

Comparative transcriptomic analysis of the brain-liver Axis reveals molecular mechanisms underlying acute cold stress response in Gynogenetic Mrigal carp

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ABSTRACT

In this study, we produced a population of gynogenetic mrigal carp (*Cirrhinus mrigala*, GMCJ) through artificial gynogenesis using UV-inactivated white crucian carp spermatozoa. The GMCJ exhibited a faster growth rate and strong resistance to low temperatures, surviving natural winter conditions with temperatures below 10 °C. To gain a deeper insight into GMCJ's molecular response to acute cold stress (a decrease in water temperature from 26 °C to 14 °C within 1 h at a rate of 0.2 °C/min), we conducted a comparative analysis of the histology and transcriptomics of the brain-liver axis. Transmission electron microscopy of the heart tissues revealed that acute cold stress result in widened myofilament spacing, along with enlarged, deformed mitochondria that developed vacuoles and ruptured. We identified differentially expressed genes (DEGs) in the brain (2463 upregulated and 3288 downregulated genes) and liver (1210 upregulated and 1224 downregulated genes). Analysis of the DEGs' correlations indicated that major pathways involved in immunity, disease, metabolism, and growth were active and interconnected. KEGG functional analysis showed that the most enriched pathways in the brain-liver axis involved the regulation of the glucagon pathway, PI3K-Akt signaling pathway, and herpes simplex infection. Numerous genes associated with the nervous-endocrine system were activated, suggesting a coordinated response of the brain-liver axis to acute stress. These findings enhance our understanding of how farmed, particularly gynogenetic fish respond to acute cold stress.

1. Introduction

Artificial gynogenesis, a process where genetically inactive sperm activates maternal DNA, is a useful method to accelerate the selective breeding of populations and variants (Komen and Thorgaard, 2007; Qin et al., 2018). Individuals produced through artificial gynogenesis often demonstrate exhibit allogynogenetic effects, with paternal genetic components integrated into the genome or retained in microchromosomes. These fish typically show superior traits such as rapid growth, hypoxia tolerance, and high disease resistance (Fu et al., 2022;

Gong et al., 2020).

Temperature is a critical environmental factor that significantly impacts an animal's survival, growth, development, and reproduction (Islam et al., 2022). In aquatic organisms, water temperature changes dramatically affect behavior and physiological responses (Little et al., 2020). Being ectotherms, fish naturally adapt to temperature changes within their species-specific thermal tolerance levels. However, exposure to temperatures below their tolerance threshold can trigger cold stress, leading to erratic swimming, loss of equilibrium, coma, or even death (Panase et al., 2018).

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Numerous studies have indicated that both acute and chronic cold stress can affect protein synthesis pace, enzyme activity, respiration rate, and oxygen consumption in cells, thereby reducing an organism's infection-fighting capacity (Abram et al., 2017; Yang et al., 2016). The molecular and physiological responses to acute and chronic cold stress, however, are distinct. Acute cold stress typically upregulates processes like secondary alcohol metabolism, drug transport, anti-oxidative and apoptotic systems, and Na⁺ -K⁺ ATPase activity across various tissues (Qian, 2021; Tseng et al., 2011). In contrast, chronic cold stress impacts pathways associated with cell adhesion, *Staphylococcus aureus* infection, the PPAR signaling pathway, *Vibrio cholerae* infection, primary immunodeficiency, and fatty acid elongation. These pathways influence the central nervous system, which in turn affects feeding behavior and metabolism in the live (Matsubara et al., 2022; Phrompanya et al., 2021). However, the effect of temperature on the brain-liver axis at the molecular level is not thoroughly explored.

The Mrigal carp (*Cirrhinus mrigala*, Hamilton) (MC, 2n = 50), a bottom-feeding fish, is economically significant in India, Bangladesh, and Pakistan (Saravanan et al., 2012). Known for its resilience and various dietary and ecological adaptations, MC was introduced to Guangdong Province in the 1990s and has been used as live bait for predatory fish. One limitation, however, is the MC's poor ability to withstand winter in central and western China, often dying when temperatures drop below 9 °C (Yu et al., 2019). Interestingly, our previous research found that gynogenetic mrigal carp, created using irradiated common carp (*Cyprinus carpio*) sperm, exhibited enhanced cold tolerance (Li et al., 2023). In this study, we successfully developed a GMCJ population by applying cold treatment to double the chromosome number in MC eggs activated by UV-treated white crucian carp (WCC, 2n = 100) sperm. The GMCJ demonstrated a faster growth rate and strong low-temperature resistance, surviving winters with temperatures below 10 °C. To better understand the molecular mechanism of their response to acute cold stress, we conducted a comparative analysis of transcriptomics in the brain-liver axis. The findings from this study may deepen our knowledge of the molecular mechanisms responding to acute cold stress and highlight the potential of gynogenetic techniques in aquaculture.

2. Materials and methods

2.1. Ethics approval and consent to participate

All experimental procedures involving fish received approval from the Animal Care and Use Committee at Hunan Normal University. These procedures adhered to the guidelines set forth in the "Measures for the Management of Laboratory Animals" by the Chinese Science and Technology Bureau.

2.2. Gynogenesis and fish rearing

The MC and WCC were sourced from the State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, China. WCC semen was diluted using Hank's solution at a ratio of 1:10. The samples were then placed in 15 cm glass culture dishes and exposed to UV light for 15 min, receiving a total UV dose of between 200 and 300 mJ/cm². Following UV exposure of the WCC sperm, MC eggs were activated and then cultured in water heated to 26–28 °C for 15 min at 3 min post-fertilization (mpf). Embryonic development was monitored stereomicroscopically every 3 h.

MC and GMCJ larvae were raised in two ponds, each measuring 2 × 2 × 1.0 m. During the rearing phase, the larval fish were kept under ambient light conditions, maintaining a pH between 6.0 and 8.0 and a dissolved oxygen content of >5.0 mg/L. Both fish groups were fed artificial feed routinely at 09:00 and 18:00 h. The quantity of food provided gradually increased in proportion to the fish's body weight. The body weight and length of the two fish groups were recorded at the

ages of 2 months, 6 months, and one year.

2.3. Morphological traits and DNA content

Thirty MC and GMCJ, sampled at three developmental stages (2 months, 6 months, and one year old), were randomly selected for morphological and measurable feature analysis (Hu et al., 2023; Wang et al., 2022). Chromosomes were prepared from kidney tissues using the method reported by Wang et al. (Wang et al., 2022). We analyzed chromosome shape and number under a light microscope, photographing fifty metaphase spreads to determine chromosome count. These preparations were examined at 330× magnification using an oil immersion lens.

The DNA content of erythrocytes from WCC, MC, and GMCJ was measured using a flow cytometer (Cell Counter Analyzer, Partec, Germany). Blood samples (1–2 mL) were collected from the caudal vein of each fish using a syringe preloaded with approximately 100 units of sodium heparin. The processing of blood samples followed procedures described in a previous study and were investigated under identical conditions (Liu et al., 2010). The DNA content of MC served as the control. A χ^2 -test, with a Yates correction, was employed to test for deviations from expected values.

2.4. Cold tolerance

2.4.1. Detection of critical thermal minimum

The critical thermal minimum (CT_{min}) is a widely used measure for evaluating cold tolerance in fish (Zhou et al., 2019). CT_{min} is determined by progressively lowering the water temperature and noting the temperature at which the fish losing balance and not actively breathing within 5 s of gill cover. For the CT_{min} assessment, we used two-month-old MC (mean body weight = 15.18 ± 1.53 g, body length = 9.98 ± 0.61 cm) and GMCJ (mean body weight = 15.86 ± 1.34 g, body length = 10.49 ± 0.53 cm). These were acclimated to 26 °C for two weeks before the study in August 2022. Three constant-temperature chambers from Shanghai Yiheng Science Corporation were utilized to control the water temperature. Fish were placed in custom-made cages corresponding to the chambers' design. The CT_{min} measurement involved reducing the water temperature from the initial point by 0.25 °C/min until equilibrium was lost, at which point the fish (n = 10 per group) were removed and weighed. For each trial, we combined ten MC and ten GMCJ (n = 20 total per tank) to minimize random errors. The average survival rates from three replicates per treatment were then calculated.

2.4.2. Acute cold tolerance

Twenty MC and twenty GMCJ specimens, weighing 84.36 ± 9.11 g and measuring 17.69 ± 0.78 cm, were randomly selected and transferred to two identically sized tanks equipped with temperature-adjustable continuous-flow systems (Ningbo Jiangnan Instrument Factory, Ningbo, China). The fish were acclimatized for one week at room temperature (26 °C) and fed commercial pelletized feed twice daily. After this acclimatization period, samples from the MC group (also known as the CG group) were collected. Simultaneously, the water temperature in the GMCJ group's tank was gradually lowered from 26 °C to 14 °C over 1 h, at a rate of 0.2 °C/min. Under low temperature stress of 14 °C for 2 h, Samples from the GMCJ group were collected at this lower temperature. The experimental temperature, set at 14 °C for the temperature was higher than the lowest tolerable temperature (12 °C) that measured. Prior to tissue collection, the fish were euthanized using 90 mg/L MS-222. Three liver tissue samples from each group were preserved in Bouin's fixative for histological analysis. The remaining liver and brain tissues were immersed in RNAlater, maintained at 4 °C for 24 h, and then stored for RNA sequencing (RNA-Seq).

Table 1
Basic information of the transcriptome.

	Samples	Clean reads	Total bases	Total mapped	Q20	GC (%)
Liver	CG1	21,115,192	6.33G	15,931,912 (75.45%)	98.12	45.19
	CG2	20,604,121	6.18G	15,435,082 (74.91%)	98.19	46.44
	CG3	21,338,831	6.4G	15,761,848 (73.86%)	98.09	45.2
	JL1	21,073,901	6.32G	16,672,975 (79.12%)	97.72	46.00
	JL2	20,922,990	6.28G	16,581,783 (79.25%)	98.24	45.47
	JL3	21,242,810	6.37G	16,247,660 (76.49%)	97.97	45.21
Brain	CGB1	20,411,124	6.12G	16,700,090 (81.82%)	97.5	45.13
	CGB2	23,136,801	6.94G	18,943,219 (81.87%)	97.48	45.05
	CGB3	22,268,569	6.68G	18,429,727 (82.76%)	97.77	45.41
	JB1	20,976,848	6.29G	17,191,533 (81.95%)	97.68	44.08
	JB2	21,201,616	6.36G	17,081,199 (80.57%)	97.6	43.69
	JB3	21,122,865	6.34G	16,490,940 (78.07%)	97.78	43.42

2.5. Transmission Electron microscopy

Transmission electron microscopy was employed to analyze the ultrastructure of 6-month-old GMCJ hearts. The heart tissues were first fixed in 2.5% glutaraldehyde, then embedded in Epon 812 using a Leica UC7 ultramicrotome (Heerbrugg, Switzerland). They were subsequently cut into slices 100 nm thick and stained with lead citrate and uranyl acetate. Finally, images of the myocardial ultrastructure were captured using a Hitachi-7650 transmission electron microscope (Hitachi, Tokyo, Japan).

2.6. RNA sequencing and data analysis

RNA extraction was conducted using TRIzol reagent. Total RNA was isolated from two tissue samples, specifically the brain and liver, of GMCJ. The RNA samples were required to meet library construction standards and pass stringent quality checks before use. Library construction and sequencing were performed by Beijing NOVogene Company. From 12 samples, we obtained a total of 2.55×10^8 clean reads (66.61 Gb). The complete clean reads were uploaded to the NCBI Sequence Read Archive (SRA) website under accession number

Table 2
Fertilization rate, hatching rate, survival rate, and water temperature of MC and GMCJ.

	MC	GMCJ
Fertilization rate	87.5%	90.4%
Hatching rate	83.4%	30.6%
Survival rate	72.4%	15.6%
Water temperature	23–25 °C	24–26 °C

PRJNA895734. Basic transcriptome data is detailed in Table 1.

Gene expression levels were quantified by calculating the fragments per kilobase of transcript per million mapped reads (FPKM). To minimize interference from expression noise, expression values from three biological replicates were screened using a mean threshold of 2 standard deviations (SD) for each gene. Differential expression analysis between the two groups was conducted using DESeq2 (version 2.13). The Benjamini-Hochberg method was applied to control the false discovery rate (FDR) and adjust the resulting *p*-values. Genes with adjusted *p* < 0.05 and a threshold normalized absolute log 2-fold change >2 were identified as DEGs.

Data were compiled from seven public databases: NCBI non-redundant protein sequences (Nr), NCBI non-redundant nucleotide sequences (Nt), protein family (Pfam), Clusters of Orthologous Groups of proteins (COG), Swiss-Prot, KEGG Ortholog database, and Gene Ontology (GO).

2.7. Quantitative real-time PCR (qPCR)

Eight DEGs, associated with metabolism and immunity, were random chosen for qRT-PCR analysis as previously described (Gong et al., 2020). The primers used in this study are listed in Table S1.

2.8. Data analysis

SPSS Statistics 19.0 (IBM Corp., NY, USA) was used to analyze the data that were presented as mean $\pm$ standard deviation (SD), and analysis of variance (ANOVA) was used to determine significant difference between MC and GMCJ. *p* < 0.05 represents a significant difference between the two group.

3. Results

3.1. Formation of GMCJ

The GMCJ population for this study was collected in May of both 2022 and 2023. Fig. 1 illustrates the GMCJ production process. To prevent the emergence of the second polar body, which facilitates

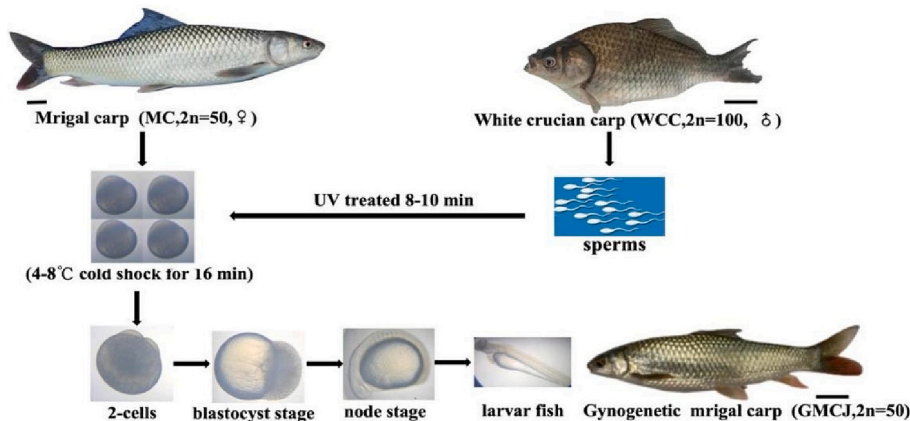


Fig. 1. GMCJ Production Process: This figure illustrates the production of GMCJ using UV-inactivated WCC sperm. Scale bar = 1 cm.

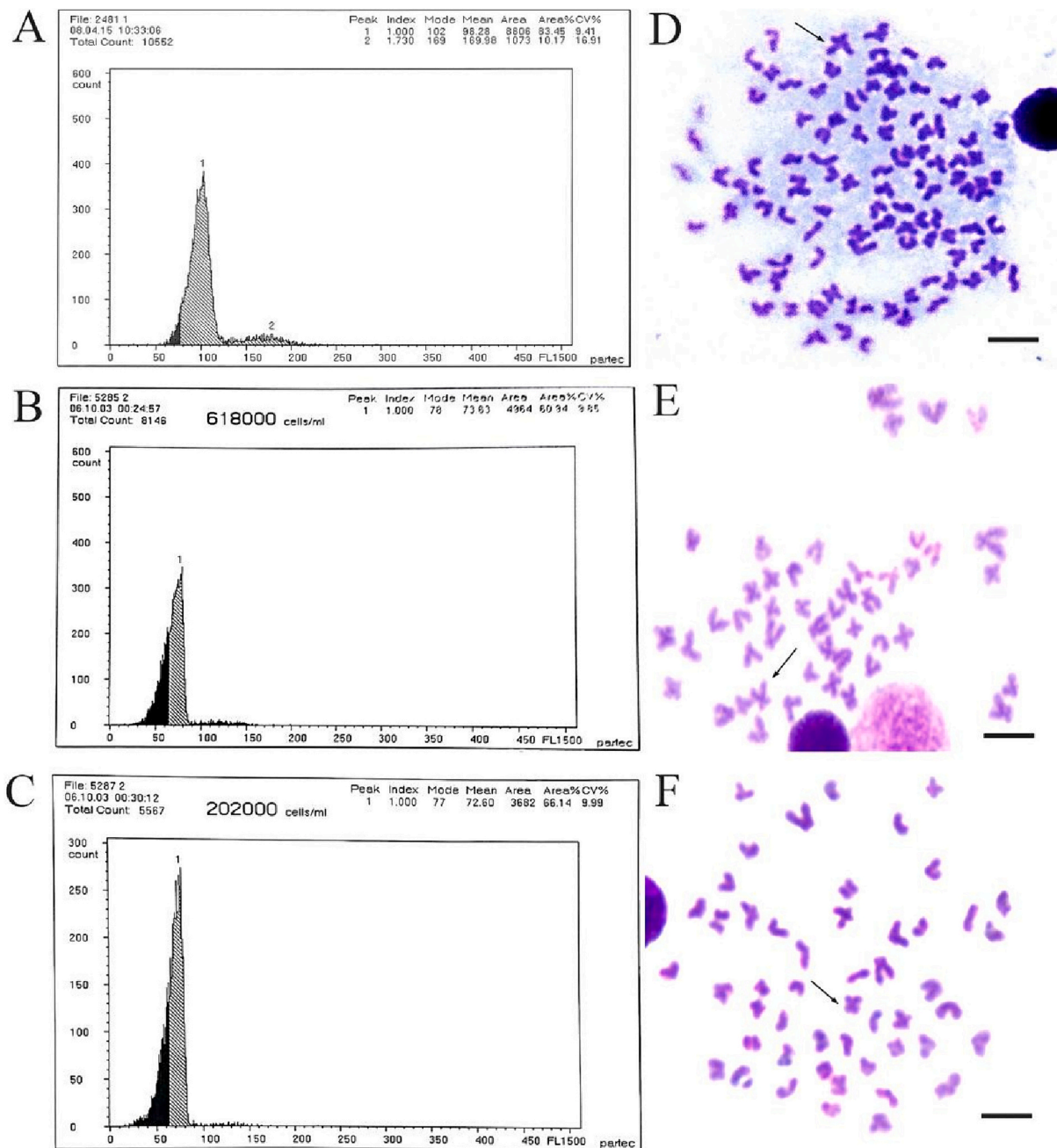


Fig. 2. DNA Content and Chromosome Analysis in WCC, MC, and GMCJ: (A) Average DNA content in WCC (98.28); (B) Average DNA content in MC (73.63); (C) Average DNA content in GMCJ (72.60); (D) 100 chromosomes in CC; (E) 50 chromosomes in MC; (F) 50 chromosomes in GMCJ, with a large pair of submetacentric chromosomes indicated by a red arrow. Scale bar = 3 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chromosomal doubling, the embryos were maintained at temperatures between 4 and 8 °C for 16 min post-fertilization. Approximately 15 to 22 h after fertilization, both MC (self-crossed) and GMCJ embryos hatched (incubation water temperature was 26 °C). The fertilization, hatching, and survival rates for the MC and GMCJ are detailed in Table 2. The GMCJ exhibited hatching and survival rates of 30.6% and 15.6% respectively, significantly lower than those of the MC, which were 83.4% and 72.4% respectively.

3.2. Morphological traits, DNA content, and chromosome numbers

The phenotype of GMCJ closely resembles that of the maternal MC under wild-type conditions. The GMCJ's measurable characteristics,

such as body height/body length (BH/BL), head length/body length (HL/BL), head height/head length (HH/HL), caudal peduncle height/caudal peduncle length (CPH/CPL), and head height/body height (HH/BH), were more similar to those of the maternal MC. Among these characteristics, the number of dorsal fins and lateral scales in GMCJ was similar to MC. The count of anal fins, lateral scales, and abdominal fins showed little variation among the three fish species, as documented in Table S2. Additionally, individuals with the GMCJ genotype demonstrated stability in these countable features.

Flow cytometry was utilized to measure DNA content. The DNA histogram showed average relative fluorescence intensities of 98.28 for WCC, 73.83 for MC, and 72.60 for GMCJ samples, respectively (refer to Fig. 2A, B, and C). The DNA content in GMCJ was comparable to that in

Table 3
Comparison and analysis of the growth rate in MC and GMCJ.

	MC		GMCJ	
	Weight	Body length	Weight	Body length
2 months	15.18 ± 1.53	9.98 ± 0.61	15.86 ± 1.34	10.49 ± 0.53
6 months	73.18 ± 5.46	17.26 ± 0.67	84.36 ± 4.11	17.69 ± 0.78
1 year	–	–	494.05 ± 45.13	30.30 ± 0.96

–:No data recorded, MC fish were perished.

MC ($p > 0.05$). For chromosomal analysis, 50 metaphase phases were examined (see Table S3). The chromosome number was $2n = 100$ for WCC (Fig. 2D) and $2n = 50$ for MC (Fig. 2E). In GMCJ, the largest submetacentric chromosome was present in its metaphase chromosome set (Fig. 2F), with the distribution of chromosome numbers indicating that GMCJ is diploid.

3.3. Cold tolerance and growth of GMCJ

The GMCJ exhibited a faster growth rate compared to MC (Table 3). Specifically, at one year of age, the average body weight and length of GMCJ were 494.05 ± 45 g and 30.30 ± 0.96 cm, respectively. Unlike the MC, which perished during the natural winter (with no data recorded), the GMCJ population survived the winter even when exposed to water temperatures as low as 8 °C. Additionally, the critical thermal minimum (CT_{min}) value for GMCJ was 12.17 ± 0.25 °C, which is lower than that of the MC (13.08 ± 0.35 °C) ($p < 0.05$). This finding suggests that the GMCJ population possesses a greater capacity to withstand low temperatures and endure more severe cold stress.

3.4. Myocardial projection Electron microscopy

Transmission electron microscopy revealed that, in the normal group, myocardial myofilaments were densely packed, and the mitochondria were of uniform size and structurally intact (Fig. 3A). In contrast, the acute hypothermia-treated group displayed increased spacing between myofilaments and mitochondria that were enlarged, deformed, and exhibited cavitation-induced ruptures (Fig. 3B). This suggests that acute hypothermia causes damage to cardiomyocytes and influences the integrity of mitochondria.

3.5. DEGs identification and functional enrichment

In total, 28,078 unigenes were co-expressed in the livers of both MC and GMCJ groups. In the GMCJ, compared to the MC group, 2434 DEGs

were identified, including 1210 upregulated and 1224 downregulated genes. In brain tissue, 44,606 unigenes were coexpressed between the two groups. A total of 2463 genes were upregulated and 3288 were downregulated in the GMCJ (Fig. S1).

GO functional analysis revealed that the top three upregulated DEG clusters in liver tissues were related to oxidation-reduction processes, ribosome biogenesis, and oxidoreductase activity. Conversely, the top three downregulated clusters involved endopeptidase inhibitor activity, enzyme modulator activity, and peptidase inhibitor activity (Fig. 4A and B). In brain tissue, the top three upregulated DEG clusters were regulation of system process, circulatory system process, and transcription factor TFIID complex. The top three downregulated clusters were transmembrane signaling receptor activity, G-protein coupled receptor activity, and receptor activity (Table 4) (Fig. 4C and D).

According to the KEGG database, upregulated genes in liver tissues were highly enriched in ribosome biogenesis in eukaryotes, valine, leucine, and isoleucine degradation, as well as cysteine and methionine metabolism (Fig. 5A). Downregulated genes were predominantly enriched in pathways related to leishmaniasis, systemic lupus erythematosus, and *Staphylococcus aureus* infection (Fig. 5B). For the brain, upregulated DEGs were significantly enriched in pathways associated with oxidative phosphorylation, Parkinson's disease, and circadian rhythms (Fig. 5C), while downregulated genes were mainly enriched in neuroactive ligand-receptor interactions, protein digestion and absorption, and ECM-receptor interactions (Fig. 5D).

Using qPCR, the trends of the expression levels of 8 genes detected by qPCR were the same as those obtained by RNA-seq data analysis, indicated the reliability of the RNA-seq data for the analysis of differentially expressed genes in this study (Fig. S2).

3.6. Brain-liver Axis analysis of DEGs

To assess the impact of acute cold stress on expression levels within the brain-liver axis, several pathways and genes associated with disease, feeding, and metabolism were identified in both brain and liver tissues (Fig. 6). For instance, in the brain and liver, the herpes simplex infection pathway showed 29 downregulated and 7 upregulated genes, and 51 downregulated and 9 upregulated genes, respectively. The PI3K-Akt pathway exhibited 67 downregulated and 11 upregulated genes in the brain, and 21 downregulated and 7 upregulated in the liver. Similarly, the glucagon pathway had 9 downregulated and 8 upregulated genes in the brain, and 6 downregulated and 8 upregulated genes in the liver (Table S4).

Notably, certain genes involved in immunity and metabolism were identified. For example, the top 15 and bottom 15 genes with the highest

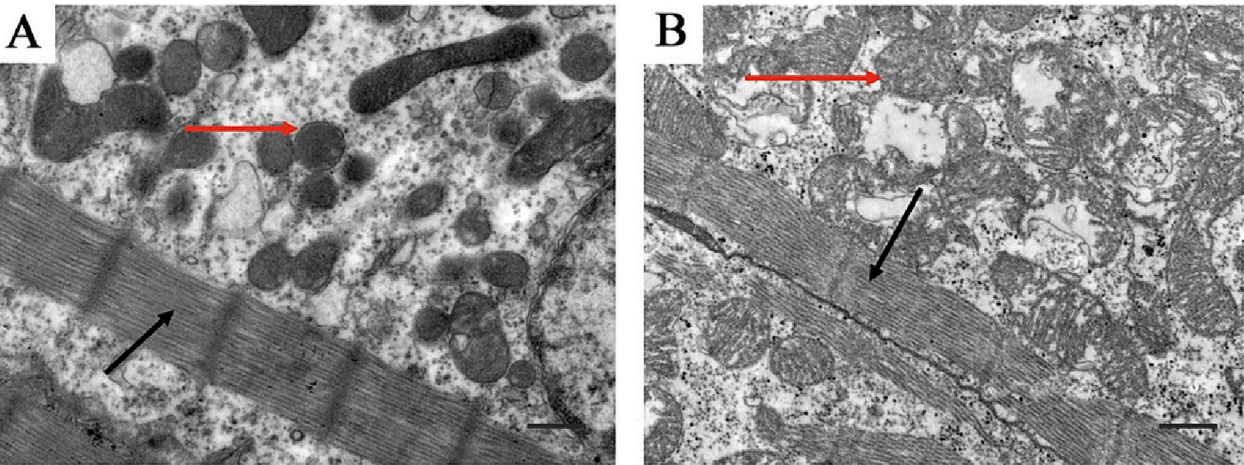


Fig. 3. Heart Tissue Microstructure in MC and GMCJ After Acute Stress: (A) Myocardial myofilaments in the normal group; (B) Myocardial myofilaments in the acute hypothermia group. Scale bar = 2 μm.

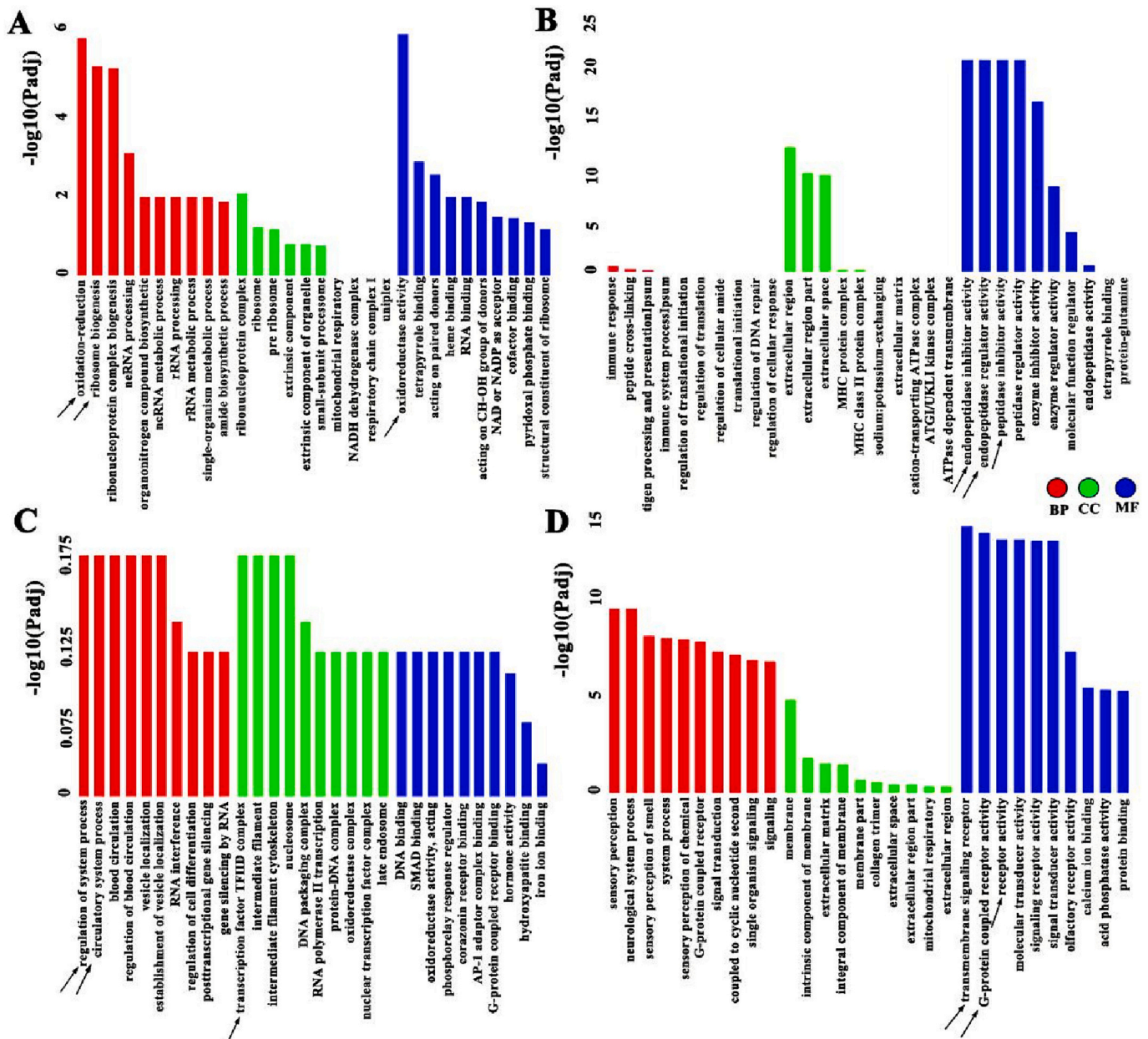


Fig. 4. GO Enrichment Analysis in Liver and Brain Transcriptomes: (A) Up-regulated genes in liver; (B) Down-regulated genes in liver; (C) Up-regulated genes in brain; (D) Down-regulated genes in brain. The horizontal axis represents GO terms, and the vertical axis shows the $-\log_{10}$ p-value.

and lowest expression levels in brain and liver, were energy, nucleotide and glycolysis metabolism-related enzymes, cell signal transduction-related factor, and immune-response genes (Table S5). Furthermore, the heat shock protein genes (HSP) and fatty acid binding protein genes (FABP) were expressed in both the brain and liver. These genes, directly regulate or provide negative feedback to brain or liver functions, including ingestion genes (e.g., NPY, leptin, Cholecystokinin A receptor, somatostatin), metabolic genes (e.g., PPAR γ , glycogen synthase, PGC-1 α), and immunity genes (e.g., complement component 3, fibronectin 1, LPAR2) (Fig. 6 and Table S6).

4. Discussion

In this study, a new strain of gynogenetic mrigal carp was developed using UV-inactivated WCC sperm. We observed that the GMCJ population endured the winter and adapted to low-temperature waters. Notably, the critical thermal minimum (CT_{min}) value of GMCJ was

lower than that of MC and marginally higher than GMC, indicating that gynogenesis in mrigal carp enhanced cold tolerance through the process of artificial gynogenesis (Li et al., 2023). This enhancement may be attributed to the insertion of DNA fragments from the parent species, coupled with the inherent greater low-temperature tolerance of common carp compared to white crucian carp (Golovanov and Smirnov, 2007). Additionally, cold acclimation is beneficial for fish to physiologically adapt to colder environments, thereby increasing their survival rates (Mao et al., 2020; Yan et al., 2005). Interestingly, the GMCJ fish exhibited faster growth than MC individuals, possibly due to the insertion of paternal DNA fragments inducing hybridization, as shown in Table 2.

The study also explored changes in apparatus and tissue structure as fish acclimatize to cold stress. Histopathological damage in the brain, gills, liver, heart, and muscle tissues has been observed in many fish species following exposure to cold stress (Phrompanya et al., 2021; Sinha et al., 2020; Song et al., 2019). For instance, a study revealed that cold-

Table 4
Summary of GO annotations of significantly subcategories.

Term Type (NO.)	Term description	GO ID	Number of DEGs
Biological_process	Oxidation-reduction process	GO:0055114	139
	Single-organism biosynthetic process	GO:0044711	109
Cellular_component	Extracellular region	GO:0005576	156
	Extracellular region part	GO:0044421	52
	Extracellular space	GO:0005615	51
Molecular_function	Peptidase inhibitor activity	GO:0030414	46
	Peptidase regulator activity	GO:0061134	46
	Endopeptidase inhibitor activity	GO:0004866	43
	Endopeptidase regulator activity	GO:0061135	43
	Enzyme inhibitor activity	GO:0004857	57
	Tetrapyrrole binding	GO:004690	40

acclimated rainbow trout (*Oncorhynchus mykiss*) displayed a hypertrophic response in the heart, accompanied by an increase in connective tissue. In our study, myocardial myofilament spacing was enlarged in the acute hypothermia group, and the mitochondria appeared enlarged, deformed, and ruptured due to cavitation. These alterations may be a response to the increased hemodynamic load on the ventricles, which results from heightened blood viscosity.

Recently, transcriptomics has gained popularity as a method for investigating the molecular mechanisms underlying cold stress. The liver, being the primary site for food metabolism in fish, plays a crucial role in synthesizing proteins, lipids, and carbohydrates(Zhang et al., 2023). AMPK is pivotal in cellular energy metabolism, acting as a critical regulator of lipid, protein, and carbohydrate synthesis and conversion, thereby maintaining equilibrium in intracellular energy metabolism (McFadden and Corl, 2009). Previous studies have shown that AMPK expression was downregulated in the livers of tilapia exposed to low temperatures(Huang et al., 2022). However, in this study, AMPK expression was activated in GMCJ, indicating an increased energy expenditure by GMCJ to sustain body metabolism under cold stress conditions.

Purines serve a protective role in organs and tissues after stress or injury and have been found to significantly contribute to the metabolic and immune responses to cold stress(Jiao et al., 2020; Kuo et al., 2022). We observed a notable upregulation in the expression of genes related to purine metabolism. Additionally, the p53 signaling pathway was significantly enriched in the livers of the giant yellow croaker (*Larimichthys crocea*) and large yellow croaker after cold stress exposure (Qian and Xue, 2016). This pathway was also active in GMCJ, suggesting that cold stress induces the activation of the p53 pathway and leads to cell apoptosis.

The hypothalamus is a central regulator in response to environmental temperature fluctuations(Phrompanya et al., 2021). In grass carp (*Ctenopharyngodon idellus*), we observed significant enrichment of the MAPK signaling pathway in the brain during exposure to severe cold (4 °C for 4 h)(Shi et al., 2020). In our study, within the MAPK signaling pathway of GMCJ, 14 genes were upregulated, and 33 genes were downregulated. High water temperatures led to reduced mRNA expression levels of SOD, CAT, GPx, and Bcl2, and increased mRNA expression of apoptosis genes (*caspase 3*, *Bax*), heat shock genes (*Hsp70* and *Hsp90*), and ER-stress genes (*grp78*, *atf6*, and *ire1*) in the fish brain (Topal et al., 2021). In GMCJ, cold water temperatures also elevated the expression levels of ATP synthase genes (*F-type ATPase*), ribosomal protein genes (*RPL24* and *RPS21*), and apoptosis genes (*caspase 3*). This could be attributed to the brain's role in maintaining body homeostasis, with mitochondrial swelling being the most prominent subcellular change following cold exposure(Liu et al., 2023). The enriched DEGs

were categorized into two groups: amino acid metabolism and biosynthesis, which included the biosynthesis of lysine in the brain, and the metabolism of arginine and proline(Liu et al., 2020). Additionally, neuroactive ligand-receptor interactions, protein digestion and absorption, and ECM-receptor interactions were active in GMCJ. These pathways are essential in regulating cell behavior and influencing neuronal function, potentially contributing to intercellular communication, cell proliferation, adhesion, migration, and the regulation of gene expression (Nersisyan et al., 2021; Wei et al., 2020).

The liver and brain are interconnected via the parasympathetic and sympathetic nervous systems, enabling bidirectional communication and allowing the central nervous system (CNS) to regulate liver function and overall body homeostasis(Mizuno and Ueno, 2017). Afferent fibers sense changes in the blood composition within the portal vein and relay this information to the CNS, while splanchnic and vagal nerve sympathetic fibers extend into the liver parenchyma and hepatic lobules(Yeo et al., 2023). Research on Tiger Barb (*Puntius tetrazona*) reveals significant changes in heat shock protein (HSP) gene expression during acute cold stress(Liu et al., 2020). In studies of Tiger Barb and Spotted Green Pufferfish, significant changes in fatty acid-binding protein (FABP) gene expression were observed under cold stress(Thirumaran and Wright, 2014). In contrast, the HSPs and FABPs were activated in both brain and liver in CMGJ. The genes underscore their pivotal roles in the response of fish to environmental challenges, thereby substantiating their significance within the stress response mechanisms of fish. The peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor, pairs with retinoid X receptor α to form a heterodimer, which then initiates the activation of genes associated with fat storage and metabolism (Bocher et al., 2002). Prior research has shown that excessive lipid accumulation in the liver, due to increased PPARγ expression, signals the brain through the vagal afferent pathway. This leads to the activation of the sympathetic nervous system (SNS) and a rise in energy expenditure(Uno et al., 2006). Our study discovered that acute cold stress similarly elevates PPARγ expression in the liver of GMCJ. This may be a response to the conversion of increased energy expenditure by GMCJ into heat and the activation of metabolism-related genes. Other studies have indicated that leptin-deficient mice exhibit significantly fewer sympathetic axons in the liver; however, the loss of sympathetic innervation can be counteracted by caloric restriction or leptin supplementation(Liu et al., 2021). In our study, we observed a reduction in leptin gene expression in the GMCJ livers, potentially leading to rapid heat loss and lower energy reserves. The expression of NRK gene was enhanced under high temperature conditions in grass carp. This gene also showed an enhanced trend in the brain of GMCJ, which may be related to the regulation of lipid metabolism and immune system(Xie et al., 2023; Zhang et al., 2022).

Acute temperature stress presents a significant challenge to fish farming, leading to reduced immune defense and disease resistance (Abram et al., 2017). In various fish species, pathways associated with *Staphylococcus aureus* infection, leishmaniasis, and pertussis have been identified, showing that cold stress adversely impacts immunity and health(Han et al., 2022; Sun et al., 2019). In the fish brain, DEGs can be categorized into circadian regulatory and circadian terms. Circadian systems are critical in regulating cellular and physiological processes in organisms, and disturbances in these systems can lead to pathological outcomes and increased disease risk(Chen et al., 2018; Liu et al., 2020). In this context, the GMCJ showed a downregulation of genes in these pathways, indicating a marked decrease in disease resistance and immune protection in response to cold exposure.

In summary, transcriptomic analysis of the liver and brain tissues of gynogenetic mrigal carp under cold stress identified DEGs and key pathways involved in regulatory processes. These findings include a disrupted immune system, reduced fatty acid production, and enhanced lipid metabolism. Numerous pathways and genes related to the nervous-endocrine system were activated, suggesting coordination of the brain-liver axis to manage acute stress. This research lays the groundwork

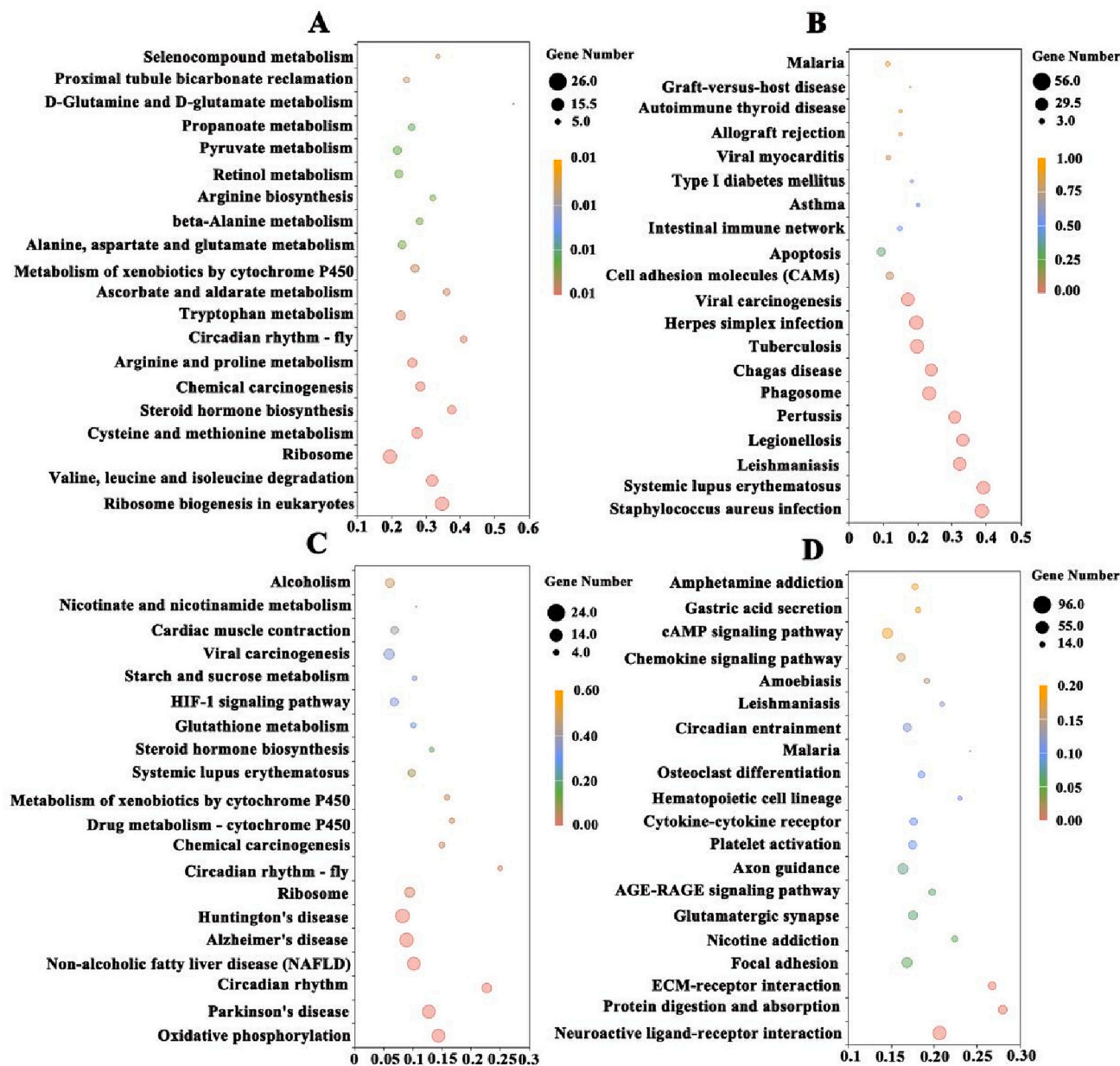


Fig. 5. KEGG Enrichment Analysis in Liver and Brain Transcriptomes: (A) Up-regulated genes in liver; (B) Down-regulated genes in liver; (C) Up-regulated genes in brain; (D) Down-regulated genes in brain.

for further investigations into the responses of mrigal carp to high-temperature stress.

CRediT authorship contribution statement

Jisen Su: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Wuhui Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Hongqing Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Zexun Zhou:** Conceptualization, Data curation, Formal analysis, Funding acquisition,

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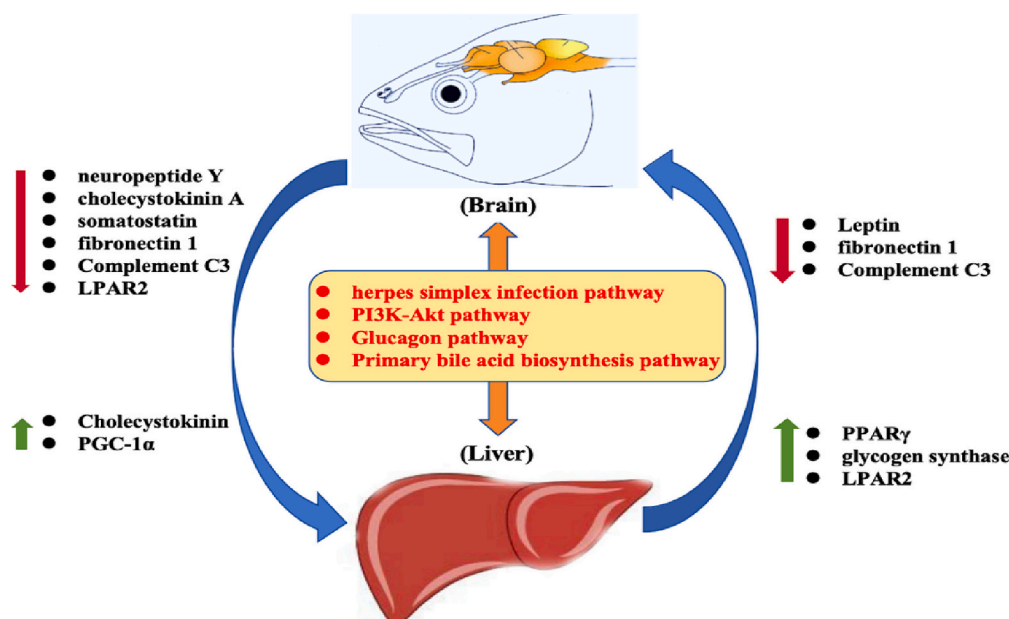


Fig. 6. Brain-Liver Axis Pathways and Genes in GM CJ Under Acute Cold Stress: This figure identifies pathways and genes involved in the brain-liver axis response of GM CJ to acute cold stress.

Zhang: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Yi Zhou:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Qinbo Qin:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Shaojun Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2024.740908>.

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