doi.org/10.36721/PJPS.2025.38.4.REG.14341.1

Comprehensive omics analysis of type 2 diabetes mellitus and cardioembolic stroke provides new biological insights and therapeutic targets

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Abstract: The objective of this study is to investigate the shared genes that are differentially expressed (DEGs) between CES and T2DM, as well as uncover the hidden molecular mechanisms involved. We retrieved the gene expression profiles for CES (GSE58294) and T2DM (GSE25724) from Gene Expression Omnibus (GEO) database. We then per formed 5 analyses: identify the overlapping DEGs between CES and T2DM, correlation analysis of hub genes; transcriptional regulation analysis of hub genes; single-cell sequencing analysis and potential therapeutic drug prediction. A total of 239 overlapping genes with the same trends were identified as DEGs between two datasets. Functional analysis emphasized the crucial role of neuronal cell development in these two diseases. Through the three algorithms of plug-in cytoHubba, five common hub genes were identified as *HNRNPD*, *APP*, *ESR1*, *RHOA* and *DICER1*. Single-cell analysis further confirmed the expression of five hub genes. In addition, TF (*FOXC1*) and miRNAs (*miR-221-3p* and *miR-222-3p*) were identified as potential key regulators between the CES and T2DM. This research reveals the shared pathogenesis of CES and T2DM. In the future, these common hub genes may provide new targets for further mechanistic research as well as new therapies for patients with CES and T2DM.

Keywords: Cardioembolic stroke, type 2 diabetes mellitus, bioinformatics analysis, protein-protein interaction network, miRNAs-gene network, TF-gene network.

Submitted on 18-03-2025 – Revised on 16-05-2025– Accepted on 05-06-2025

INTRODUCTION

Stroke is the third-leading cause of death globally and the primary cause of permanent disability. The majority of strokes (87%) are of the ischemic type. Among those ischemic subtypes, the prognosis and lethality rate of cardioembolic strokes (CES) is commonly worse than others. CES typically originates from cardiac thromboses, cardiac masses, or paradoxical emboli from venous thrombosis and accounts for approximately 20% of ischemic strokes (Spence, 2018; Qin Y *et al*., 2025).

Type 2 diabetes mellitus (T2DM), the predominant form of diabetes mellitus (DM), is a major risk factor for cardiovascular disorders, including ischemic stroke and atherosclerosis (Borse *et al*., 2021). It is projected that by 2040, approximately one in ten adults globally will be affected by T2DM.. Chronic hyperglycemia in blood is the main characteristic of T2DM patients (Forbes and Cooper, 2013), followed with high viscosity, high stagnation and high coagulation, which can cause slower blood flow, thrombosis or even embolism in microvessels (Teigen *et al*., 2022). Moreover, long-term hyperglycemia will accelerate the formation of arteriosclerosis with high risk of ischemic stroke (Kronfli *et al*., 2021). These clinical conditions are the common factors of comorbidities between CES and T2DM patients (Aguilar-Ballester *et al*., 2021). Although some clinical trials had shown the correlationship between CES and T2DM, the underlying pathological mechanisms were still unclear. Hence, in the present study, we utilized a series of bioinformatic approaches to screen common hub genes and to explore transcriptional regulatory networks composed of microRNAs (miRNAs) and transcriptional factor (TF) between CES and T2DM. Our current work has discovered the overlapping genes and the related signaling pathways between CES and T2DM, which can be used as new diagnostic biomarkers and therapeutic targets for either condition or their co-occurrence.

MATERIALS AND METHODS

Gene expression profile data selection

Transcriptome and single-cell datasets for ischemic stroke and type 2 diabetes were obtained from the GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo) database. GSE58294 contains 69 ischaemic stroke samples and 23 control samples, GSE225948 contains 4 ischaemic stroke samples and 4 control samples; GSE195986 contains 7 type 2 diabetes samples and 4 control samples and GSE25724 contains 6 type 2 diabetes samples and 3 control samples (Cui and Li, 2023). As all datasets were publicly available, ethics committee approval and

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informed patient consent were not required. Additionally, a clinical trial number was not applicable.

DEGs selection

The GEO2R online analysis tool (http://www.ncbi.nlm.nih.gov/geo/geo2r/) was employed to extract and analyze differentially expressed genes (DEGs). The selection criteria for DEGs were set as *p*-value < 0.05 and |log2FC | > 0.583(Zhou *et al*., 2023).

Protein-protein interaction (PPI) network construction and identification of hub genes

The exploration of gene interaction for constructing the PPI network involved the utilization of the Search Tool for the Retrieval of Interacting Genes (STRING) (https://string-db.org/, version 11.0). Select "Multiple proteins" section to import the intersecting target gene dataset and choose "Homo sapiens" under the "Organism" option. Interactions with a confidence score greater than 0.4 were considered statistically significant according to the reference (Deng *et al*., 2023). Interaction score was the minimum required (the choice of confidence was based on the number of targets to choose, if the target was relatively few, lower confidence was chosen to retain more proteins for subsequent analysis).

To search for important hub genes, the cytoHubba plug-in in Cytoscape (https://cytoscape.org/, version 3.7.2) was used to screen the top 20 hub genes in each algorithm. Common hub genes were determined by identifying the intersection of results across the different algorithms.

Enrichment analysis of hub genes

To further uncover the function of the hub genes, we used the R language Cluster Profiler package (3.18.0) to perform enrichment analysis based on GO and KEGG for hub genes (set as species "Homo sapiens"). The enrich plot package (1.10.2) was used to visualize the results (Top 10). For GO and KEGG analysis, P-value indicated statistically significant differences.

Analysis of hub genes by transcription factors (TFs) and miRNA- regulatory network

TFs are a kind of protein that can bind to specific DNA sequences and regulate gene expression. We utilized the Network Analyst database (https://www.networkanalyst.ca/, version 3.0) to construct a regulatory network of TFs and genes. These TF targets were derived from the JASPAR TF binding site profile database.

The co-regulatory network of TFs and genes was visually analyzed using the JASPAR database. The JASPAR database was used to generate a visual analysis of the TF-gene co-regulatory network. Based on the common hub genes, TFs that co-regulated the functional pathways and gene expression between T2DM and CES, were identified from JASPAR database to form TFs-gene regulatory network.

MicroRNAs (miRNAs) are one class of short non-coding RNA that regulate gene expression by degrading target mRNA or inhibiting translation (Bartel, 2004). We attempted to draw the gene-miRNA interaction network to analyze the regulation association between 5 common hub genes and miRNA through an online tool, Network Analyst with the miRTarBase database (v8.0) (Huang *et al*., 2022). Five hub genes were submitted to Network Analyst database (v3.0) to generate TFs-miRNA regulatory network. The Cytoscape program visualized the literature-curated regulatory interaction information obtained from Reg Network.

Gene-drug screening

Gene target-based drug screening has emerged as a novel approach for studying drug molecular identification, which facilitates the expansion of drug options and streamlines the drug development process. Gene target based drug screening has become a new approach for drug molecular identification study, which helps to expend the scope of relevant drugs and reduce the process of drug development. The construction of regulatory networks such as drug-gene was facilitated by utilizing the Network Analyst tool (https://www.networkanalyst.ca/, version 3.0)(Protein-drug interactions: Specify organism: *H. sapiens* (human); Set ID type: Official Gene Symbol; The protein and drug target information was collected from the Drug Bank database(Version 5.0).

Data processing for single-cell RNA sequencing (scRNA-seq)

We obtained scRNA-seq data for ischemic stroke and type 2 diabetes from the GEO database and analyzed them using the 'Seurat' R package. Genes were screened based on the following criteria: (1) Each gene was expressed in at least 3 tumour cells. (2) The number of genes detected in each cell was between 200 and 6000. (3) The number of unique molecular identifiers (UMIs) counted per cell was more than 1000. (4) The percentage of mitochondrial genes per cell was less than 20. The Harmony algorithm was executed in the 'Harmony' R package to eliminate batch correction. The first 2500 highly variable genes were identified for dimensionality reduction clustering and cell subgroup annotation was performed manually.

RESULTS

Identification of DEGs between CES and T2DM

The GSE58294 (CES) and GSE25724 (T2DM) datasets were downloaded from the NCBI GEO database. With the threshold of a *p*-value < 0.05 and $|\log_2 FC| > 0.583$, 3140 DEGs (1499 upregulated and 1641 downregulated) were identified in the GSE58294 dataset (fig. 1A) and top 20 were listed in table 1. The most representative DEG is *HNRNPD*, a member of the nuclear heterogeneous ribonucleoproteins (HNRNPs) family. It is a central regulator in telomere biogenesis, cell signaling and

regulating gene expression at the transcription and translation levels and involved in the occurrence and development of many diseases such as cancer, cardiovascular disease and viral infection (Geuens *et al*., 2016). HNRNPD is specifically expressed during neurodevelopment and regulates genes involved in neuronal differentiation and maturation. Its expression is regulated by N-methyl-D-aspartic acid receptor (NMDAR) activation, which leads to a series of intracellular cascade effects regulating nerve cell survival, differentiation and plasticity. These 3707 DEGs (1498 upregulated and 2209 downregulated) were identified in the GSE25724 dataset (fig. 1B) and top 20 were listed in table 2. Recent studies showed that protein kinase cepsilon zeta (PRKCZ) was involved in insulin secretion of pancreatic islets in the pathogenesis of T2DM. Besides, Venn diagram analysis was employed to assess 672 overlapping differentially expressed genes (DEGs) between GSE58294 and GSE25724 (fig. 1C). Following that, we excluded genes with opposite expression trends in GSE58294 and GSE25724 and finally obtained 239 DEGs for further investigation.

PPI network construction and hub genes identification

Based on the STRING database, the interactions of the overlapping DEGs were evaluated by Cytoscape software to construct PPI network, resulting with 164 nodes and 307 edges (fig. 2A) and the interaction number of top 20 genes was shown in fig. 2B.

Through these three algorithms of plug-in cytoHubba (namely MCC, degree and radiality), we had calculated the top 20 hub genes. After taking the intersection of these genes in each algorithm, we found 5 common hub genes, including *HNRNPD*, *APP*, *ESR1*, *RHOA* and *DICER1* (fig. 3A-C). Their full names and related functions were provided in table 3.

RhoA is an important molecular switch that regulates cytoskeletal dynamics and exhibits multiple functions in different cells of the nervous system (Xu *et al*., 2021). Studies have shown that RhoA plays a key role in axon development and regeneration as well as dendritic development (Wang *et al*., 2022). Amyloid precursor protein (APP) is the first protein associated with sporadic Alzheimer's disease. It has been shown that the absence of APP leads to harm in neuronal circuits and a decline in the quantity of synaptic connections. Moreover, it significantly hinders communication between nerve cells, which has a profound impact on learning abilities, At the same time, the involvement of the APP family is pivotal in the formation of the nervous system, as well as in learning, memory formation and social communication.

Analysis of the functional characteristics of 5 hub genes

GO and KEGG Pathway enrichment analysis was performed to examine the associated biological functions and pathways involved in the 5 hub genes. GO enrichment analysis showed that the 5 hub genes were mainly enriched in glial cell differentition (P=1.95E-05), positive regulation of T cell migration (P=3.23E-05) and positive regulation of lymphocyte migration (P=4.71E-05) (fig. 4A-C). The results of top 10 functional enrichment pathways showed that these genes were mainly enriched in endocrine and other factor-regulated calcium reabsorption (P= 0.024) and prolactin signaling pathway (P=0.032) (fig. 4D). These findings suggest that inflammatory responses and endocrine factors may play critical roles in the pathogenesis of both CES and T2DM.

It has been found that the lack of nutritional support of astrocytes is associated with local inflammation driven by microglia and is considered a critical pathogenic mechanism in the progression of metabolic diseases such as obesity and diabetes (Rosenbaum *et al*., 2022). A study by RANA *et al*. showed that neuroinflammation caused by hyperglycemia is related to microglia activation. When exposed to high glucose, microglia may polarize into an activated state, in which M1 is superior to M2, leading to oxidative stress and the production of inflammatory factors, thus resulting in reduced synaptic plasticity and impaired learning and memory. These changes contribute to DM cognitive dysfunction (Elmore *et al*., 2018).

In the early stage after cerebral ischemic injury, astrocytes are activated and rapidly migrate to the site of injury. On one hand, astrocytes maintain intracellular environmental homeostasis by releasing anti-inflammatory factors, ingestion of excessive glutamate and formation of early glial scar and play a protective role in the brain (Barthels and Das, 2020). On the other hand, during the recovery period of cerebral ischemia and hypoxia, astrocytes secrete inflammatory factors to promote inflammatory response, release excitatory amino acids and cause injury to brain tissue due to glial restriction caused by excessive proliferation of glial scars (Cekanaviciute and Buckwalter, 2016).

Microglia are the natural immune cells of the central nervous system, which are the main mediators of neuroinflammation (Guruswamy and ElAli, 2017). In the activated state, microglia can secrete pro-inflammatory or anti-inflammatory cytokines and affect the course of disease (Xu *et al*., 2020).

In conclusion, the activation, polarization and mediated inflammatory response of microglia play an important role in the occurrence, development and outcome of brain injury in ischemic stroke and type 2 diabetes.

Construction of transcription factors (TFs)-gene regulatory network

Based on the JASPAR TFs binding site profile database, TFs-gene regulatory network was constructed using the Network Analyst 3.0 platform, based on five hub genes (*HNRNPD*, *APP*, *ESR1*, *RHOA* and *DICER1*).

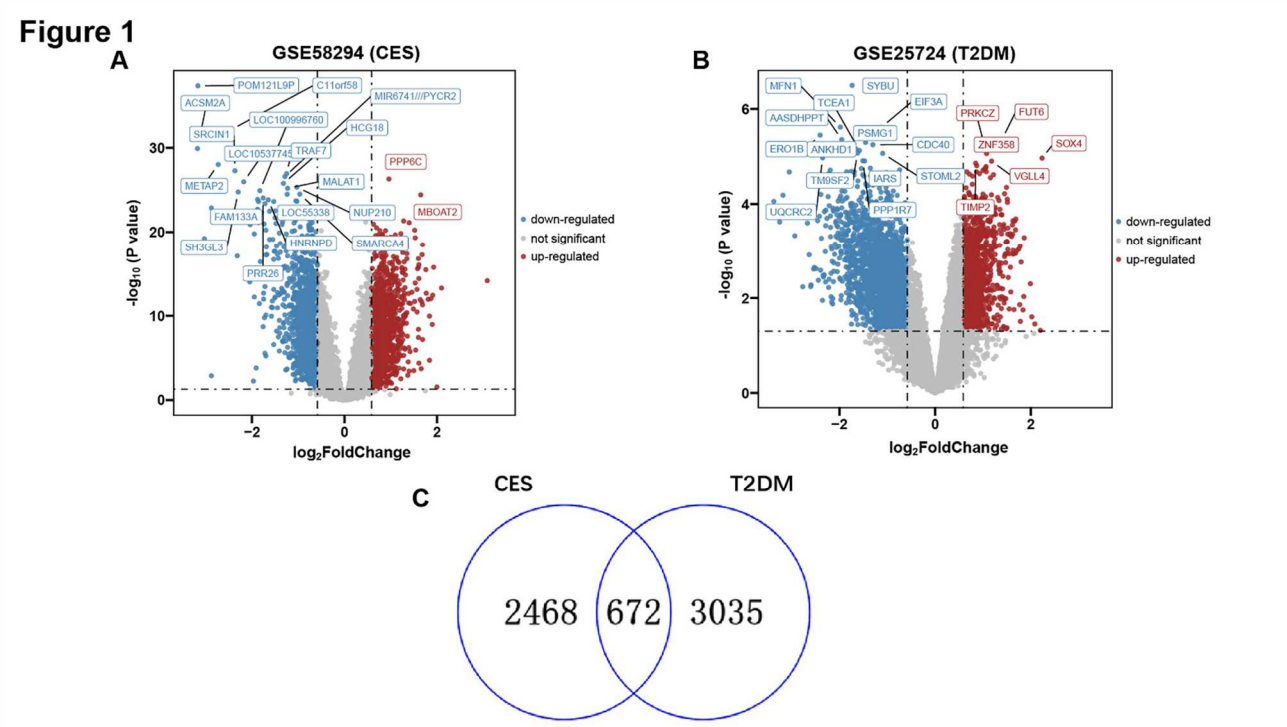


Fig. 1: The volcano diagram and venn diagram of DEGs between CES and T2DM. A: The volcano map of GSE58294. B: The volcano map of GSE25724. C: Venn diagram of DEGs in GSE58294 and GSE25724 gene chips.

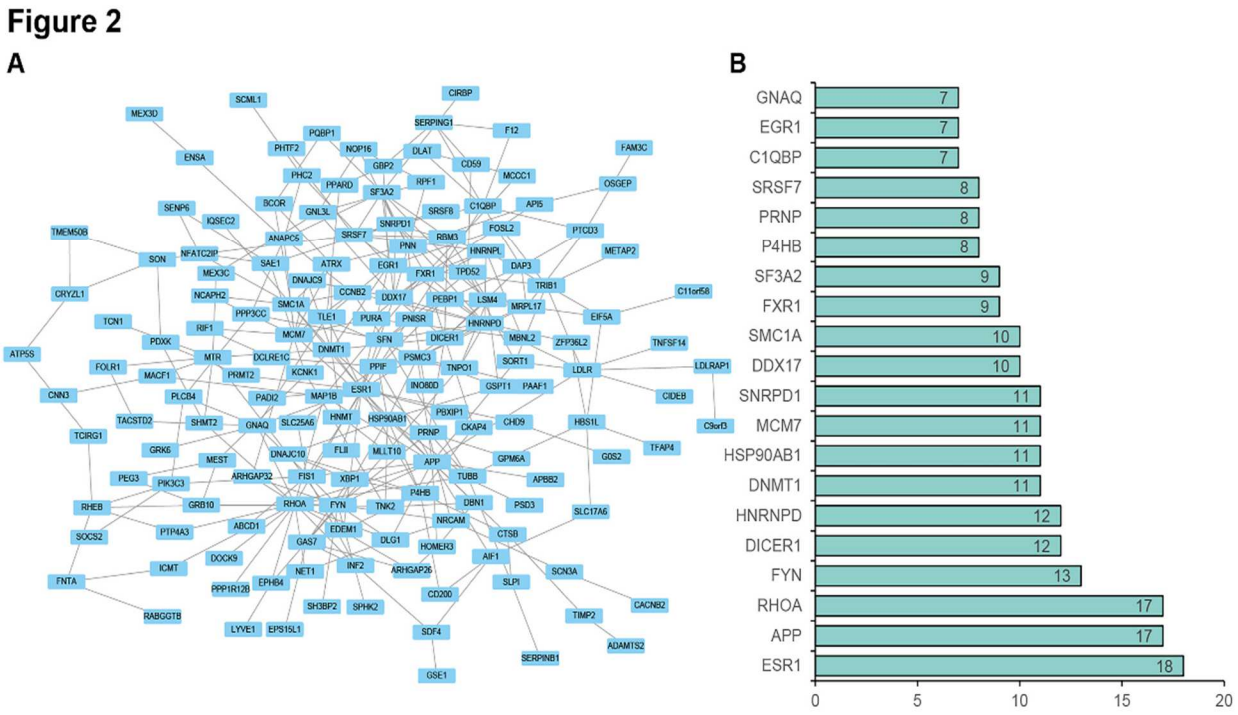


Fig. 2: PPI network. A: The PPI among the overlapping DEGs. B: The interaction number of each DEGs.

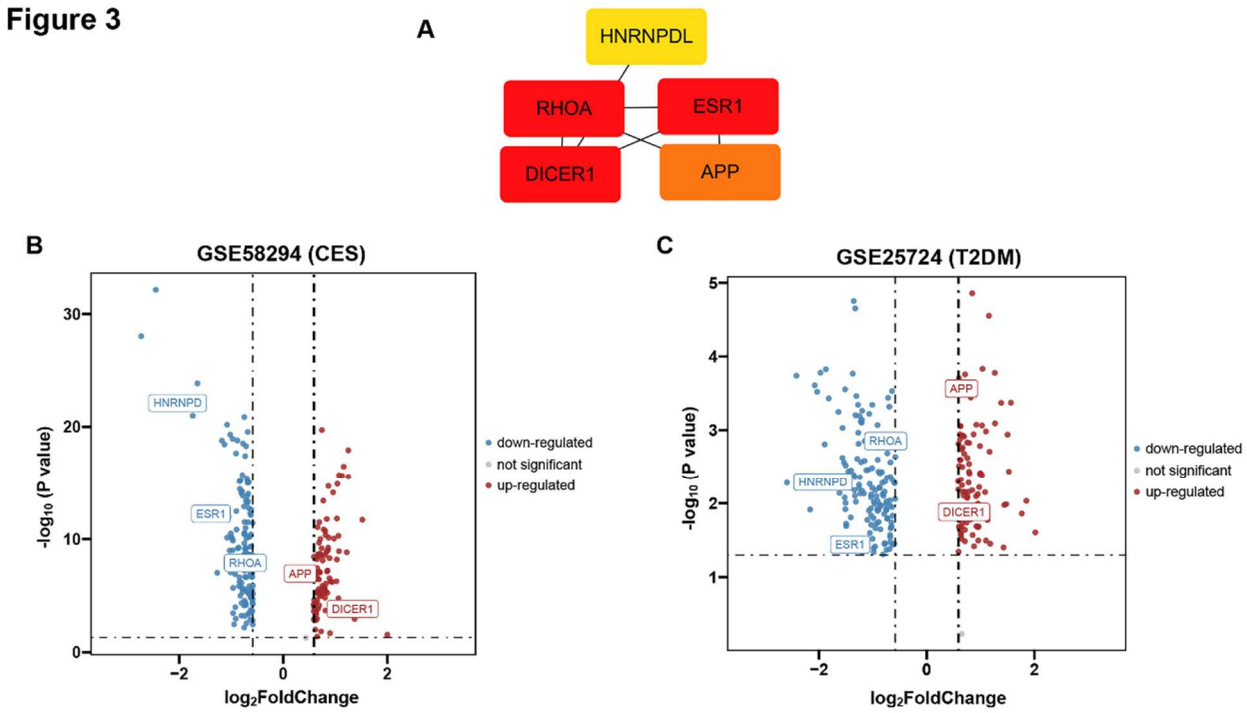


Fig. 3: A: PPI network of 5 hub genes. B-C: The volcano map of 5 hub genes. D-E: Functional enrichment analysis of five hub genes.

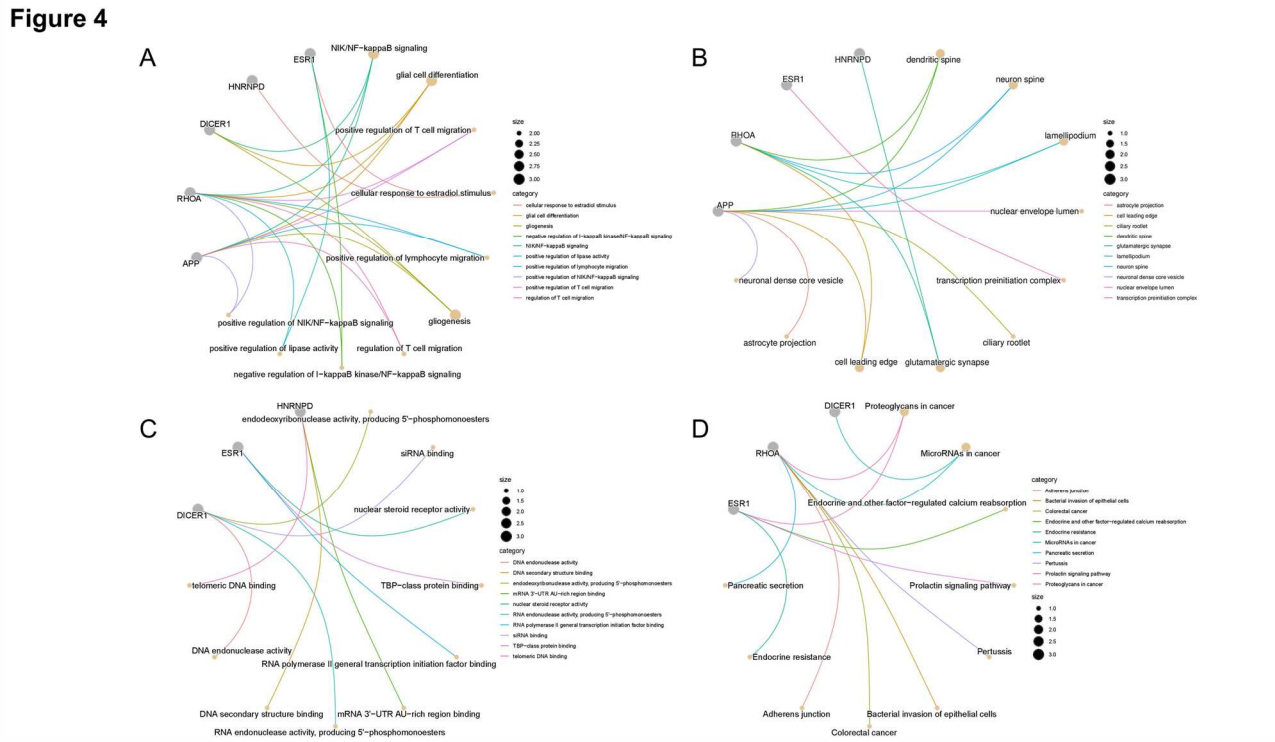


Fig. 4: Results of biological functions and pathways analysis in 5 hub genes between CES and T2DM. A-C: GO terms (biological process、cellular component and molecular function) enrichment of 5 hub genes based on Cluster Profiler analysis. D: Functional enrichment of the top 10 significant pathways.

Figure 5

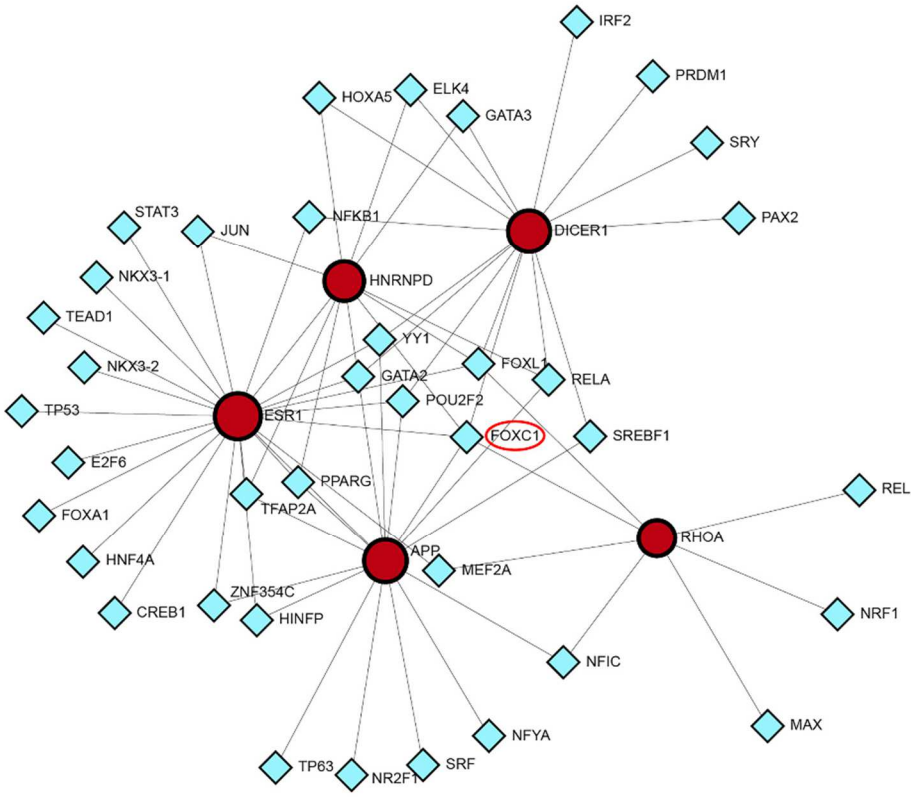


Fig. 5: Integration of gene-TFs interaction networks. Note: The highlighted red color node represented the five hub genes and other nodes represented TFs. The network consists of 43 loci and 70 edges.

Figure 6

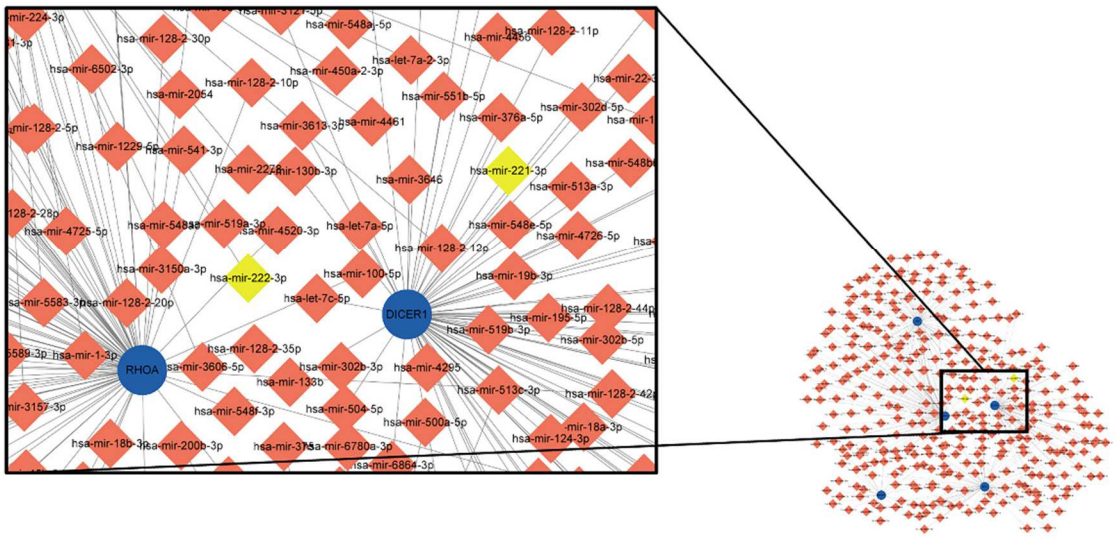


Fig. 6: The network presents the gene-miRNA coregulatory network. The network consists of 307 nodes, 348 edges, and 5 seeds The nodes in yellow color are the five hub genes, red nodes represent miRNA and blue nodes indicate Top2 miRNA (miR-221-3p and miR-222-3p).

Figure 7

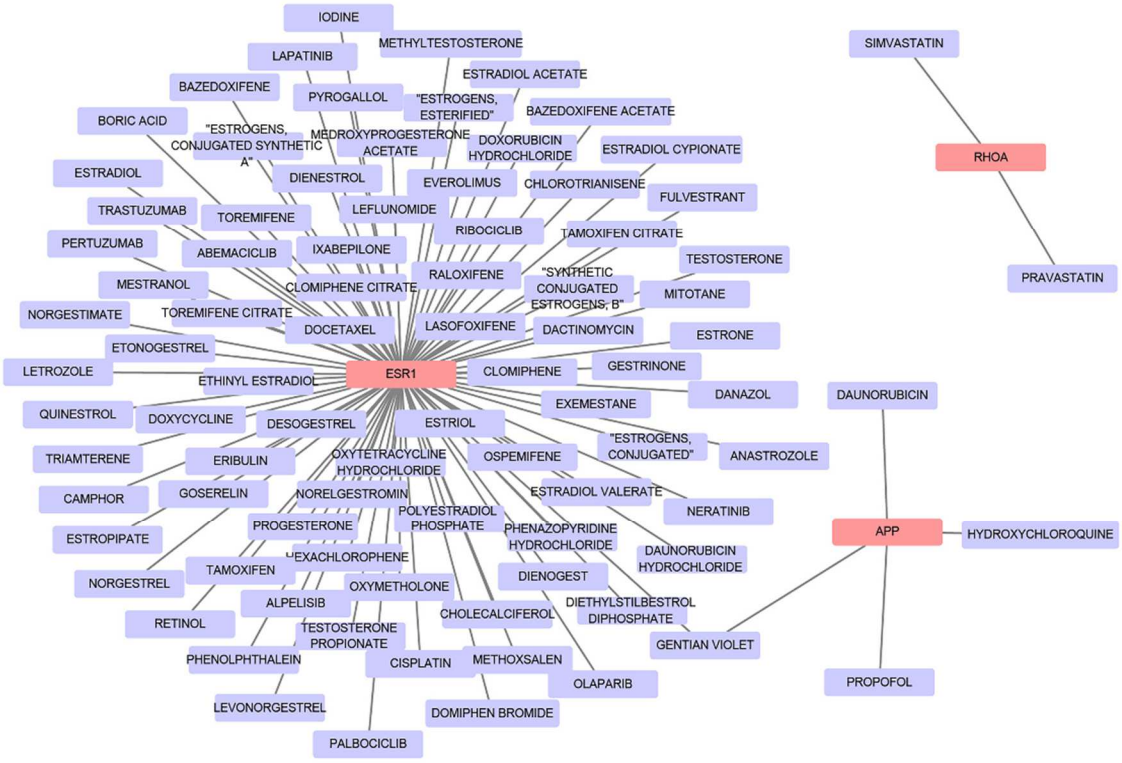
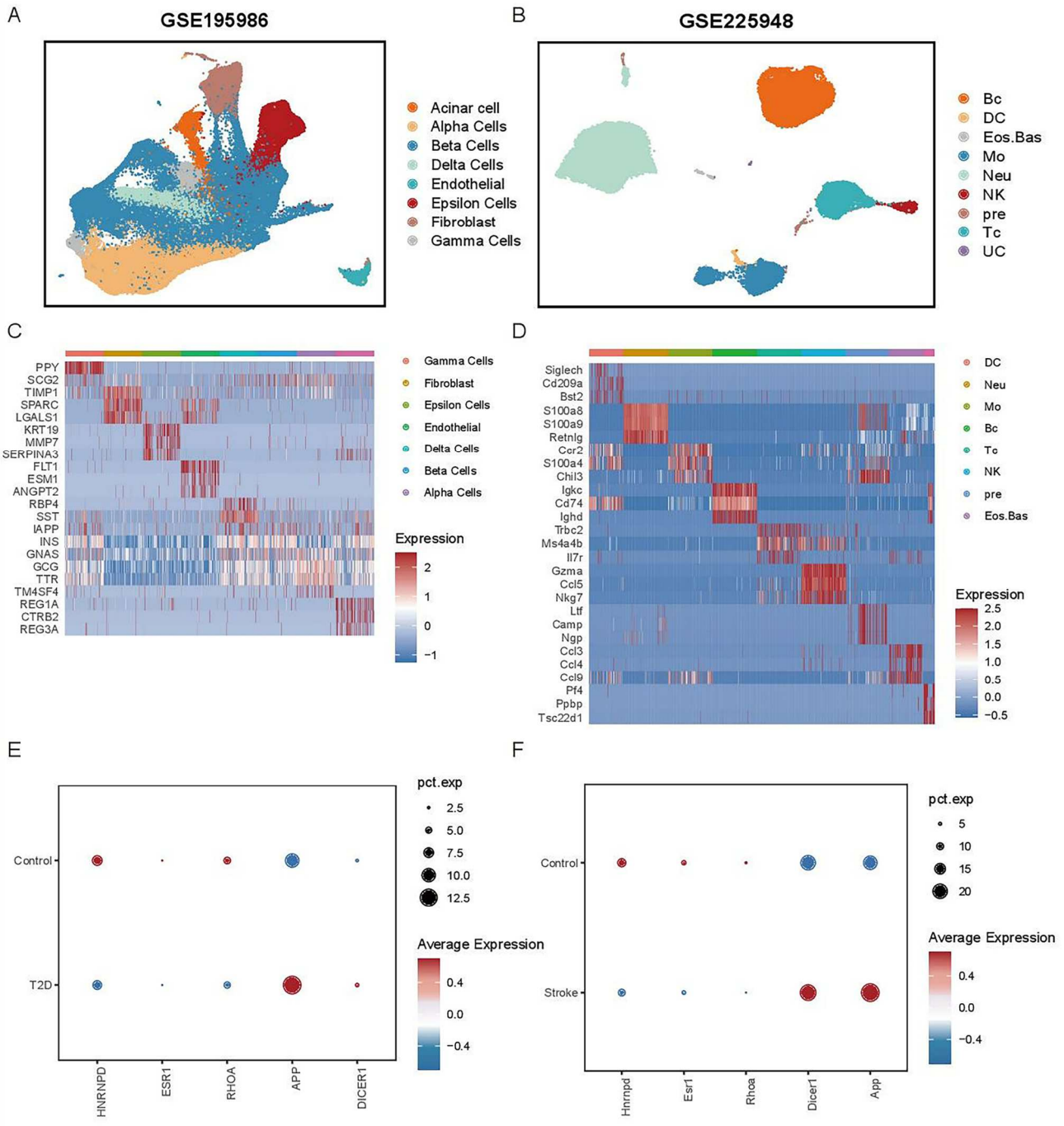


Fig. 7: Integration of interaction networks drugs and five hub genes.

Table 1: Top20 Gene in CES.

Gene	p-value	log2FoldChange
POM121L9P	4.74E-38	-3.17
C11orf58	7.18E-33	-2.45
ACSM2A	1.24E-30	-3.18
METAP2	9.32E-29	-2.73
SRCIN1	5.16E-28	-2.37
TRAF7	1.12E-27	-1.25
MIR6741///PYCR2	2.52E-27	-1.29
PPP6C	4.90E-27	0.962
LOC105377458	1.06E-26	-2.18
HCG18	1.61E-26	-1.32
NUP210	4.54E-26	-1.03
MALAT1	6.08E-26	-1.23
LOC100996760	1.17E-25	-1.83
SH3GL3	1.67E-25	-2.3
SMARCA4	3.03E-25	-0.966
LOC55338	3.12E-25	-1.24
MBOAT2	3.68E-25	1.65
PRR26	8.69E-25	-1.76
FAM133A	1.26E-24	-1.88
HNRNPD	1.41E-24	-1.65



A: UMAP plot showing cellular subpopulations of GSE195986;

B: UMAP plot showing cellular subpopulations of GSE225948;

C: Heatmap showing marker genes for each cellular subpopulation of GSE195986;

D: Heatmap showing marker genes for each cellular subpopulation of GSE225948;

E: Dotplot of single-cell differential analysis showing genes in the normal and type 2 diabetes groups for relative expression;

F: Dot plot of single-cell differential analysis showing relative expression of genes in normal and stroke groups.

Fig. 8: Single-cell transcriptome analysis.

Table 2: Top20 Gene in T2DM.

Gene	p-value	log2FoldChange
SYBU	3.23E-07	-1.735
MFN1	2.43E-06	-1.98079
EIF3A	2.46E-06	-1.12249
ERO1B	3.58E-06	-2.40367
FUT6	4.4E-06	1.397189
AASDHPPT	4.45E-06	-1.95131
PSMG1	5.09E-06	-1.45342
CDC40	5.69E-06	-1.30208
ZNF358	6.06E-06	1.21299
ANKHD1	6.96E-06	-1.65566
TCEA1	7.53E-06	-1.58314
TM9SF2	8.54E-06	-1.62078
STOML2	8.64E-06	-1.09713
PRKCZ	8.73E-06	1.073444
UQCRC2	1.07E-05	-2.35085
SOX4	1.09E-05	2.233183
PPP1R7	1.24E-05	-1.51083
IARS	1.25E-05	-1.47361
VGLL4	1.26E-05	1.179108
TIMP2	1.39E-05	0.845295

Table 3: The profile of five hub genes in detail.

Gene symbol	Description	Degree	Function
HNRNPD	Heterogeneous Nuclear Ribonucleoprotein D	3	Belongs to the subfamily of heterogeneous nuclear ribonucleoproteins, which are associated with pre-mRNAs and appear to influence pre-mRNA processing and other aspects of mRNA metabolism and transport.
APP	Amyloid Beta Precursor Protein	3	Encodes a cell surface receptor and transmembrane precursor protein that is cleaved by secretases to form a number of peptides.
ESR1	Estrogen Receptor 1	3	Encodes an estrogen receptor and ligand-activated transcription factor.
RHOA	Ras Homolog Family Member A	2	Encodes a member of the Rho family of small GTPases, which cycle between inactive GDP-bound and active GTP-bound states and function as molecular switches in signal transduction cascades.
DICER1	Dicer 1, Ribonuclease III	1	Encodes a protein possessing an RNA helicase motif containing a DEXH box in its amino terminus and an RNA motif in the carboxy terminus.

Table 4: Top 5 TFs (ranked by Degree)

Id	Label	Degree	Betweenness
2296	FOXC1	5	84.58
2300	FOXL1	4	58.53
2624	GATA2	4	22.47
7528	YY1	3	18.29
5452	POU2F2	3	18.29

Table 5: Top 5 miRNA (ranked by Degree)

Id	Label	Degree	Betweenness
MIMAT0000279	hsa-mir-222-3p	4	4175.71
MIMAT0000278	hsa-mir-221-3p	4	3782.2
MIMAT0000099	hsa-mir-101-3p	3	4435.01
MIMAT0017991	hsa-mir-3613-3p	3	2137.37
MIMAT0000425	hsa-mir-130a-3p	3	1281.81

The network included 43 loci with 70 edges. These loci were combined by 5 seed genes and 38 transcription factors. The TF-gene regulatory network was constructed in fig. 5. The top5 interacting TFs were FOXC1, GATA2, FOXL1, YY1 and POU2F2, ranked by degree and betweenness as shown in table 4.

The family known as the Forkhead box (Fox) comprises numerous transcription factor and is increasingly acknowledged as playing a crucial role in maintaining immune balance (Peng, 2010). FOXC1 possesses an inhibitory domain for transcription, which is closely associated with various pathological processes such as oxidative stress, apoptosis and inflammation (Ito *et al*., 2014). Studies reported that recombinant Sirtuin6 (SIRT6) activator MDL-811 activated SIRT6 to make zeste homolog 2 (EZH2) deacetylated and promoted FOXC1 expression (He *et al*., 2021). Finally, these changes resulted in the reduction of brain tissue damage after ischemia/reperfusion brain injury or LPS-induced neuroinflammation and improved prognosis.

Analysis of gene-miRNA regulatory network

The gene-miRNA regulatory network was also constructed using the NetworkAnalyst 3.0 platform, based on five hub genes (*HNRNPD*, *APP*, *ESR1*, *RHOA* and *DICER1*). The network included 307 nodes, 348 edges and 5 seeds (fig. 6). We listed top 5 relevant miRNA according to the rank of degree, including hsa-*miR-222-3p*, *hsa-miR-221-3p*, hsa-*miR-101-3p*, *hsa-miR-3613-3p* and *hsa-miR-130a-3p* as shown in table 5. Subsequently, *miR-221-3p* and *miR-222-3p* were selected to interact with most of DEGs. miR-221-3p was interacted with *DICER1*, *ESR1*, *HNRNPD* and *RHOA*, miR-222-3p was interacted with *APP*, *ESR1*, *HNRNPD* and *DICER1.*

The screening of target drugs

To facilitate further research on therapeutic strategy, we performed a drug-targeting enrichment analysis based on these hub genes, including 4 drugs for *APP*, 83 for *ESR1* and 2 for *RHOA* (fig. 7). No drugs targeting DICER1 and HNRNPD genes were found in the database. We speculated that ESR1 might be the most promising drug target and gentian violet was considered to be able to target both *ESR1* and *APP* genes. It was reported that gentian violet could eradicate methicillin-resistant *Staphylococcus aureus* in skin wound infections (Grønseth *et al.*, 2023). In addition, Gentian violet could promote wound healing of foreign body granuloma and other skin diseases such as pyoderma gangrenosa, epidermolysis bullosa and calcification(Pona *et al*., 2020).

Single-cell transcriptome analysis of key genes

To further validate our findings, we analyzed single cell data from public databases GSE195986 (type 2 diabetes), GSE225948 (stroke). Firstly, normal and type 2 diabetic tissues were down-clustered into 8 cell subpopulations including Acinar Cells, Alpha Cells, Bata Cells, Delta Cells, Endothelial Cells, Epsilon Cells, Fibroblasts and Gamma Cells (fig. 8A). Stroke and non-stroke samples were also subjected to dimensionality reduction clustering into B cells (BC), Dendritic cells (DC), Eosinophils-Basophils (EosBas), Monocytes (Mo), NK cells (NK), hematopoietic precursors (pre), T cells (Tc), unclassified (UC) and other 9 cell subpopulations (fig. 8B). The heatmap showed the expression of marker genes in each subpopulation after the reduced clustering (fig. 8C&D), which proved that the results of reduced clustering were reliable.

Single-cell transcriptome analysis showed that HNRNPD, ESR1 and RHOA genes were highly expressed in nondiabetic samples, APP and DICER1 genes were highly expressed in type 2 diabetic samples (fig. 8E, fig. S1) and HNRNPD, ESR1 and RHOA genes were highly expressed in nondiabetic samples, APP and DICER1 genes were highly expressed in stroke samples (fig. 8F, fig. S1).

DISCUSSION

Accumulating clinical evidence elucidated the significant correlation between CES and T2DM, as the early stage of T2DM seemed to be closely related to CES (Georgakis *et al.*, 2021; Sarfo *et al.*, 2022; Zhu *et al.*, 2021). Due to insulin resistance and hypofunction of islet β cells in diabetes patients, abnormal metabolism of sugar, fat and protein in the body also accelerated arteriosclerosis (Wang *et al.*, 2013). In addition, hyperglycemia-induced hypertension was conducive to the formation of thrombus and promoted the occurrence of ischemic stroke. It was reported that the accident risk of acute cerebrovascular disease was 2-4 times higher in diabetic patients than in non-diabetic patients, especially ischemic stroke, accounting for 10%-15% of all deaths from T2DM patients (Mosenzon *et al.*, 2023; Oza *et al.*, 2017).

Although previous studies explored the central genes associated with CES and T2DM separately, few studies investigated their common pathological mechanisms based on bioinformatics analysis. Due to the high comorbidities between CES and T2DM, we first identified the hub gene between two disorders to further elucidate the common pathogenesis. In this study, two independent gene chip databases (CES and T2DM) were selected from the GEO database and we obtained 239 common DEGs with the same trend between CES and T2DM for a further series of bioinformatics analysis.

According to the cytoHubba plug-in of Cytoscape, five hub genes (*HNRNPD*, *APP, ESR1, RHOA, DICER1*) were screened. Single-cell analysis further confirmed that HNRNPD, ESR1 and RHOA genes were highly expressed in nondiabetic, non-stroke samples, whereas APP and DICER1 genes were highly expressed in type 2 diabetes and stroke samples. At the same time, we performed enrichment analysis of 5 hub genes, These results suggested that inflammatory response and endocrine

factors were involved in the occurrence and development of these two disorders.

The protein level of plasma Aβ expressed by APP was studied to be correlated to the presence and progression of small vessel disease (SVD) markers, suggesting that Aβ pathology might contribute to SVD development and progression(van Leijsen *et al.*, 2018) Aβ aggregation has been implicated in neurodegeneration, inflammation, brain atrophy and cognitive impairment(Hardy and Selkoe, 2002). According to epidemiological studies, it was deduced that risk factors like hypertension, diabetes, atherosclerosis and stroke had a notable impact on cognitive impairment (van Leijsen *et al.*, 2018). ESR1 has also been associated with the progression of atherosclerosis and/or accelerating the transition from subclinical atherosclerosis to plaque rupture and acute thrombotic CVD events (stroke) (Shearman *et al.*, 2003). It was reported that RhoA was also involved in the regulation of vascular tone, inflammation and oxidative stress. Activation of RhoA/Rho kinase plays a crucial role in the development of numerous cardiovascular disorders, primarily atherosclerosis, stroke and other non-cardiovascular conditions like DM. In the clinical report on genes polymorphisms with ischemic stroke susceptibility and post-stroke mortality, DICER1 seemed to be positively correlated to the development of ischemic stroke (Kim *et al.*, 2018). In addition, specific deletion of DICER1 in β-cell could result into the impaired insulin secretion and diabetes (Kalis *et al.*, 2011).

Current studies revealed that miRNAs were considered as innovative biomarkers for various cardiovascular diseases, including congestive heart failure, coronary artery disease, diabetes mellitus and stroke (Cakmak *et al.*, 2015). In this study, we also constructed a miRNA-target gene network and two miRNAs (*miR-221-3p* and *miR-222-3p*) were selected to interact with all DEGs. *MiR-221-3p* was highly expressed in acute myocardial infarction and used as a biomedical marker for early prediction (Coskunpinar *et al.*, 2016). In addition, *miR-221-3p*/*miR-222-3p* also participated in the biological pathways of immune regulation, endothelial integrity and neurogenesis (Yasmeen *et al.*, 2019). Furthermore, there was an observed correlation between FOXC1 and the occurrence of stroke. FOXC1 could also regulate the developing vasculature through platelet-derived growth factor. It was speculated that FOXC1 might decrease trophoblast cell damage caused by high glucose in gestational diabetes mellitus and that FOXC1 could be a promising target for therapeutic intervention in patients with gestational diabetes (Cao and Zhang, 2022).

There were some limitations in this study. Firstly, the common DEGs and related biomedical mechanism were only based on the clinical evidence with small sample sizes, thus more clinical data are expected in further trials; We attempted to perform the modified *p*-value analysis to reduce the false positive samples from 239 to 143 DEGs, which did not change the final results of bioinformatics analysis; Secondly, we only applied three algorithms to measure enrichment of common hub genes, which was the limitation of online analysis. Lastly, the clinical data of diabetes patients were not stratified based on disease duration or clinical indicators, the association of T2DM with neurophysiological process and neural apoptosis could not be studied in more degrees.

CONCLUSION

In summary, we identified the common DEGs between CES and T2DM and performed a series of bioinformatics analyses including functional enrichment and PPI network analysis. We discovered that CES and T2DM shared numerous underlying mechanisms that might be influenced by distinct hub genes (*HNRNPD*, *APP*, *ESR1*, *RHOA* and *DICER1*). Based on the miRNA-gene and TF-gene interaction network, TF (FOXC1) and miRNAs (miR-221-3p, miR-222-3p) were identified as potential key regulators of CES and T2DM. These common signaling pathways might provide new diagnostic biomarkers and new targets for the management of single disease or T2DM complicated with CES. However, our research also has some limitations. First of all, this is a retrospective study that requires external verification to verify our findings; Secondly, the function of the hub gene needs to be further verified in an in vitro model, which will be the focus of our future work.

Author contributions

Hao Liu: Conceptualization (equal), formal analysis (equal), writing–original draft and editing (equal), funding acquisition (lead). Yue Hao: Conceptualization (equal), formal analysis (equal), resources (equal), methodology (lead), Resources (equal), writing-review and editing (equal). Jun-Tao Zhang methodology (equal), resources (equal). Li-Fen Guo: methodology (equal), resources (equal). Heng-Qian He methodology (equal), resources (equal). Li-Qin Ying methodology (equal), resources (equal). Si-Yu Xian: methodology (equal), resources (equal). Qin-Kang Lu: Conceptualization (equal), resources (equal), supervision (equal), writing–review and editing (equal).

Funding

This study was supported in part by grants from the National Natural Science Foundation of China (82101392), Ningbo Clinical Research Center for Ophthalmology (2022L003), Ningbo Key Laboratory for neuroretinopathy medical research, Ningbo Clinical Research Center for Ophthalmology , the Project of Ningbo Leading Medical &Health Disipline (2016-S05) and Technology Innovation 2025 Major Project of Ningbo (2021Z054) and Ningbo "Innovation Yongjiang 2035" Key Technology Breakthrough program (2024Z233)

Conflict of interests

All authors declare that they have no conflict of interests.

Ethical approval and consent to participate

Since datasets were free from the public database, ethics committee approval and patient consent were not required to be conducted in this study and clinical trial number is not applicable.

REFERENCES

Aguilar-Ballester M, Hurtado-Genovés G, Taberner-Cortés A, Herrero-Cervera A, Martínez-Hervás S and González-Navarro H (2021). Therapies for the treatment of cardiovascular disease associated with type 2 diabetes and dyslipidemia. *Int. J. Mol. Sci*., **22**(2).

Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism and function. Cell, **116**(2): 281-297.

Barthels D and Das H (2020). Current advances in ischemic stroke research and therapies. *Biochim. Biophys. Acta. Mol. Basis Dis*., **1866**(4): 165260.

Borse SP, Chhipa AS, Sharma V, Singh DP and Nivsarkar M (2021). Management of type 2 diabetes: Current strategies, unfocussed aspects, challenges and alternatives. *Med. Princ. Pract*., **30**(2): 109-121.

Cakmak HA, Coskunpinar E, Ikitimur B, Barman HA, Karadag B, Tiryakioglu NO, Kahraman K and Vural VA (2015). The prognostic value of circulating microRNAs in heart failure: Preliminary results from a genome-wide expression study. *J. Cardiovasc. Med. (Hagerstown)*, **16**(6): 431-437.

Cao S and Zhang S (2022). Forkhead-box C1 attenuates high glucose-induced trophoblast cell injury during gestational diabetes mellitus via activating adenosine monophosphate-activated protein kinase through regulating fibroblast growth factor 19. *Bioengineered*, **13**(1): 1174-1184.

Cekanaviciute E and Buckwalter MS (2016). Astrocytes: Integrative regulators of neuroinflammation in stroke and other neurological diseases. *Neurotherapeutics*, **13**(4): 685-701.

Coskunpinar E, Cakmak HA, Kalkan AK, Tiryakioglu NO, Erturk M and Ongen Z (2016). Circulating miR-221-3p as a novel marker for early prediction of acute myocardial infarction. *Gene*, **591**(1): 90-96.

Cui K and Li Z (2023). Identification and analysis of type 2 diabetes-mellitus-associated autophagy-related genes. *Front. Endocrinol. (Lausanne)*, **14**: 1164112.

Deng YX, Liu K, Qiu QX, Tang ZY, Que RM, Li DK, Gu XR, Zhou GL, Wu YF, Zhou LY, Yin WJ and Zuo XC (2023). Identification and validation of hub genes in drug induced acute kidney injury basing on integrated transcriptomic analysis. *Front. Immunol*., **14**: 1126348.

Elmore MRP, Hohsfield LA, Kramár EA, Soreq L, Lee RJ, Pham ST, Najafi AR, Spangenberg EE, Wood MA, West BL and Green KN (2018). Replacement of microglia in the aged brain reverses cognitive, synaptic and neuronal deficits in mice. *Aging Cell*, **17**(6): e12832.

Forbes JM and Cooper ME (2013). Mechanisms of diabetic complications. *Physiol. Rev.*, **93**(1): 137-188.

Geuens T, Bouhy D and Timmerman V (2016). The hnRNP family: Insights into their role in health and disease. *Hum Genet*, **135**(8): 851-867.

Grønseth T, Ovchinnikov KV, Carlsen H, Benth J, Diep DB, von Unge M and Silvola JT (2023). Lugol's solution and Gentian violet eradicate methicillin-resistant Staphylococcus aureus biofilm in skin wound infections. *Int. Wound J*., **20**(1): 120-130.

Guruswamy R and ElAli A (2017). Complex roles of microglial cells in ischemic stroke pathobiology: New insights and future directions. *Int. J. Mol. Sci*., **18**(3): 496.

Hardy J and Selkoe DJ (2002). The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science*, **297**(5580): 353-356.

He T, Shang J, Gao C, Guan X, Chen Y, Zhu L, Zhang L, Zhang C, Zhang J and Pang T (2021). A novel SIRT6 activator ameliorates neuroinflammation and ischemic brain injury via EZH2/FOXC1 axis. *Acta. Pharm. Sin. B*., **11**(3): 708-726.

Huang HY, Lin YC, Cui S, Huang Y, Tang Y, Xu J, Bao J, Li Y, Wen J, Zuo H, Wang W, Li J, Ni J, Ruan Y, Li L, Chen Y, Xie Y, Zhu Z, Cai X and Huang HD (2022). miRTarBase update 2022: An informative resource for experimentally validated miRNA-target interactions. *Nucleic. Acids Res*., **50**(D1): D222-d230.

Ito YA, Goping IS, Berry F and Walter MA (2014). Dysfunction of the stress-responsive FOXC1 transcription factor contributes to the earlier-onset glaucoma observed in Axenfeld-Rieger syndrome patients. *Cell Death Dis*., **5**(2): e1069.

Kalis M, Bolmeson C, Esguerra JL, Gupta S, Edlund A, Tormo-Badia N, Speidel D, Holmberg D, Mayans S, Khoo NK, Wendt A, Eliasson L and Cilio CM (2011). Beta-cell specific deletion of Dicer1 leads to defective insulin secretion and diabetes mellitus. *PLoS One*, **6**(12): e29166.

Kim JO, Bae J, Kim J, Oh SH, An HJ, Han IB, Oh D, Kim OJ and Kim NK (2018). Association of microRNA Biogenesis Genes Polymorphisms with Ischemic Stroke Susceptibility and Post-Stroke Mortality. *J. Stroke*, **20**(1): 110-121.

Kronfli A, Boukerche F, Medina D, Geertsen A, Patel A, Ramedani S, Lehman E and Aziz F (2021). Immediate postoperative hyperglycemia after peripheral arterial bypass is associated with short-term and long-term poor outcomes. *J. Vasc. Surg*., **73**(4): 1350-1360.

Mosenzon O, Cheng AY, Rabinstein AA and Sacco S (2023). Diabetes and stroke: What are the connections? *J. Stroke*, **25**(1): 26-38.

Oza R, Rundell K and Garcellano M (2017). Recurrent ischemic stroke: Strategies for prevention. *Am. Fam. Physician*, **96**(7): 436-440.

Peng SL (2010). Forkhead transcription factors in chronic

inflammation. *Int. J. Biochem. Cell Biol*., **42**(4): 482-485.

Pona A, Quan EY, Cline A and Feldman SR (2020). Review of the use of gentian violet in dermatology practice. *Dermatol. Online J*., **26**(5): 13030/qt79g6z0cf.

Qin Y, Zhou Y, Xiong J, Lu C, Zhou J, Su X and Han J (2025). *Limosilacto bacillus reuteri* RE225 alleviates gout by modulating the TLR4/MyD88/NF-κB inflammatory pathway and the Nrf2/HO-1 oxidative stress pathway, and by regulating gut microbiota. J Sci *Food Agric*., **105**(2): 1185-1193.

Rosenbaum JL, Melhorn SJ, Schoen S, Webb MF, De Leon MRB, Humphreys M, Utzschneider KM and Schur EA (2022). Evidence that hypothalamic gliosis is related to impaired glucose homeostasis in adults with obesity. *Diabetes Care*, **45**(2): 416-424.

Sarfo FS, Ovbiagele B, Akinyemi J, Akpa O, Akpalu A, Wahab K, Ogbole G, Obiako R, Komolafe M, Owolabi L, Osaigbovo G, Jenkins C, Fakunle A, Adeoye A, Lackland D, Arnett D, Tiwari HK, Olunuga T, Uvere E and Owolabi M (2022). Differential associations between pre-diabetes, diabetes and stroke occurrence among West Africans. *J. Stroke Cerebrovasc Dis*., **31**(11): 106805.

Shearman AM, Cupples LA, Demissie S, Peter I, Schmid CH, Karas RH, Mendelsohn ME, Housman DE and Levy D (2003). Association between estrogen receptor alpha gene variation and cardiovascular disease. *JAMA*, **290**(17): 2263-2270.

Spence JD (2018). Cardioembolic stroke: Everything has changed. *Stroke Vasc Neurol.,* **3**(2): 76-83.

Teigen IA, Riaz M, Åm MK, Christiansen SC and Carlsen SM (2022). Vasodilatory effects of glucagon: A possible new approach to enhanced subcutaneous insulin absorption in artificial pancreas devices. *Front Bioeng. Biotechnol*., **10**: 986858.

Van Leijsen EMC, Kuiperij HB, Kersten I, Bergkamp MI, van Uden IWM, Vanderstichele H, Stoops E, Claassen J, van Dijk EJ, de Leeuw FE and Verbeek MM (2018). Plasma Aβ (amyloid-β) levels and severity and progression of small vessel disease. *Stroke*, **49**(4): 884-890.

Wang F, Wan A and Rodrigues B (2013). The function of heparanase in diabetes and its complications. *Can. J. Diabetes*, **37**(5): 332-338.

Wang Q, Song LJ, Ding ZB, Chai Z, Yu JZ, Xiao BG and Ma CG (2022). Advantages of Rho-associated kinases and their inhibitor fasudil for the treatment of neurodegenerative diseases. *Neural. Regen. Res*., **17**(12): 2623-2631.

Xu J, Wen J, Fu L, Liao L, Zou Y, Zhang J, Deng J, Zhang H, Liu J, Wang X, Zuo D and Guo J (2021). Macrophage-specific RhoA knockout delays Wallerian degeneration after peripheral nerve injury in mice. *J. Neuroinflammation*, **18**(1): 234.

Xu S, Lu J, Shao A, Zhang JH and Zhang J (2020). Glial cells: Role of the immune response in ischemic stroke. *Front Immunol*., **11**: 294.

Yasmeen S, Kaur S, Mirza AH, Brodin B, Pociot F and Kruuse C (2019). miRNA-27a-3p and miRNA-222-3p as novel modulators of phosphodiesterase 3a (PDE3A) in cerebral microvascular endothelial cells. *Mol. Neurobiol*., **56**(8): 5304-5314.

Zhou H, Mu L, Yang Z and Shi Y (2023). Identification of a novel immune landscape signature as effective diagnostic markers related to immune cell infiltration in diabetic nephropathy. *Front Immunol*., **14**: 1113212.

Zhu T, Cui J and Goodarzi MO (2021). Polycystic ovary syndrome and risk of type 2 diabetes, coronary heart disease and stroke. *Diabetes*, **70**(2): 627-637.

Zou L, Yan S, Guan X, Pan Y and Qu X (2013). Hypermethylation of the PRKCZ gene in type 2 diabetes mellitus. *J. Diabetes Res*., **2013**: 721493.