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Molecular Characterization and Response of Silver Carp (*Hypophthalmichthys molitrix*) *GLUT1* under Hypoxia Stress

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Abstract: As an important freshwater species with economic and ecological benefits, silver carp (*Hypophthalmichthys molitrix*) exhibits poor tolerance to hypoxia. Glucose transporters (GLUTs) are core membrane proteins that transport glucose to tissues and regulate essential life activities. Its expression is regulated by HIF-1α and cells in hypoxic conditions to maintain energy demand through GLUTs inducing enhanced glucose transport. We cloned *H. molitrix glut1* (*SLC2A1*) and analyzed its sequence using bioinformatics tools. The *glut1* cDNA was 2104 base pairs long and encoded a 490 amino acid protein. Phylogenetic analysis revealed that sliver carp *glut1* is evolutionarily conserved and exhibited the highest sequence similarity with *Ctenopharyngodon idella glut1*. *Glut1* expression was the highest and lowest in the gills and liver, respectively. Hypoxic stress significantly increased *glut1* expression in the brain ($p < 0.05$); in the gills, it was the highest and lowest in the semi-asphyxia and asphyxia groups, respectively; in the liver, it was significantly higher under hypoxia than that of the normoxia group; and in the heart, it was significantly higher in the floating head, semi-asphyxia, and asphyxia groups than in the normoxia group ($p < 0.05$). The proposed mechanism may thus provide the basis for elucidating the molecular basis of silver carp's hypoxia stress response mediated by *glut1*.

Keywords: glucose transporter 1; *Hypophthalmichthys molitrix*; hypoxia stress; glucose metabolism

Key Contribution: Studying the expression patterns of the *glut1* gene in eight different tissues and exploring the tissue response of liver, brain, heart, and gills under hypoxic stress are the objectives of this study. These results contribute to further studying the response mechanism of the *glut1* gene to hypoxic stress.

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1. Introduction

Silver carp, *Hypophthalmichthys molitrix*, is one of the main freshwater fish in China, ranking second in terms of aquaculture yield in 2022, playing an important role in China's freshwater aquaculture industry [1]. Silver carp have long been an important source of protein for the general public in China due to its excellent quality, low price, rich nutrition, and sufficient supply. With the development of industrialization, excessive nitrogen and phosphorus in the water can lead to the outbreak of algae. As a filter-feeding fish, silver carp mainly feed on phytoplankton, thereby inhibiting the outbreak of cyanobacteria and regulating water quality [2–4]. Silver carp have been introduced in over 88 countries to provide ecological benefits [5].

Dissolved oxygen (DO) is closely related to the survival and growth of fish. When the water dissolved oxygen is lower than 2 mg/L, the fish lack oxygen and their reproduction is adversely affected [6]. Silver carp have poor tolerance to low oxygen and are prone to

death due to hypoxia during high-density breeding in summer. Therefore, the response mechanism of silver carp to hypoxia should be investigated.

Reactive oxygen species (ROS) are reactive oxygen radicals that are naturally produced by oxygen metabolism. During sustained hypoxia, ROS levels will sharply increase, leading to an increase in oxidative stress and cell apoptosis [7–9]. It has been reported that hypoxia can produce physiological and biochemical changes in the gills, liver, brain and heart of silver carp [10–12]. Under hypoxic stress, the blood indicators (red blood cells, white blood cells, hemoglobin) and antioxidant oxidative stress system (GSH-PX, CAT, SOD) of tissues are activated, indicating that the body improves oxygen delivery by increasing hemoglobin concentrations and responds to the damage caused by hypoxic stress by increasing the antioxidant capacity. Studies have shown that tissue sections indicate severe hypoxic stress may cause tissue and organ damage and even death [12]. The GO enrichment analysis of differential proteins in the liver and brain of silver carp under hypoxic stress shows that the glycolipid transporter is one of the most important components [10].

GLUT1, known as red blood cell glucose transporter protein, is a member of the GLUT family and was identified by Zhong et al. in crucian carp embryonic cells using suppression subtractive hybridization technology. It is encoded by the SLC2A1 (solute carrier family 2-facilitated glucose transporter member 1) gene, and its expression is regulated by transcription factors, such as *HIF-1 α* and *c-Myc* [13]. Under hypoxia, the DNA binding site of *HIF-1α* is activated by *HIF-L*, which increases *glut1* expression, thus enhancing glucose uptake and cell metabolism, improving tolerance to hypoxia [14]. *C-Myc* promotes the transcription of *glut1* and increases the glucose uptake of cells under hypoxic conditions [15]. After reoxygenation, *c-Myc* inhibits *HIF-1* expression by regulating mitochondrial coding genes, thereby reducing the transcription of *glut1*. Previous studies have shown that GLUTs are induced to enhance glucose transport and maintain cellular energy requirements [16–18]. However, studies on the regulation or tissue-specific expression pattern of *glut1* under hypoxic stress are still limited [19,20]. Therefore, it is economically and ecologically important to explore the response mechanism of silver carp to *glut1* under hypoxic stress.

In this study, *glut1* was cloned and identified, and the specific expression patterns of *glut1* in different tissues of silver carp were analyzed. At the same time, the transcriptional response of *glut1* in the brain, gill, liver and heart tissues of silver carp under different oxygen concentrations was studied.

2. Materials and Methods

2.1. Experimental Fish

The fish were cultured at the Silver Carp Genetics and Breeding Center of the Chinese Academy of Fisheries, Yangtze River Research Institute, Ministry of Agriculture and Rural Affairs (Jingzhou, Hubei, China). Before the experiment, in a recirculating freshwater system equipped with a water tank, the fish were temporarily housed for two weeks (water temperature 23.5 ± 0.5 °C, dissolved oxygen > 6 mg/L). The fish were fed with pellet feeds twice daily and stop feeding 24 h before the start of the experiment.

2.2. Experimental Design

After the adaption conditions, 120 silver carp with a body length of 22.1 ± 0.8 cm and a weight of 200 ± 12.4 g were selected and randomly divided into a normoxia group (normal oxygen group), floating head group, semi-asphyxia group, and asphyxia group (10 fish/tank, 3 tank/group).

The temperature of the water in the tanks was maintained at 23.0 ± 0.53 °C. The DO level (6.42 ± 0.3 mg/L) in the normoxia tanks was the same as that of the water used for the experiment, and the tanks were sealed with a plastic film. During the experiment, we used portable multi-parameter instruments (HACH, HQ40d, Loveland, CO, USA) every 20 min to monitor the DO values, and the values were adjusted as required to maintain the hypoxia status. In the floating head group (DO level: 0.76 ± 0.03 mg/L), five fish were randomly

sampled after all fish exhibited the floating head phenomenon (after 1.5 h of hypoxic treatment). In the semi-asphyxia group (DO level: 0.58 ± 0.06 mg/L), after half of the fish had died (after 2 h of hypoxic treatment), the alive carp were collected as samples. In the asphyxia group (DO level: 0.53 ± 0.01 mg/L), after all fish had died (after 2.5 h of hypoxic treatment), five were randomly selected from each tank as the asphyxia group samples. Before sampling, the fish were anesthetized using 100 ppm tricaine methane sulfonate (MS-222, Sigma, Livonia, MI, USA), and the fish entered an anesthesia state for approximately two minutes. Liver, brain, heart and gill tissues of the silver carp were collected from the hypoxia experimental groups. Each processing group completed sampling within 15 min. The tissue samples were placed into separate cryopreservation tubes, quick-frozen in liquid nitrogen, and stored at −80 °C in an ultra-low-temperature refrigerator until use. All experimental protocols on silver carp for this study were in accordance with the guiding principles approved by the Chinese Academy of Fishery Sciences (Wuhan, China), Yangtze River Fisheries Research Institute Animal Care.

2.3. Total RNA Extraction and cDNA Synthesis

Eight different tissues (brain, gill, heart, intestine, liver, kidney, muscle, and spleen) collected at normal oxygen levels were used in the tissue expression experiments. Four different tissues (brain, gill, heart, liver) collected from three hypoxia treatment groups were used to analyze the response of tissue under hypoxia.

Trizol (Invitrogen, Waltham, MA, USA) was used to extract the total RNA. For quality, to determine the purity and concentration of RNA, an NanoPhotometer® NP80 (IMPLEN, Munchen, Germany) was used, and a 1% agarose gel electrophoresis was used to determine the integrity of the total RNA. A reverse transcription of the RNA was performed to generate cDNA using a HiScript cDNA Synthesis Kit (Nanjing Vazyme Biotechnology Co., Ltd., Nanjing, China), and the cDNA was stored at −20 °C until further experiments.

2.4. Glut1 Cloning and Sequencing

The cDNA and first strand of the rapid amplification of cDNA ends (RACE) cDNA were synthesized using high-quality RNA as the template and a Goldenstar TM RT6 cDNA Synthesis Kit (Beijing Qingke Biology Co., Ltd., Beijing, China) and SMARTer® RACE cDNA 5′/3′ Kit (Dalian, Takara), respectively, according to the manufacturer's instructions, and the products were stored at −20 °C. Next, we designed the core segment primers using the primer Premier 5.0 according to the nucleotide sequence of *glut1* in the silver carp transcriptome database (BioProject ID: PRJNA705843) (Table 1). PCR was performed using the cDNA reverse transcribed from the RNA extracted from the silver carp gill tissue and used as a template. The reaction volume was 25 μL, and the reaction conditions were as follows: pre-denaturation at 98 °C for 2 min; 35 cycles of 98 °C for 10 s, 65 °C for 10 s, and 72 °C for 20 s; final extension at 72 °C for 2 min; and incubation at 4 °C for 10 min. The amplicons were subjected to 1% agarose gel electrophoresis and sent to Tianyihuiyuan Biotech Company (Wuhan, Hube, China) for sequencing. The sequencing results were splintered and compared with the *glut1* sequence present in the transcriptome library. Next, we designed 3′-RACE-specific primers based on the sequenced target fragment. The 3′-RACE cDNA was used as the template to amplify the 3′-end of the target fragment using a SMARTer® RACE kit (Takara Co., Ltd., Dalian, China) as per the manufacturer's instructions. Primer GSP1 and connector primer UPM (Table 1) were used for the initial round of polymerase chain reaction (PCR), and the reaction conditions were as follows: an initial denaturation step at 94 °C for 3 min, followed by 25 cycles consisting of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 90 s. Subsequently, an additional 25 cycles were performed with denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 90 s. The PCR reaction was concluded with a final extension step at 72 °C for 10 min. Next, the 5 μL first-round PCR product was diluted 50 times, and the 2 μL diluted product was used for nested PCR. GSP2 and UPM SHORT (RACE primer) primers were used for this step. The reaction conditions were as

follows: 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 90 s; and a final extension at 72 °C for 10 min. The amplicons were subjected to gel electrophoresis and purified using a DNA gel purification kit (Takara Co., Ltd., Dalian, China). The purified product was cloned into a pMD-18T vector (Takara Co., Ltd., Dalian, China) and transformed into *Escherichia coli* DH5α competent cells. The positive monoclonal strain was selected for sequence verification.

Table 1. Sequences of primers used in this study.

Primer Name	Primer Sequence (5′–3′)	Function
Glut1-1	F: CCCTTGGTTTCTGGGACTT R: ACTGGACGGTTCGTGGTAG	ORF amplification
Glut1-3GSP1	ACTATCGGAGCTGGAGTCGTCA	3RACE
Glut1-3GSP2	CTGGACCGCAAACTTTATCGTG	3RACE
UPM	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	
UPM Short	CTAATACGACTCACTATAGGGC	
Glut1-qPCR-F	TTGCCTTGCTGGAGAAATACG	qPCR
Glut1-qPCR-R	CCCACGATAAAGTTTGCGGTC	qPCR
β-actin-F	GAACCCCAAAGCCAACAG	qPCR
β-actin-R	CAGAGTCCATCACGATACCAG	qPCR

2.5. Bioinformatics Analysis of glut1

To obtain the *glut1* cDNA sequences, positive colonies of TA clones were sequenced and assembled. The ORF finder (https://www.ncbi.nlm.nih.gov/orffinder, accessed on 2 September 2022) was used to deduce the *glut1* open reading frame (ORF) and its coding sequence of amino acids. NCBI BLAST was used to analyze its homologous sequence (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 8 September 2022). ProtParam (https://web.expasy.org/protparam/, accessed on 10 September 2022) was used to predict the physical and chemical properties of protein, and we identified the protein isoelectric point (pI) and relative molecular mass. NetPhos 3.1 was used to identify phosphorylation sites (http://www.cbs.dtu.dk/services/NetPhos/, accessed on 20 September 2022). Protein glycosylation sites were predicted using NGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/, accessed on 23 September 2022). A phylogenetic tree was constructed using the neighbor connection (NJ) method, and 1000 guided replicas were performed using MEGA 11 (MEGA Limited, Auckland, New Zealand).

2.6. Fluorescence Quantitative PCR (qPCR) Analysis

Specific primers (Table 1) were designed to investigate the expression of *glut1* in these tissues (*glut1*-qPCR-F, *glut1*-qPCR-R); *β-actin* was used as the internal control. The reaction mixture was as follows: 10.0 μL of 2 × ChamQ Universal SYBR qPCR Master Mix, 0.4 μL of primer 1 (10 μmol/L), 0.4 μL of primer 2 (10 μmol/L), 0.8 μL of template cDNA, and 8.4 μL of ddH_2O. The reaction mixture was centrifuged, added to a 96-well plate, and placed in a fluorescence quantitative PCR to determine the relative expression levels of the gene in different tissues. Each sample had three replicates. The reaction conditions were as follows: 95 °C for 5 min; 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s; 60 °C for 1 min; and 95 °C for 15 s.

2.7. Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA), with $p > 0.05$ considered statistically significant. GraphPad Prism 9.0 was used for figure design.

3. Results

3.1. Glut1 Sequence Analysis

The *glut1* cDNA sequence was 2104 base pairs (bp) in length, contained a 1473 bp ORF encoding 490 amino acids, a 75 bp 5′-untranslated region (UTR), and a 556 bp 3′-UTR

(Figure 1, Table S1). The relative molecular weight of the *glut1* protein was 53.92 kD, and the predicted pI was 8.47 as per the ProtParam software; the protein contained 34 and 37 strongly acidic (Asp + Glu) and basic amino acids (Arg + Lys), respectively. The amino acid sequence included 22 serine (Ser), 10 threonine (Thr), and 4 tyrosine (Tyr) phosphorylation sites. The instability index (II) of *glut1* was 36.36, the average hydrophilic index (GRAVY) was 0.487, and the fat index was 101.69, which indicated that the protein was stable and hydrophobic.

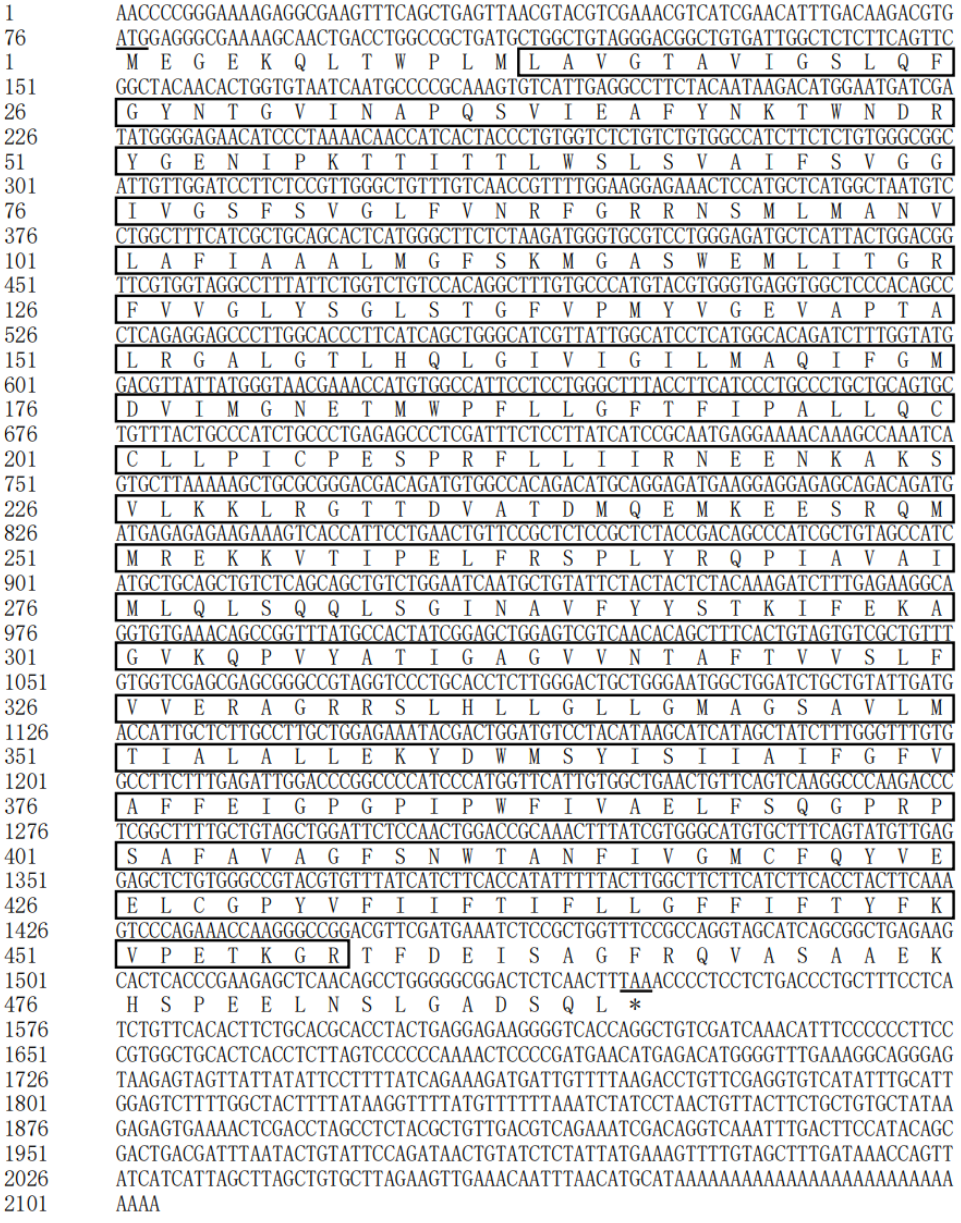

Figure 1. Full-length cDNA of *glut1* and the amino acid sequence of *glut1*. Codons of initiation (ATG) and termination (TAA) are highlighted; the MFS structural domain is also shown.

3.2. Homology and Phylogenetic Tree Analysis of Silver Carp glut1

Homology analysis sequence alignment of the amino acid sequences of *glut1* in different species showed that the amino acid sequences of silver carp *glut1* were highly homologous with those of other species, and this sequence was relatively conserved (Table 2, Figure 2). The *glut1* phylogenetic tree (Figure 3) revealed that silver carp first clustered with the *Megalobrama* into a small branch, and then clustered into a large branch with other fish, indicating that the silver carp had the closest genetic relationship with *Ctenopharyngodon*

idella; finally, this branch clustered into a branch with mammals. Silver carp exhibited the maximum distance from insects (fruit flies), followed by mammals (humans, mice, and sheep). These results confirmed that the *glut1* sequence was highly conserved.

Table 2. Homology analysis of the amino acid sequence of *glut1* from different species.

Species	Accession Number	Sequence Identity
Megalobrama amblycephala	KM977633.1	97.3%
Ctenopharyngodon idella	MG818478.1	96.7%
Sinocyclocheilus anshuiensis	XP_016312675.1	94.7%
Anabarilius grahami	ROL50799.1	94.4%
Labeo rohita	XP_050968139.1	93.7%
Onychostoma macrolepis	KAF4112506.1	93.3%
Pimephales promelas	XP_039548111.1	92.9%
Myxocyprinus asiaticus	XP_051567510.1	91.8%
Danio rerio	XP_002662574.1	91.2%
Cyprinus carpio	AAF75683.1	89.4%
Salmo trutta	XP_029550223.1	87.2%
Oncorhynchus mykiss	AAF75681.1	86.8%
Oreochromis niloticus	NP_001266656.1	81.8%
Sparus aurata	XP_030275602.1	81.5%
Gadus morhua	XP_030230417.1	81.4%
Mus musculus	NP_035530.2	80.3%
Homo sapiens	NP_006507.2	79.9%
Ovis aries	XP_027824429.1	79.5%
Gallus gallus	AAB02037.1	78.8%
Drosophila melanogaster	NP_001097467.1	46%

3.3. Expression Pattern of glut1 in Different Tissues of Silver Carp

We performed qPCR to determine the expression of *glut1* in the brain, gill, heart, intestine, liver, kidney, muscle, and spleen tissues of silver carp (Figure 4). The expression of *glut1* was detected in all eight tissues, with the highest level in the gill tissue, followed by the spleen tissue, and the lowest in the liver tissue compared with other tissues. The significance difference analysis did not reveal a significant difference in *glut1* expression in the intestine, kidney, muscle, and heart tissues ($p > 0.05$). Compared with brain tissue, significant differences in *glut1* expression in the other six tissues were not observed ($p < 0.05$), except in heart tissue.

3.4. Analysis of glut1 Expression in Tissues under Different Oxygen Concentrations

Changes in *glut1* expression in silver carp tissues under different oxygen concentrations are shown in Figure 5. *glut1* expression was the lowest and relatively stable in all tissues in the normoxia group. Moreover, *glut1* expression in the brain tissue was highest in the floating head group compared with the normoxia group ($p < 0.05$). Furthermore, with the decrease in oxygen concentration, *glut1* expression decreased, but it was significantly higher than the normoxia group ($p < 0.05$). In the case of gill tissue, *glut1* expression was the highest in the semi-asphyxia group and was lowest in the asphyxia group as the oxygen concentration decreased compared with the normoxia group ($p < 0.05$). Throughout the entire experimental process, the expression of *glut1* in the liver tissues of the three hypoxic treatment groups was significantly higher than the normoxic group. The expression of *glut1* in the heart tissue increased with hypoxic stress, and the floating head and semi-asphyxia groups.

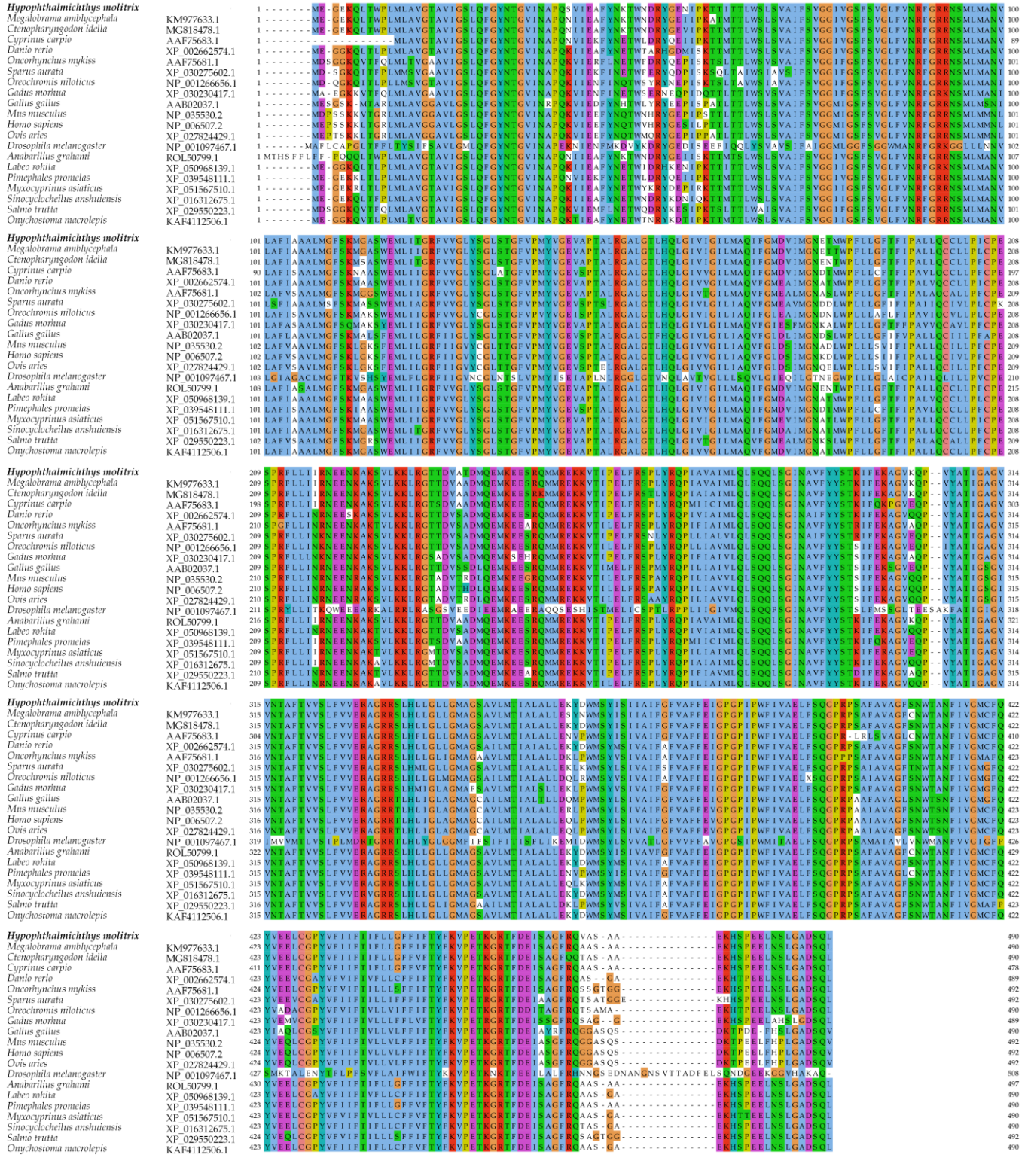

Figure 2. Amino acid sequence alignment of *glut1* from different species. The accession number of the GenBank is displayed on the left side of the sequence.

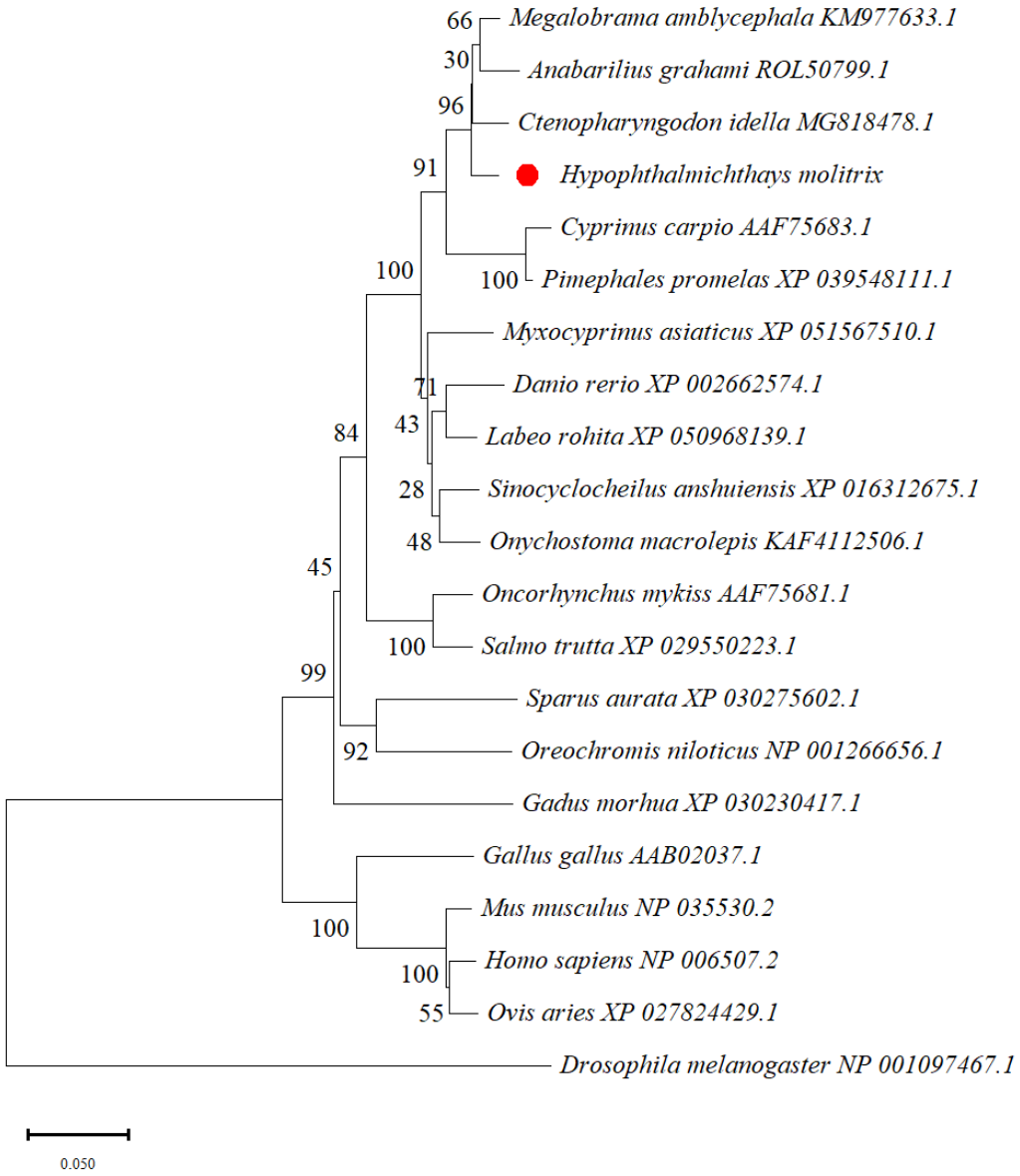

Figure 3. Neighbor-joining phylogenetic tree of *glut1* from silver carp and other species constructed using MEGA 11. The red dot represents the *glut1* gene of silver carp. The GenBank accession numbers of the protein sequences used for analysis are as follows: *Megalobrama amblycephala* (KM977633.1); *Anabarilius grahami* (ROL50799.1); *Ctenopharyngodon idella* (MG818478.1); *Cyprinus carpio* (AAF75683.1); *Pimephales promelas* (XP_039548111.1); *Myxocyprinus asiaticus* (XP_051567510.1); *Danio rerio* (XP_002662574.1); *Labeo rohita* (XP_050968139.1); *Sinocyclocheilus anshuiensis* (XP_016312675.1); *Onychostoma macrolepis* (KAF4112506.1). *Oncorhynchus mykiss* (AAF75681.1); *Salmo trutta* (XP_029550223.1); *Sparus aurata* (XP_030275602.1); *Oreochromis niloticus* (NP_001266656.1); *Gadus morhua* (XP_030230417.1); *Gallus gallus* (AAB02037.1); *Mus musculus* (NP_035530.2); *Homo sapiens* (NP_006507.2); *Ovis aries* (XP_027824429.1); *Drosophila melanogaster* (NP_001097467.1).

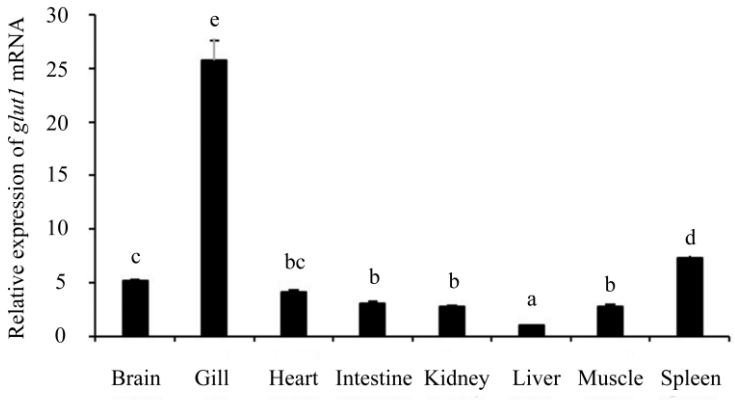

Figure 4. *Glut1* expression in different tissues of silver carp. β-actin was the positive control. Data were shown as mean ± SE (n = 3). The data denoted with different lowercase letters indicate a significant difference ($p < 0.05$).

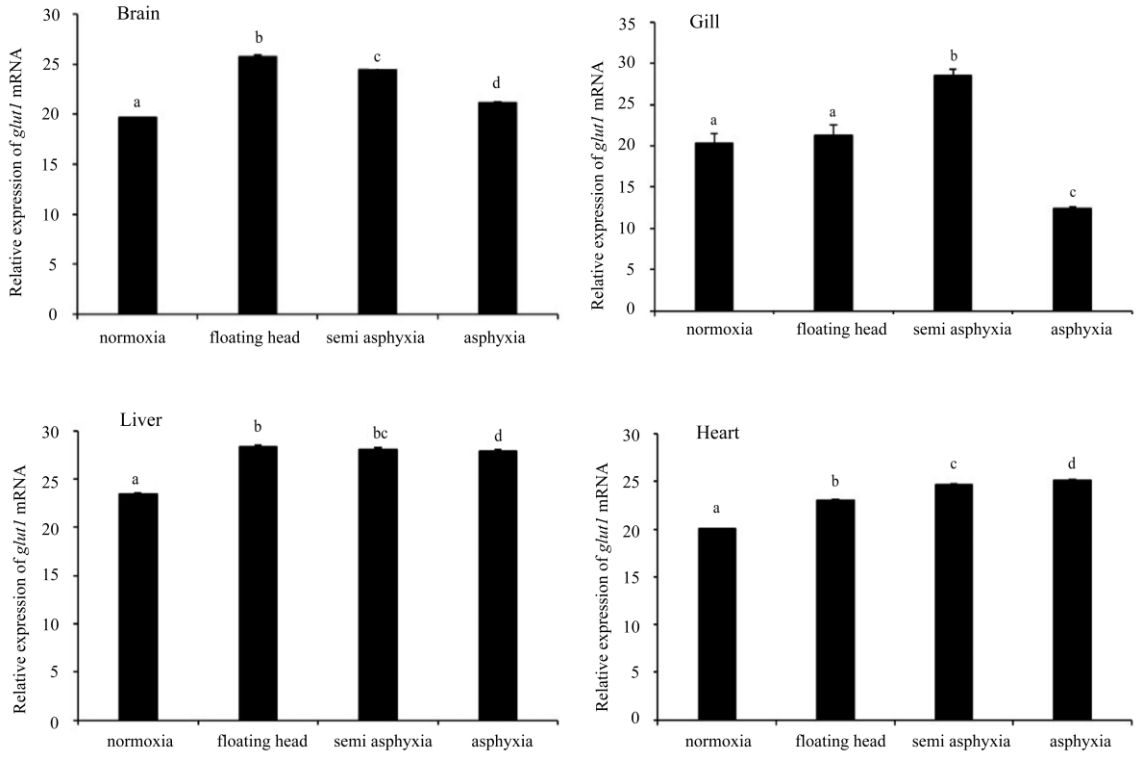

Figure 5. *Glut1* mRNA expression levels in different tissues of silver carp under different oxygen concentrations. Significant differences are indicated by different lowercase letters in the superscript ($p < 0.05$); no significant differences are indicated by the same letter ($p > 0.05$).

4. Discussion

Dissolved oxygen (DO) is one of the limiting factors for the growth, reproduction, and survival of fish throughout their entire life cycle [21]. This study investigated the effects of different hypoxic conditions (floating head, semi asphyxia, asphyxia) on the *glut1* gene in silver carp. We analyzed the amino acid sequence of silver carp *glut1*, and the results show that silver carp have the highest homology with *Ctenopharyngodon idella*, with over 90% homology with other fish and mammals. This indicates that *glut1* is relatively conserved, implying that the function of this gene is similar in fish and mammals. The phylogenetic tree elucidated that silver carp and *C. idella* first cluster into a small branch and then cluster into a large branch with other fish, suggesting that silver carp and *C. idella are* closely related [22].

The expression pattern of silver carp *glut1* was elucidated. The results revealed that the *glut1* gene is expressed widely in different tissues (Figure 4). The *glut1* gene is highly expressed in the gills, brain, and heart, especially in the gills. In a previous study, *glut1* was most expressed in the adult gill tissues of *L. vannamei*. Another study reported similar observations for the gills of *Atlantic cod*, which could be because fish gills not only regulate the respiration of the fish, but are closely related to the acid–base balance, ammonia nitrogen excretion, ion balance, immune defense and osmotic pressure regulation, and are very important components of fish [23–26]. The expression of *glut1* in the liver is relatively low, but some studies have shown that the *glut1* gene is closely related to the glycolysis pathway. As a vital organ for glucose regulation, the liver has also attracted our attention. Meanwhile, the liver is also of great research value as an important response organ for fish to hypoxia [10,27]. In addition, the expression levels of *glut1* in the spleen is second only to the gills, which may be related to the fact that the spleen is an important immune organ of the fish and secretes immune cells under various adverse stimuli [28–30].

Previous studies have shown that four organs, liver, brain, heart and gill, play an important role in the adapted process of fish to hypoxia; therefore, they were selected to clarify the impact of hypoxia on the expression of *glut1*. The expression levels of *glut1* in four tissues of silver carp under different oxygen concentrations was observed. In the floating head and semi-asphyxia groups, the overall expression levels of *glut1* show an upward trend, possibly because during the regulation process, the body provides energy by regulating glucose metabolism to maintain normal physiological activities under hypoxic stress [31–34].

Except for the gills, the other three tissues were still upregulated *glut1* relative to normal oxygen levels in the asphyxia group. As the oxygen concentration decreased, the expression of *glut1* in the gill tissue during the asphyxia process was significantly lower than in the normoxia group, indicating that the expression of *glut1* in the gill tissues was inhibited under hypoxic conditions, possibly related to severe damage to the gill tissues during the asphyxia process. This result is similar to the results reported by Chen et al. and Sun et al. [35,36]. Therefore, the change in *glut1* expression in silver carp could be a regulatory mechanism for coping with hypoxic stress in water.

In this study, we cloned the *glut1* gene of silver carp for the first time, and revealed its changes in expression under hypoxic stress, indicating that its upregulated expression in response to hypoxia is an adaptive mechanism of the body to hypoxia, which provides a reference for subsequent research on the hypoxia tolerance of silver carp.

5. Conclusions

In recent years, research on silver carp under hypoxia stress has mainly focused on cDNA cloning and gene expression under hypoxic stress; however, it is unclear which tissues of the silver carp are more sensitive to hypoxia and how they can adapt to a hypoxic environment through autoregulation. In this study, a potential regulatory protein, *glut1*, for hypoxia tolerance in silver carp was investigated through transcriptome sequencing. Multiple sequence analyses revealed that silver carp *glut1* is highly conserved. Furthermore, qPCR elucidated that *glut1* is widely expressed in various silver carp tissues. These results provide a theoretical basis for understanding the molecular mechanism of the glucose transporter gene, *glut1*, in response to hypoxic stress in silver carp.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8080425/s1, Table S1: cDNA and amino acid sequences of *GLUT1* in *Hypophthalmichthys molitrix*.

Author Contributions: Z.Z. Writing—original draft preparation, writing—review and editing, software; X.L.: methodology, formal analysis, investigation, data curation; G.Z. resources, funding acquisition; H.L. conceptualization, supervision, project administration, writing—review and editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Laboratory Animal Ethical Inspection, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences approved the study (protocol code: 2023zhangzewen01).

Informed Consent Statement: The data presented in this study are available in the article.

Data Availability Statement: The raw data that support this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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