencing and microarray studies, *Nucleic Acids Res*, **43**, e47, https://doi.org/10.1093/NAR/GKV007 **(2015)**

27. Rojas M., Chávez-Castillo M., Bautista J., Ortega Á., Nava M., Salazar J., Díaz-Camargo E., Medina O., Rojas-Quintero J. and Bermúdez V., Alzheimer's disease and type 2 diabetes mellitus: Pathophysiologic and pharmacotherapeutics links, *World J Diabetes*, **12**, 745, https://doi.org/10.4239/WJD.V12.I6.745 **(2021)**

28. Sardina F., Conte A., Paladino S., Pierantoni G.M. and Rinaldo C., HIPK2 in the physiology of nervous system and its implications in neurological disorders, *Biochim Biophys Acta Mol Cell Res*., https://doi.org/10.1016/J.BBAMCR.2023.119465 **(2023)**

29. Sharma A.K. and Khanna D., Diabetes mellitus associated cardiovascular signalling alteration: A need for the revisit, *Cellular Signalling*, https://doi.org/10.1016/j.cellsig.2013.01.022 **(2013)**

30. Sharma A.K., Taneja G., Kumar Ashish, Sahu M., Sharma G., Kumar Asim, Sardana S. and Deep A., Insulin analogs: Glimpse on contemporary facts and future prospective, https://doi.org/10.1016/j.lfs.2019.01.011 **(2019)**

31. Sharma V., Singh J., Kumar A., Kansara S., Akhtar M.S., Khan M.F., Aldosari S.A., Mukherjee M. and Sharma A.K., Integrative experimental validation of concomitant miRNAs and transcription factors with differentially expressed genes in acute myocardial infarction, *Eur J Pharmacol*., **971**, 176540, https://doi.org/10.1016/J.EJPHAR.2024.176540 **(2024)**

32. Solis E., Hascup K.N. and Hascup E.R., Alzheimer's Disease: The Link Between Amyloid-β and Neurovascular Dysfunction, *J Alzheimers Dis*, **76**, 1179, https://doi.org/10.3233/JAD-200473 **(2020)**

33. Sood S., Gallagher I.J., Lunnon K., Rullman E., Keohane A., Crossland H., Phillips B.E., Cederholm T., Jensen T., van Loon L.J.C., Lannfelt L., Kraus W.E., Atherton P.J., Howard R.,

Gustafsson T., Hodges A. and Timmons J.A., A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status, *Genome Biol*., https://doi.org/10.1186/S13059-015-0750-X **(2015)**

34. Szklarczyk D., Gable A.L., Lyon D., Junge A., Wyder S., Huerta-Cepas J., Simonovic M., Doncheva N.T., Morris J.H., Bork P., Jensen L.J. and Von Mering C., STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, *Nucleic Acids Res*, **47**, D607–D613, https://doi.org/10.1093/NAR/GKY1131 **(2019)**

35. Volpe C.M.O., Villar-Delfino P.H., Dos Anjos P.M.F. and Nogueira-Machado J.A., Cellular death, reactive oxygen species (ROS) and diabetic complications, *Cell Death & Disease*, **9**, https://doi.org/10.1038/s41419-017-0135-z **(2018)**

36. Wong N.D. and Sattar N., Cardiovascular risk in diabetes mellitus: epidemiology, assessment and prevention, *Nature Reviews Cardiology*, **20**, 685–695, https://doi.org/10.1038/s41569-023-00877-z **(2023)**

37. Yong J., Johnson J.D., Arvan P., Han J. and Kaufman R.J., Therapeutic opportunities for pancreatic β-cell ER stress in diabetes mellitus, *Nature Reviews Endocrinology*, **17**, 455–467, https://doi.org/10.1038/s41574-021-00510-4 **(2021)**

38. Yu G., Wang L.G., Han Y. and He Q.Y., clusterProfiler: an R package for comparing biological themes among gene clusters, *OMICS*, **16**, 284–287, https://doi.org/10.1089/OMI.2011.0118 **(2012)**

39. Zhang H., Wei W., Zhao M., Ma L., Jiang X., Pei H., Cao Y. and Li H., Interaction between Aβ and Tau in the Pathogenesis of Alzheimer's Disease, *Int J Biol Sci*., **17**, 2181, https://doi.org/10.7150/IJBS.57078 **(2021)**

40. Zheng Y., Ley S.H. and Hu F.B., Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, *Nature Reviews Endocrinology*, **14**, 88–98, https://doi.org/10.1038/nrendo.2017.151 **(2017)**.

(Received 08th July 2025, accepted 14th August 2025)