Identification and characterization of biomarkers associated with endoplasmic reticulum protein processing in cerebral ischemia-reperfusion injury

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ABSTRACT

Background. Cerebral ischemia (CI), ranking as the second leading global cause of death, is frequently treated by reestablishing blood flow and oxygenation. Paradoxically, this reperfusion can intensify tissue damage, leading to CI-reperfusion injury. This research sought to uncover biomarkers pertaining to protein processing in the endoplasmic reticulum (PER) during CI-reperfusion injury.

Methods. We utilized the Gene Expression Omnibus (GEO) dataset GSE163614 to discern differentially expressed genes (DEGs) and single out PER-related DEGs. The functions and pathways of these PER-related DEGs were identified *via* Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Core genes were pinpointed through protein-protein interaction (PPI) networks. Subsequent to this, genes with diagnostic relevance were distinguished using external validation datasets. A single-sample gene-set enrichment analysis (ssGSEA) was undertaken to pinpoint genes with strong associations to hypoxia and apoptosis, suggesting their potential roles as primary inducers of apoptosis in hypoxic conditions during ischemia-reperfusion injuries.

Results. Our study demonstrated that PER-related genes, specifically ADCY5, CAMK2A, PLCB1, NTRK2, and DLG4, were markedly down-regulated in models, exhibiting a robust association with hypoxia and apoptosis.

Conclusion. The data indicates that ADCY5, CAMK2A, PLCB1, NTRK2, and DLG4 could be pivotal genes responsible for triggering apoptosis in hypoxic environments during CI-reperfusion injury.

Subjects Biochemistry, Bioinformatics, Molecular Biology, Neurology
Keywords Cerebral ischemia-reperfusion injury, Endoplasmic reticulum protein processing, Gene expression profiling, Protein-protein interactions, Hypoxia-inducible factor 1 alpha

INTRODUCTION

Factors such as external force, thrombosis, and thromboembolic arterial occlusion can lead to cerebral ischemia (CI) (*Bramlett & Dietrich, 2004*), also known as stroke, the second primary cause of death worldwide (*Katan & Luft, 2018*). Prompt restoration of blood circulation is vital for CI treatment. Characterized by the interruption of blood and oxygen

supply, ischemia can induce local tissue hypoxia. When blood flow is restored, reperfusion may exacerbate tissue damage and trigger a severe inflammatory reaction, a phenomenon termed "reperfusion injury" (*Eltzschig & Eckle, 2011*). Ischemia-reperfusion injury can provoke various cellular death events, including apoptosis, necrosis, autophagy-associated cell death, and mitochondrial dysfunction (*Eltzschig & Eckle, 2011*; *Khoshnam, Winlow & Farzaneh, 2017*; *Neag et al., 2022*). Restoration of blood oxygen supply after prolonged ischaemia may further exacerbate brain damage and neurological deficits associated with local inflammation and excess reactive oxygen (ROS) species-induced neuronal cell death (*Jurcau & Simion, 2021*; *Zhang et al., 2022*).

Protein processing in the endoplasmic reticulum (PER) refers to the process where newly synthesized polypeptide chains in eukaryotes initially enter the endoplasmic reticulum (ER), undergo post-translational modifications like glycosylation and disulfide bond formation in the ER cavity, fold and assemble into multi-subunit complexes, and are subsequently transported to the Golgi apparatus (*Kaufman, 2002*; *Malhotra & Kaufman, 2007*). Misfolded proteins bind to BiP and are then degraded by ER-associated degradation (ERAD). Ischemia and hypoxia can cause an accumulation of unfolded proteins in the ER cavity, thus initiating the unfolded protein response (UPR) (*Malhotra & Kaufman, 2007*; *Pan et al., 2021*). The UPR, ERAD, autophagy, and hypoxia signal transduction collectively dictate the extent of ER stress (ERS), which in turn determines the intracellular balance or initiates the death process. The hypoxia-Inducible Factor 1 (HIF-1) Pathway is one of the longest-studied signals (*Semenza, 2007*). HIF-1 induces high concentrations of pro- or anti-apoptotic proteins under different oxygenated conditions, inducing apoptosis at the cellular or local level (*Greijer & Van der Wall, 2004*). Therefore HIF-1 signalling has been extensively studied in recent years as a key target in the treatment of cardiovascular diseases (*Liu et al., 2020*).

In the context of CI-reperfusion, a combination of calcium overload, ROS accumulation, and inflammatory response can trigger ERS. The severity of ERS determines cellular fate: ERS promotes cell survival by initiating UPR in the early stage of CI-reperfusion, whereas severe and persistent ERS and UPR lead to cell apoptosis (*Wang et al., 2022*). Apoptosis remains the predominant mode of cell death in CI-reperfusion (*Zhang et al., 2022*), and interfering with neuronal apoptotic signalling and thus neuroprotection provides a theoretical basis for the clinical prevention and treatment of CI-reperfusion.

It has been reported that inhibiting ERS can reduce the volume of cerebral infarction, suggesting that targeting ERS may be an effective strategy for mitigating brain injury. However, no efficacious targets have yet been identified for effectively alleviating CI-reperfusion injury. Unravelling these complexities may provide assistance in treatment strategies for stroke patients, increasing patient survival and improving the quality of life of stroke survivors. Therefore, this study utilized bioinformatics to analyze public databases of rat models of CI-reperfusion injury, aiming to screen and determine potential therapeutic targets.

MATERIALS AND METHODS

Data acquisition and pre-processing

The GSE163614 dataset was retrieved from the National Center for Biotechnology Information (NCBI) database. ENSRNOG identifiers were mapped to gene symbols utilizing the annotation file Rattus_norvegicus.mRatBN7.2.107.gtf. This dataset comprised three CI/reperfusion injured rats and three control rats (*Yi et al., 2021*).

Datasets GSE78731 (*Oh et al., 2018*) and GSE97537 (*Zou et al., 2019*) were also sourced from the Gene Expression Omnibus (GEO) within the NCBI database. Probe identifiers were transformed into gene symbols using the platform-specific file. Entries with a single probe mapping to multiple genes were excluded. When multiple probes were mapped to one gene, their data were averaged. From GSE78731, five Middle Cerebral Artery Occlusion (MCAO) and five control samples were extracted, and from GSE97537, seven MCAO and five sham samples were derived.

Differential gene expression analysis

Employing the 'limma' package in R, differential expression between the three CI/reperfusion injured rats and three controls in the GSE163614 dataset was conducted. DEGs were identified using a threshold of $|log2(Fold Change)| > log2(1.2)$ and FDR < 0.05. This analysis was followed by the generation of volcano and heat maps.

Isolation of PER-related genes

PER-related pathways were sourced from the single-sample Gene-Set Enrichment Analysis (ssGSEA) official website. PER scores were computed *via* ssGSEA, and the *t*-test function was applied to compare the MCAO and sham groups. Associations between the PER score and DEGs were determined using R's 'cor' function. Genes exhibiting $|cor| > 0.7$ were designated as PER-associated DEGs. These genes were further assessed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment *via* the clusterProfiler package.

Identification of central genes in PER-related genes

A protein-protein interaction (PPI) network of the PER-related DEGs was constructed using the STRING database. Essential interactions were discerned using a minimum score of 0.7. Visualization and degree analysis of the PPI were executed using Cytoscape.

Identification of diagnostically relevant per-associated genes

For datasets GSE78731 and GSE97537, crucial genes linked to PER were scrutinized. Receiver operating characteristic (ROC) curves were produced for these DEGs, and their diagnostic efficacy was evaluated based on the Area Under the Curve (AUC).

Correlation of key PER genes with hypoxia

To further explore the possible mechanisms of CI-reperfusion, the HIF-1 signalling pathway gene set was downloaded from the KEGG official website. HIF-1 signalling scores for MCAO and sham groups were determined using the ssGSEA approach. The correlation between key diagnostic genes and the HIF-1 pathway score was analyzed *via* the Spearman correlation method.

Table 1 Primer sequences for 5 genes.

Gene	Primer name	Sequence (5′->3′)	Length	Tm	Location
ADCY5	Forward Primer	TCTCCTGCACCAACATCGTG	20	62.2	1157-1176
	Reverse Primer	CATGGCAACATGACGGGGA	19	62.4	1326-1308
CAMK2A	Forward Primer	GCTCTTCGAGGAATTGGGCAA	21	62.7	42-62
	Reverse Primer	CCTCTGAGATGCTGTCATGTAGT	23	61.2	244-222
PLCB1	Forward Primer	GGACTGACCCTCAGGGATTTT	21	61.1	131-151
	Reverse Primer	AAGCCACGAGATTCAAATGGG	21	60.6	364-344
NTRK2	Forward Primer	TCGTGGCATTTCCGAGATTGG	21	62.7	155-175
	Reverse Primer	TCGTCAGTTTGTTTCGGGTAAA	22	60.1	385-364
DLG4	Forward Primer	CACAACCTCTTATTCCCAGCAC	22	60.9	909-930
	Reverse Primer	CATGGCTGTGGGGTAGTCG	19	62.1	987-969
GAPDH	Forward Primer	GGAGCGAGATCCCTCCAAAAT	21	61.6	108-128
	Reverse Primer	GGCTGTTGTCATACTTCTCATGG	23	60.9	304-282

Cell cultivation

The PC12 cell line was sourced from the Cell Bank of the Shanghai Institute of Cell Biology, affiliated with the Chinese Academy of Sciences. The cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) at 37 °C. Under normoxic conditions, PC12 cells were maintained in an atmosphere consisting of 95% air and 5% CO_2. For simulating ischemic conditions, an oxygen and glucose deprivation/reperfusion (OGD/R) model was employed. Cells were exposed to glucose-free DMEM in a hypoxic environment of 95% N_2 and 5% CO_2 for a duration of 2 h. Post hypoxia, cells were reintroduced to regular glucose conditions and an oxygenated environment for a recovery period of 24 h.

qRT-PCR analysis

For assessing gene expression alterations, quantitative reverse transcription PCR (qRT-PCR) was utilized. Total RNA from PC12 cells was extracted and then reverse-transcribed using the Takara RNA PCR Kit (AMV) Ver 3.0 (TaKaRa Bio Inc., Shiga, Japan). The synthesized cDNA was subjected to PCR amplification using SYBR Green I as the fluorescent dye on an ABI PRISM 7300 real-time PCR system. The thermal cycling conditions were initiated with a denaturation step at 95 °C for 30 s, followed by 45 amplification cycles, each consisting of 95 °C for 10 s (denaturation) and 62 °C for 31 s (annealing/extension). To ensure reproducibility and accuracy, all experiments were performed in duplicate and repeated twice. The specific primer sequences used for each target gene are provided in Table 1. All primer sequences were validated by BLAST.

RESULTS

Screening of genes associated with CI/reperfusion injury and investigation of their relationship with PER-associated pathways

The main technical roadmap of this study is shown in Fig. S1. Differentially expressed genes (DEGs) from three rats with CI/reperfusion injury and three control rats in the
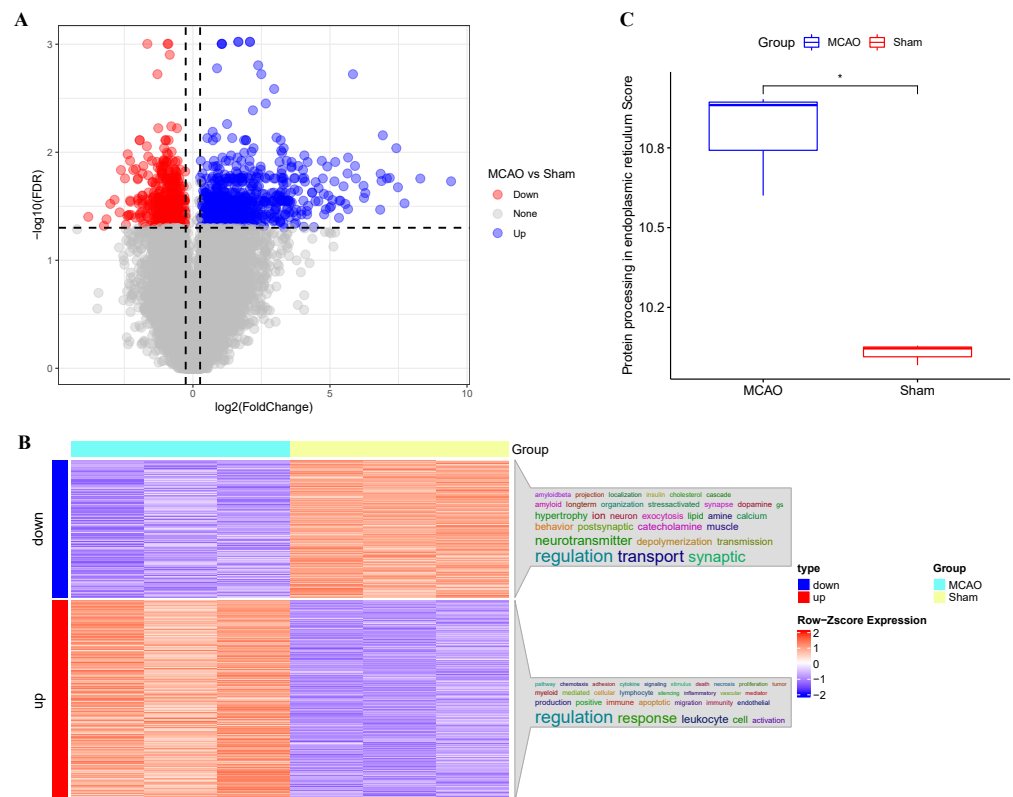
Figure 1 Screening of genes associated with CI/reperfusion injury and their relationship with PER-associated pathways. (A) Volcano map for the difference analysis of three rats with CI/reperfusion injury and three control rats; (B) heat map of DEGs and enrichment analysis; (C) comparison of PER-associated pathways between rats with CI/reperfusion injury and the control group (*$P < 0.05$).

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GSE163614 dataset were analyzed. Both volcano (Fig. 1A) and heat maps were generated. Using the anno_GO_keywords function, an enrichment analysis word cloud (Fig. 1B) was produced, revealing 651 upregulated genes (red) and 446 downregulated genes (blue). In addition, the top 10 genes with the most significant differences in expression up- and down-regulation were summarised in Table S1, respectively. The ssGSEA analysis indicated a stronger association of CI/reperfusion injury with PER in the MCAO group, as evidenced by a higher PER score (Fig. 1C).

Functional enrichment analysis of PER-associated gene pathways

DEGs were correlated with the PER score, and gene sets related to a |cor| > 0.7 threshold were isolated. This resulted in 648 genes positively correlated with the PER score and 446 negatively correlated genes (Table S2). Subsequent GO and KEGG enrichment analyses (using an FDR < 0.05 threshold) identified 1056 biological processes (BP), 132 cellular components (CC), 42 molecular functions (MF), and 73 KEGG signalling pathways. The top 10 enriched items are displayed as a bar graph in Fig. 2, the more the colour tends to be red, the smaller the p.adjust value, indicating a higher degree of enrichment. Importantly,
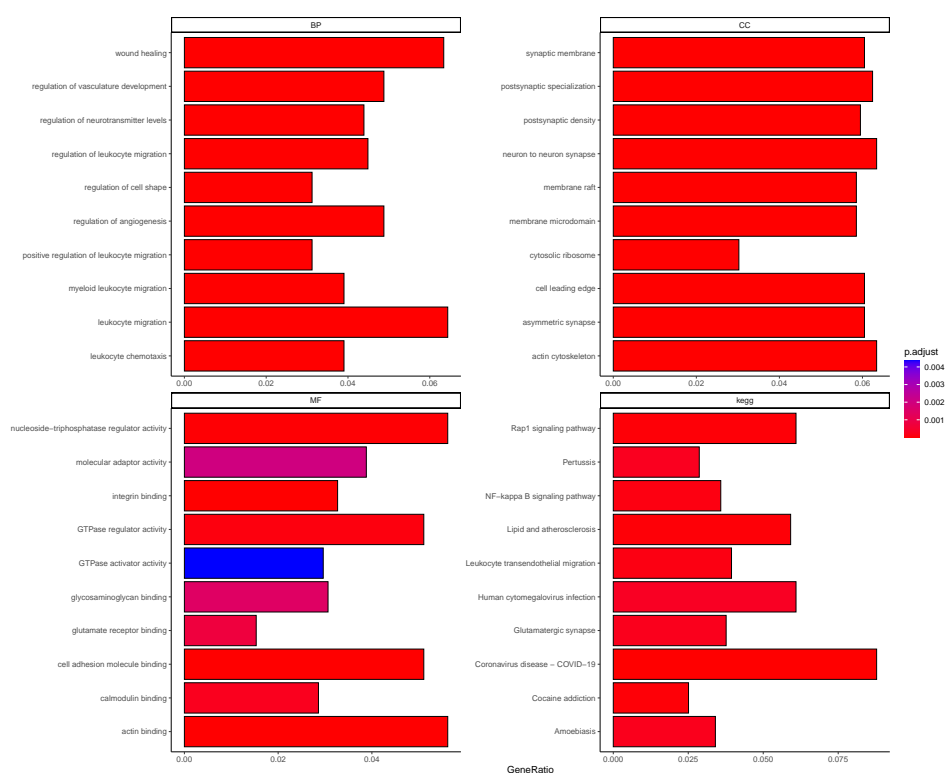
Figure 2 **Different GO and KEGG enrichment anaysis.** Smaller adjusted p values tend towards red, indicating higher enrichment; larger adjusted p values tend towards blue, indicating lower enrichment.

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the PER-associated genes showed significant involvement in neurotraumatic processes, such as leukocyte migration, wound healing, and angiogenesis regulation (Table S3).

Identification of central genes in PER-associated gene set

The Protein-Protein Interaction (PPI) network of the PER-associated genes was built *via* the STRING database (*Mering et al., 2003*) to predict potential biological functions among proteins and further explored using Cytoscape (Fig. 3A), followed by a degree analysis (Fig. 3B). The 20 most pivotal nodes, both positively and negatively correlated, were highlighted as key genes (Table 2). A node with a higher degree value suggests its centrality within the network.

Diagnostic potential of key PER-associated genes

For diagnostic relevance, we examined the key PER genes across the independent datasets GSE78731 and GSE97537. Seventeen of the top 20 genes showcased significant degree correlations (Figs. 4A and 4B, Table 2): ITGB1, RPS5, RPS3A, RPS27, RPL11, RPL5, RPS27L, FN1, PTPRC, RPLP0, STAT3, RPL6, RPL10A, RPL26, RPL27, RPSA, RPS15, RPS24, RPS4X, TP53, DLG4, PRKCB, GRIN2A, GNAO1, THY1, NTRK2, PRKCA, GRIA3, PLCB1, AGT, CAMK2A, DLG1, SHC3, HSPA4L, PAK1, ADCY5, APOE, NR3C1, ADRB1, RGS8. ROC curves of these 17 genes evaluated their diagnostic efficacy (Figs. 4C and 4D),
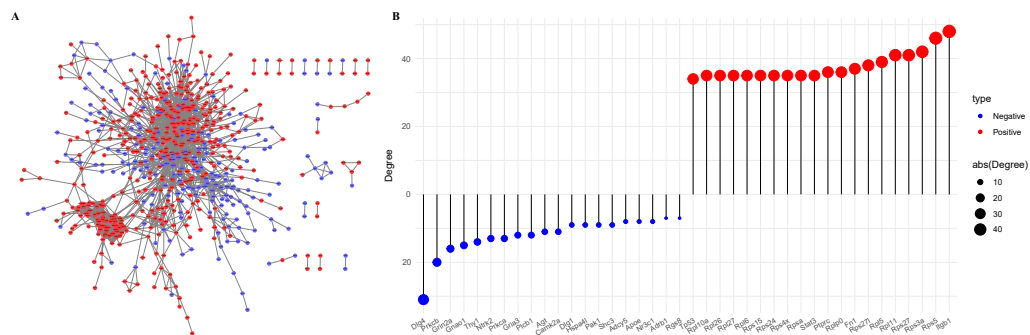
Figure 3   Identification of key genes in PER-associated genes. (A) PPI network of PER-associated genes, with red indicating positive correlation and blue indicating negative correlation; (B) dot-bar map of the top 20 positive and negative degree genes in the network.

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Table 2   Top 20 hub genes for relatedness.

Symbol	Degree	Correlation	Symbol	Degree	Correlation
ITGB1	48	Positive	DLG4	−31	Negative
RPS5	46	Positive	PRKCB	−20	Negative
RPS3A	42	Positive	GRIN2A	−16	Negative
RPS27	41	Positive	GNAO1	−15	Negative
RPL11	41	Positive	THY1	−14	Negative
RPL5	39	Positive	NTRK2	−13	Negative
RPS27L	38	Positive	PRKCA	−13	Negative
FN1	37	Positive	GRIA3	−12	Negative
PTPRC	36	Positive	PLCB1	−12	Negative
RPLP0	36	Positive	AGT	−11	Negative
STAT3	35	Positive	CAMK2A	−11	Negative
RPL6	35	Positive	DLG1	−9	Negative
RPL10A	35	Positive	SHC3	−9	Negative
RPL26	35	Positive	HSPA4L	−9	Negative
RPL27	35	Positive	PAK1	−9	Negative
RPSA	35	Positive	ADCY5	−8	Negative
RPS15	35	Positive	APOE	−8	Negative
RPS24	35	Positive	NR3C1	−8	Negative
RPS4X	35	Positive	ADRB1	−7	Negative
TP53	34	Positive	RGS8	−7	Negative

with each gene demonstrating AUCs exceeding 0.7, suggesting their diagnostic promise. Further validation in the GSE163614 dataset supported these findings (Fig. 5).

Evaluating the association of diagnostic key PER genes with hypoxia

Decreased cerebral blood flow can instigate hypoxia, precipitating ischemic neuronal injury and favouring anaerobic tissue metabolism (*Kalogeris et al., 2012*). To contextualize hypoxia, the HIF-1 signalling pathway was extracted from KEGG. Its scores across groups
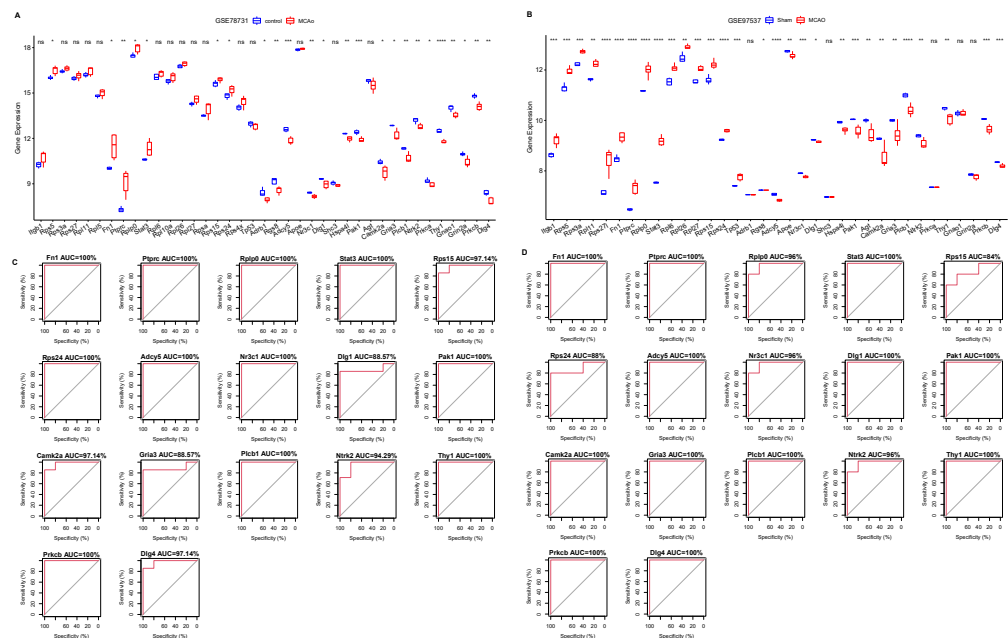
Figure 4 Screening of key genes with diagnostic value. Comparison of expression levels of the top 20 degree genes with positive/negative correlations in GSE78731 (A) and GSE97537 (B); ROC curves in GSE78731 (C) and GSE97537 (D). ***$P < 0.05$; ****$P < 0.01$; *****$P < 0.001$.

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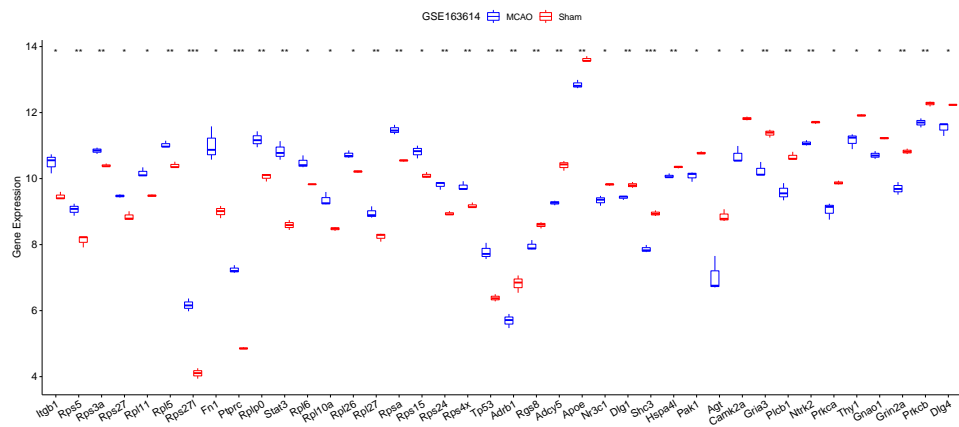
Figure 5 Notable differences were found among the top 20 degree genes with positive/negative correlation between the MCAO and sham groups in GSE163614. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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were computed using ssGSEA, revealing an elevated score in the MCAO group (Fig. 6A). Correlation analyses between the 17 diagnostic genes and the HIF-1 signalling pathway score identified six genes—PRKCB, ADCY5, CAMK2, PLCB1, NTRK2, and DLG4—that displayed significant associations with hypoxia (Fig. 6B).
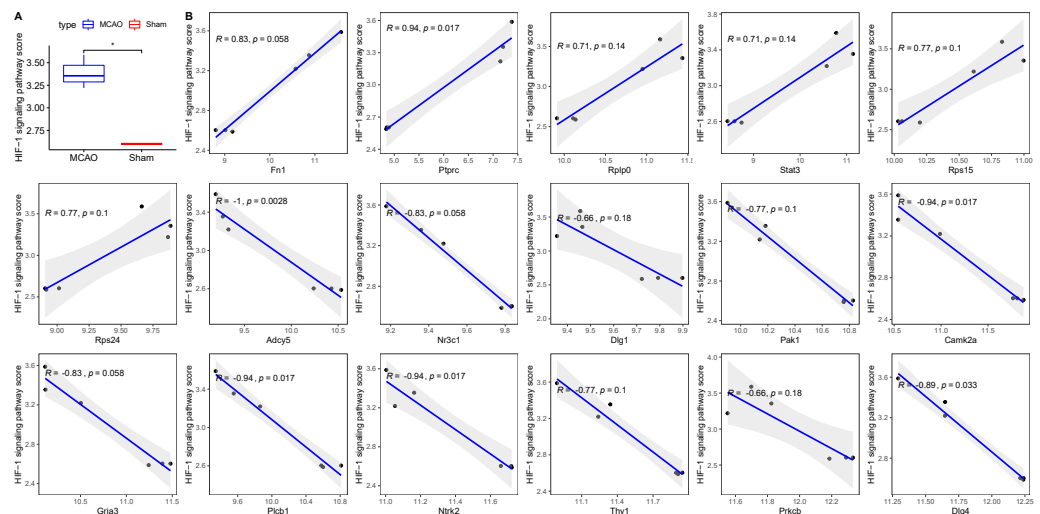
Figure 6 **Relationships between 17 key PER genes with diagnostic value and hypoxia.** (A) HIF-1 signaling pathway score in rats with CI/reperfusion injury and control group; (B) correlation analysis between 17 key genes and HIF-1 signaling pathway score.
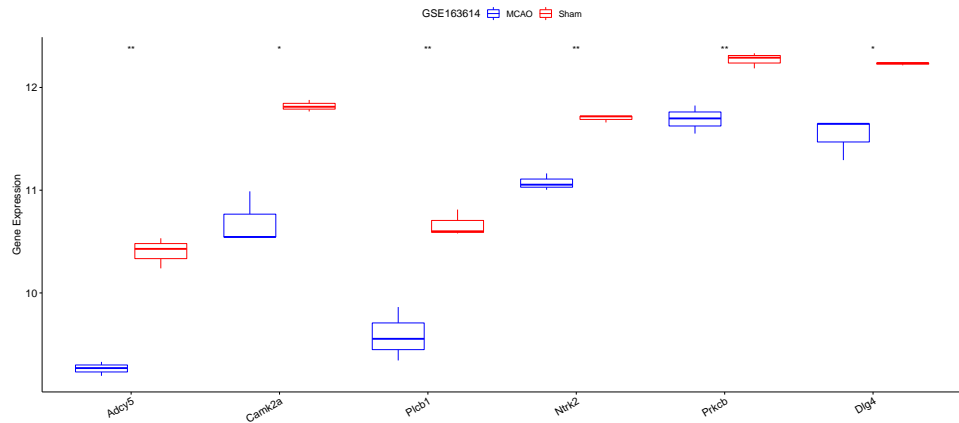
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Investigating the interplay between key hypoxia-linked genes and apoptosis

Exacerbated endoplasmic reticulum stress (ERS) activation during CI/reperfusion can stimulate apoptosis *via* pathways such as CHOP, Caspase12, and JNK (*Wang et al., 2022*). We probed the connections between crucial hypoxia-associated genes and apoptosis. The MCAO group displayed a marked under-expression of these genes compared to the Sham group (Fig. 7). Subsequent analyses showed an elevated apoptosis score in the MCAO group (Fig. 8A). Furthermore, ADCY5, CAMK2A, PLCB1, NTRK2, and DLG4 showed significant negative correlations with apoptosis score (Fig. 8B). These genes appear to be instrumental in hypoxia-induced apoptosis during CI/reperfusion injury. PCR-based validation further highlighted their reduced expression in cells subjected to glucose deprivation/reperfusion (OGD/R), which suggests their consistent downregulation during such metabolic stress (Fig. 9).

DISCUSSION

CI is a leading cause of disability and mortality worldwide (*Siniscalchi et al., 2014*). Typically initiated by an embolism or thrombosis in a major cerebral artery (*Miller et al., 2017*), CI is primarily managed through thrombolysis and restoration of blood flow (*Jean et al., 1998*). Although acute ischemia inflicts neuronal damage, the subsequent restoration of blood flow can intensify this injury, culminating in significant brain dysfunction (*Rosenberg, Estrada & Dencoff, 1998*). The challenge, therefore, is to prevent the amplification of brain injury following ischemia/reperfusion.

In the current study, the ssGSEA algorithm was used for the first time to analyse the PER scores of MACO rats. The results showed that the PER score was increased in the MCAO

Figure 7 **Expression analysis of six key hypoxia-associated genes in the GSE163614 dataset.** $***P <$ 0.05; $****P < 0.01$.
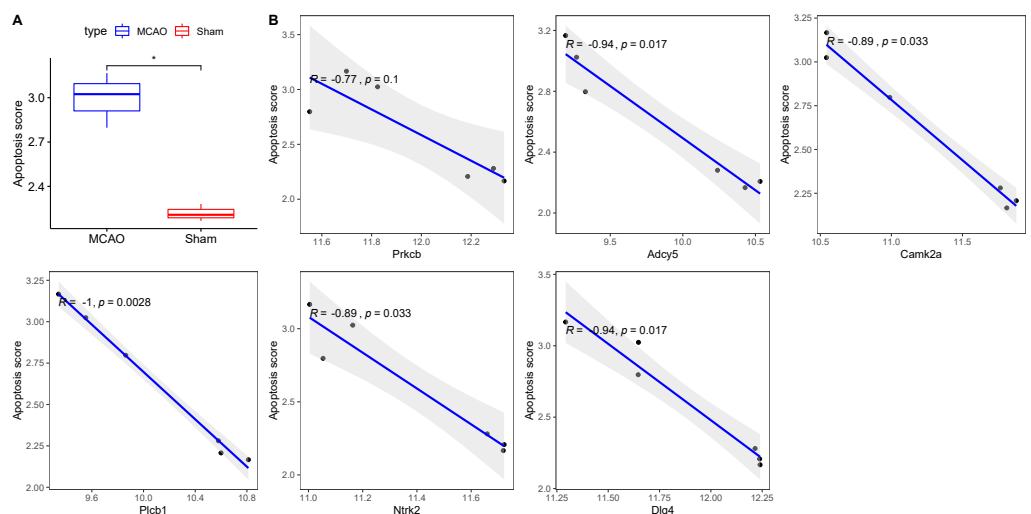
Figure 8 **Correlations between key hypoxia-associated genes and apoptosis.** (A) Comparison of apoptosis score between rats with CI/reperfusion injury and control group; (B) correlation analysis between six key hypoxia-associated genes and apoptosis score.

group, which suggests a close association between MCAO and PER. Subsequent DEG and correlation analyses were employed to identify genes with a strong PER affiliation, leading to the construction of a PPI. Within this PPI, 40 core genes were discerned. Utilizing two external validation datasets, we affirmed the expression and diagnostic relevance of these core nodes for the MCAO group, pinpointing 17 essential diagnostic genes. Considering the established link between ischemia/reperfusion-induced apoptosis and hypoxia, we further discerned five genes (ADCY5, CAMK2A, PLCB1, NTRK2, and DLG4) from the initial 17 that displayed significant associations with both hypoxia and apoptosis. These
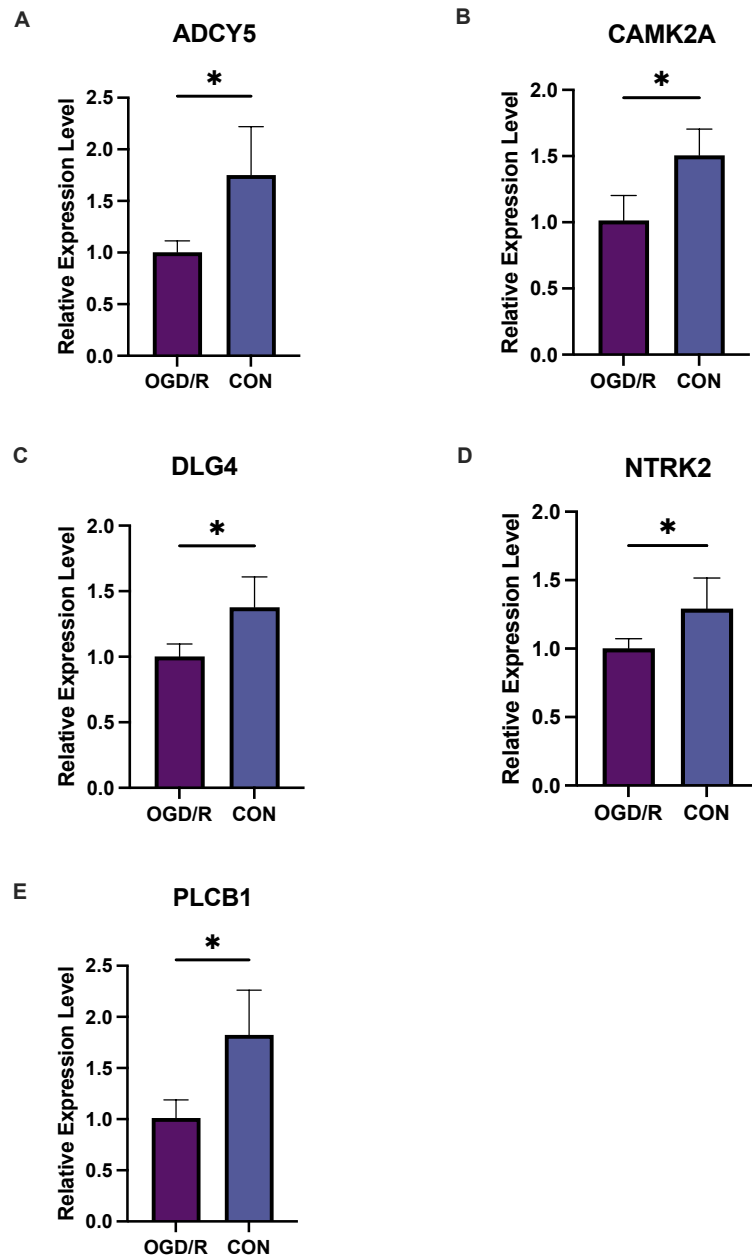
Figure 9 **Five key genes show low expression in PC12 cells from an oligosaccharide/reperfusion (OGD/R) injury model.** The relative expression levels of (A) ADCY5, (B) CAMK2A, (C) DLG4, (D) PLCB1, and (E) NTRK2 in PC cells were examined using PCR. $*P < 0.05$; $***P < 0.001$.

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genes are hypothesized to be paramount in instigating apoptosis in hypoxic conditions during ischemia/reperfusion injury.

ADCY5, an adenylate cyclase family member, facilitates the conversion of adenine-5′-triphosphate to 3′, 5′-cyclic adenosine-5′-triphosphate (cAMP) (*Linder, 2006*). Though ADCY5 is ubiquitously present in the brain and has been linked to various neuro-related

conditions (*Ferrini et al., 2021*; *Kaiyrzhanov et al., 2021*), ADCY5 is specifically expressed in the brain and selectively possesses high expression levels in sites such as the striatum and olfactory nodes, and mutations in its gene may affect neurodevelopmental phenotypes, which in turn may lead to motor dysfunction. Its association with CI/reperfusion remains unexplored. We conjecture that ADCY5 may also influence the quality of survival of survivors by modulating motor function in CI/reperfusion.

CAMK2, a calcium-responsive serine/threonine kinase (*Chao et al., 2011*), comprises four distinct subunits (CAMK2A, 2B, 2D, and 2G) each possessing specific tissue distributions and functionalities (*Wang et al., 2020a*). Especially noteworthy are the CAMK2A and CAMK2B variants, which dominate brain expression (*Cook et al., 2018*; *Wang et al., 2020a*). Not only is CAMK2A implicated in cognitive functions (*Lee et al., 2021*), neurodevelopment (*Akita et al., 2018*), and certain cancer types (*Wang et al., 2020a*; *Yu et al., 2021*), but a study parallel to ours also unveiled its potential to augment synaptic plasticity in MCAO mice (*Shen et al., 2021*). In addition, it has been shown that CAMK2A may confer neuroprotection to neurons in CI/reperfusion through NF-KB signalling (*Ye et al., 2019*).PLCB1, primarily localized in cerebral regions (*Caricasole et al., 2000*; *McOmish et al., 2008*), has been associated with various neuropsychiatric disorders (*Girirajan et al., 2013*; *Schoonjans et al., 2016*; *St Pourcain et al., 2014*; *Udawela et al., 2011*; *Vasco, Cardinale & Polonia, 2012*). The NTRK2 gene, encoding the tropomyosin receptor kinase B (TrkB), plays an instrumental role in neuronal development and survival (*Amatu, Sartore-Bianchi & Siena, 2016*), findings which resonate with our study. This alludes to the potential therapeutic benefits of augmenting TRKB expression to attenuate ischemia/reperfusion injury. It has been shown that PLCB1, a key biological marker in cognitive improvement, modulates PLCB1 expression abundance in the cerebral cortex and contributes to the improvement of associated cognitive dysfunction (*Mabondzo et al., 2023*).

DLG4 is responsible for synthesizing the post-synaptic density protein 95 (PSD-95), a member of the membrane-associated guanylate kinases family. As a predominant scaffold protein in excitatory postsynaptic density, it is instrumental in synaptic plasticity (*Bustos et al., 2017*; *Rodríguez-Palmero et al., 2021*). Its aberrant expression has been observed in numerous neurological disorders (*Arbuckle et al., 2010*; *Bustos et al., 2017*; *De Bartolomeis et al., 2014*; *Savioz, Leuba & Vallet, 2014*; *Zhang et al., 2014*), with previous research suggesting that enhancing PSD95 expression may bolster synapse count and minimize neuronal death (*Wang et al., 2020b*). Previous studies have shown that the use of electrical stimulation of the parietal nucleus of the cerebellum can modulate neurotransmitter release from synaptic vesicles by methylating DLG4 and activating neuronal and neurovascular coupling, thereby stimulating interneuronal neuroprotection and reducing brain injury (*Gao et al., 2023*).

Our study delineates five key genes (ADCY5, CAMK2A, PLCB1, NTRK2, and DLG4) that may be instrumental in prompting apoptosis in hypoxic scenarios during CI/reperfusion injury. While some genes' roles in mitigating CI/reperfusion injury have been established, others require further validation. Nonetheless, their extensive brain expression and crucial functions are indisputable. It is imperative to further investigate the notion that

modulation of these genes' expression can influence cell apoptosis *via* PER, culminating in CI/reperfusion injury.

REFERENCES

Akita T, Aoto K, Kato M, Shiina M, Mutoh H, Nakashima M, Kuki I, Okazaki S, Magara S, Shiihara T. 2018. *De novo* variants in CAMK 2A and CAMK 2B cause neurodevelopmental disorders. *Annals of Clinical and Translational Neurology* **5**:280–296 DOI 10.1002/acn3.528.

Amatu A, Sartore-Bianchi A, Siena S. 2016. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open* **1**:e000023 DOI 10.1136/esmoopen-2015-000023.

Arbuckle MI, Komiyama NH, Delaney A, Coba M, Garry EM, Rosie R, Allchorne AJ, Forsyth LH, Bence M, Carlisle HJ. 2010. The SH3 domain of postsynaptic density

95 mediates inflammatory pain through phosphatidylinositol-3-kinase recruitment. *EMBO Reports* **11**:473–478 DOI 10.1038/embor.2010.63.

Bramlett HM, Dietrich WD. 2004. Pathophysiology of cerebral ischemia and brain trauma: similarities and differences. *Journal of Cerebral Blood Flow & Metabolism* **24**:133–150 DOI 10.1097/01.WCB.0000111614.19196.04.

Bustos FJ, Ampuero E, Jury N, Aguilar R, Falahi F, Toledo J, Ahumada J, Lata J, Cubillos P, Henríquez B. 2017. Epigenetic editing of the Dlg4/PSD95 gene improves cognition in aged and Alzheimer's disease mice. *Brain* **140**:3252–3268 DOI 10.1093/brain/awx272.

Caricasole A, Sala C, Roncarati R, Formenti E, Terstappen GC. 2000. Cloning and characterization of the human phosphoinositide-specific phospholipase C-beta 1 (PLC β1). *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression* **1517**:63–72 DOI 10.1016/S0167-4781(00)00260-8.

Chao LH, Stratton MM, Lee I-H, Rosenberg OS, Levitz J, Mandell DJ, Kortemme T, Groves JT, Schulman H, Kuriyan J. 2011. A mechanism for tunable autoinhibition in the structure of a human Ca2+/calmodulin-dependent kinase II holoenzyme. *Cell* **146**:732–745 DOI 10.1016/j.cell.2011.07.038.

Cook SG, Bourke AM, O'Leary H, Zaegel V, Lasda E, Mize-Berge J, Quillinan N, Tucker CL, Coultrap SJ, Herson PS. 2018. Analysis of the CaMKII α and β splice-variant distribution among brain regions reveals isoform-specific differences in holoenzyme formation. *Scientific Reports* **8**:5448 DOI 10.1038/s41598-018-23779-4.

De Bartolomeis A, Latte G, Tomasetti C, Iasevoli F. 2014. Glutamatergic postsynaptic density protein dysfunctions in synaptic plasticity and dendritic spines morphology: relevance to schizophrenia and other behavioral disorders pathophysiology, and implications for novel therapeutic approaches. *Molecular Neurobiology* **49**:484–511 DOI 10.1007/s12035-013-8534-3.

Eltzschig HK, Eckle T. 2011. Ischemia and reperfusion—from mechanism to translation. *Nature Medicine* **17**:1391–1401 DOI 10.1038/nm.2507.

Ferrini A, Steel D, Barwick K, Kurian MA. 2021. An update on the phenotype, genotype and neurobiology of ADCY5-related disease. *Movement Disorders* **36**:1104–1114 DOI 10.1002/mds.28495.

Gao J, Pang X, Zhang L, Li S, Qin Z, Xie X, Liu J. 2023. Transcriptome analysis reveals the neuroprotective effect of Dlg4 against fastigial nucleus stimulation-induced ischemia/reperfusion injury in rats. *BMC Neuroscience* **24**:40 DOI 10.1186/s12868-023-00811-6.

Girirajan S, Dennis MY, Baker C, Malig M, Coe BP, Campbell CD, Mark K, Vu TH, Alkan C, Cheng Z. 2013. Refinement and discovery of new hotspots of copy-number variation associated with autism spectrum disorder. *The American Journal of Human Genetics* **92**:221–237 DOI 10.1016/j.ajhg.2012.12.016.

Greijer A, Van der Wall E. 2004. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. *Journal of Clinical Pathology* **57**:1009–1014 DOI 10.1136/jcp.2003.015032.

Jean WC, Spellman SR, Nussbaum ES, Low WC. 1998. Reperfusion injury after focal cerebral ischemia: the role of inflammation and the therapeutic horizon. *Neurosurgery* **43**:1382–1396.

Jurcau A, Simion A. 2021. Neuroinflammation in cerebral ischemia and ischemia/reperfusion injuries: from pathophysiology to therapeutic strategies. *International Journal of Molecular Sciences* **23**:14 DOI 10.3390/ijms23010014.

Kaiyrzhanov R, Zaki MS, Maroofian R, Dominik N, Rad A, Vona B, Houlden H. 2021. A novel homozygous ADCY5 variant is associated with a neurodevelopmental disorder and movement abnormalities. *Movement Disorders Clinical Practice* **8**:1140–1143 DOI 10.1002/mdc3.13310.

Kalogeris T, Baines CP, Krenz M, Korthuis RJ. 2012. Cell biology of ischemia/reperfusion injury. *International Review of Cell and Molecular Biology* **298**:229–317 DOI 10.1016/B978-0-12-394309-5.00006-7.

Katan M, Luft A. 2018. Global burden of stroke. In: *Seminars in neurology.* Thieme Medical Publishers, 208–211.

Kaufman RJ. 2002. Orchestrating the unfolded protein response in health and disease. *The Journal of Clinical Investigation* **110**:1389–1398 DOI 10.1172/JCI0216886.

Khoshnam SE, Winlow W, Farzaneh M. 2017. The interplay of MicroRNAs in the inflammatory mechanisms following ischemic stroke. *Journal of Neuropatholgy & Experimental Neurology* **76**:548–561 DOI 10.1093/jnen/nlx036.

Lee L-C, Su M-T, Huang H-Y, Cho Y-C, Yeh T-K, Chang C-Y. 2021. Association of CaMK2A and MeCP2 signaling pathways with cognitive ability in adolescents. *Molecular Brain* **14**:1–14 DOI 10.1186/s13041-020-00716-z.

Linder J. 2006. Class III adenylyl cyclases: molecular mechanisms of catalysis and regulation. *Cellular and Molecular Life Sciences* **63**:1736–1751 DOI 10.1007/s00018-006-6072-0.

Liu M, Galli G, Wang Y, Fan Q, Wang Z, Wang X, Xiao W. 2020. Novel therapeutic targets for hypoxia-related cardiovascular diseases: the role of HIF-1. *Frontiers in Physiology* **11**:774 DOI 10.3389/fphys.2020.00774.

Mabondzo A, Harati R, Broca-Brisson L, Guyot A-C, Costa N, Cacciante F, Putignano E, Baroncelli L, Skelton MR, Saab C. 2023. Dodecyl creatine ester improves cognitive function and identifies key protein drivers including KIF1A and PLCB1 in a mouse model of creatine transporter deficiency. *Frontiers in Molecular Neuroscience* **16**:1118707 DOI 10.3389/fnmol.2023.1118707.

Malhotra JD, Kaufman RJ. 2007. The endoplasmic reticulum and the unfolded protein response. *Seminars in Cell & Developmental Biology* **18(6)**:716–731 DOI 10.1016/j.semcdb.2007.09.003.

McOmish CE, Burrows EL, Howard M, Hannan AJ. 2008. PLC-β1 knockout mice as a model of disrupted cortical development and plasticity: Behavioral endophenotypes and dysregulation of RGS4 gene expression. *Hippocampus* **18**:824–834 DOI 10.1002/hipo.20443.

Mering Cv, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. 2003. STRING: a database of predicted functional associations between proteins. *Nucleic Acids Research* **31**:258–261 DOI 10.1093/nar/gkg034.

Miller JB, Merck LH, Wira CR, Meurer WJ, Schrock JW, Nomura JT, Siket MS, Madsen TE, Wright DW, Panagos PD. 2017. The advanced reperfusion era: implications for emergency systems of ischemic stroke care. *Annals of Emergency Medicine* **69**:192–201 DOI 10.1016/j.annemergmed.2016.06.042.

Neag M-A, Mitre A-O, Burlacu C-C, Inceu A-I, Mihu C, Melincovici C-S, Bichescu M, Buzoianu A-D. 2022. miRNA involvement in cerebral ischemia-reperfusion injury. *Frontiers in Neuroscience* **16**:901360 DOI 10.3389/fnins.2022.901360.

Oh SH, Choi C, Noh JE, Lee N, Jeong YW, Jeon I, Shin JM, Kim JH, Kim HJ, Lee JM, Kim HS, Kim OJ, Song J. 2018. Interleukin-1 receptor antagonist-mediated neuroprotection by umbilical cord-derived mesenchymal stromal cells following transplantation into a rodent stroke model. *Experimental & Molecular Medicine* **50**:1–12 DOI 10.1038/s12276-018-0041-1.

Pan B, Sun J, Liu Z, Wang L, Huo H, Zhao Y, Tu P, Xiao W, Zheng J, Li J. 2021. Longxuetongluo Capsule protects against cerebral ischemia/reperfusion injury through endoplasmic reticulum stress and MAPK-mediated mechanisms. *Journal of Advanced Research* **33**:215–225 DOI 10.1016/j.jare.2021.01.016.

Rodríguez-Palmero A, Boerrigter MM, Gómez-Andrés D, Aldinger KA, Marcos-Alcalde Í, Popp B, Everman DB, Lovgren AK, Arpin S, Bahrambeigi V. 2021. DLG4-related synaptopathy: a new rare brain disorder. *Genetics in Medicine* **23**:888–899 DOI 10.1038/s41436-020-01075-9.

Rosenberg G, Estrada E, Dencoff J. 1998. Matrix metalloproteinases and TIMPs are associated with blood–brain barrier opening after reperfusion in rat brain. *Stroke* **29**:2189–2195 DOI 10.1161/01.STR.29.10.2189.

Savioz A, Leuba G, Vallet PG. 2014. A framework to understand the variations of PSD-95 expression in brain aging and in Alzheimer's disease. *Ageing Research Reviews* **18**:86–94 DOI 10.1016/j.arr.2014.09.004.

Schoonjans A-S, Meuwissen M, Reyniers E, Kooy F, Ceulemans B. 2016. PLCB1 epileptic encephalopathies; Review and expansion of the phenotypic spectrum. *European Journal of Paediatric Neurology* **20**:474–479 DOI 10.1016/j.ejpn.2016.01.002.

Semenza GL. 2007. Hypoxia-inducible factor 1 (HIF-1) pathway. *Science's STKE* **2007**:cm8–cm8.

Shen W, Jin L, Zhu A, Lin Y, Pan G, Zhou S, Cheng J, Zhang J, Tu F, Liu C. 2021. Treadmill exercise enhances synaptic plasticity in the ischemic penumbra of MCAO mice by inducing the expression of Camk2a *via* CYFIP1 upregulation. *Life Sciences* **270**:119033 DOI 10.1016/j.lfs.2021.119033.

Siniscalchi A, Gallelli L, Malferrari G, Pirritano D, Serra R, Santangelo E, De Sarro G. 2014. Cerebral stroke injury: the role of cytokines and brain inflammation. *Journal of Basic and Clinical Physiology and Pharmacology* **25**:131–137 DOI 10.1515/jbcpp-2013-0121.

St Pourcain B, Skuse DH, Mandy WP, Wang K, Hakonarson H, Timpson NJ, Evans DM, Kemp JP, Ring SM, McArdle WL. 2014. Variability in the common genetic architecture of social-communication spectrum phenotypes during childhood and adolescence. *Molecular Autism* **5**:1–12 DOI 10.1186/2040-2392-5-1.

Udawela M, Scarr E, Hannan AJ, Thomas EA, Dean B. 2011. Phospholipase C beta 1 expression in the dorsolateral prefrontal cortex from patients with schizophrenia at different stages of illness. *Australian & New Zealand Journal of Psychiatry* **45**:140–147 DOI 10.3109/00048674.2010.533364.

Vasco VRL, Cardinale G, Polonia P. 2012. Deletion of PLCB1 gene in schizophrenia-affected patients. *Journal of Cellular and Molecular Medicine* **16**:844–851 DOI 10.1111/j.1582-4934.2011.01363.x.

Wang S-Q, Liu J, Qin J, Zhu Y, Tin VP-C, Yam JWP, Wong MP, Xiao Z-J. 2020a. CAMK2A supported tumor initiating cells of lung adenocarcinoma by upregulating SOX2 through EZH2 phosphorylation. *Cell Death & Disease* **11**:410 DOI 10.1038/s41419-020-2553-6.

Wang W, Liu X, Yang Z, Shen H, Liu L, Yu Y, Zhang T. 2020b. Levodopa improves cognitive function and the deficits of structural synaptic plasticity in hippocampus induced by global cerebral ischemia/reperfusion injury in rats. *Frontiers in Neuroscience* **14**:586321 DOI 10.3389/fnins.2020.586321.

Wang L, Liu Y, Zhang X, Ye Y, Xiong X, Zhang S, Gu L, Jian Z, Wang H. 2022. Endoplasmic reticulum stress and the unfolded protein response in cerebral ischemia/reperfusion injury. *Frontiers in Cellular Neuroscience* **16**:864426 DOI 10.3389/fncel.2022.864426.

Ye J, Das S, Roy A, Wei W, Huang H, Lorenz-Guertin JM, Xu Q, Jacob TC, Wang B, Sun D. 2019. Ischemic injury-induced CaMKII δ and CaMKII γ confer neuroprotection through the NF-κB signaling pathway. *Molecular Neurobiology* **56**:2123–2136 DOI 10.1007/s12035-018-1198-2.

Yi D, Wang Q, Zhao Y, Song Y, You H, Wang J, Liu R, Shi Z, Chen X, Luo Q. 2021. Alteration of N (6)-Methyladenosine mRNA Methylation in a Rat Model of Cerebral Ischemia-Reperfusion Injury. *Frontiers in Neuroscience* **15**:605654 DOI 10.3389/fnins.2021.605654.

Yu T-J, Liu Y-Y, Li X-G, Lian B, Lu X-X, Jin X, Shao Z-M, Hu X, Di G-H, Jiang Y-Z. 2021. PDSS1-mediated activation of CAMK2A-STAT3 signaling promotes metastasis in triple-negative breast cancer. *Cancer Research* **81**:5491–5505 DOI 10.1158/0008-5472.CAN-21-0747.

Zhang Q, Jia M, Wang Y, Wang Q, Wu J. 2022. Cell death mechanisms in cerebral ischemia–reperfusion injury. *Neurochemical Research* **47**:3525–3542 DOI 10.1007/s11064-022-03697-8.

Zhang J, Saur T, Duke AN, Grant SG, Platt DM, Rowlett JK, Isacson O, Yao W-D. 2014. Motor impairments, striatal degeneration, and altered dopamine-glutamate interplay in mice lacking PSD-95. *Journal of Neurogenetics* **28**:98–111 DOI 10.3109/01677063.2014.892486.

Zou J-B, Chai H-B, Zhang X-F, Guo D-Y, Tai J, Wang Y, Liang Y-L, Wang F, Cheng J-X, Wang J. 2019. Reconstruction of the lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveal functional lncRNAs in cerebral infarction. *Scientific Reports* **9**:12176 DOI 10.1038/s41598-019-48435-3.