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# Molecular characterization of nine suppressors of cytokine signaling (SOCS) genes from yellow catfish *Pelteobagrus fulvidraco* and their changes in mRNA expression to dietary carbohydrate levels

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ABSTRACT

Suppressors of cytokine signaling (SOCS) are important molecules that mediates the regulation of glucose homeostasis. Here, we cloned and characterized the full-length cDNA sequences of nine genes of the SOCS family (SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH) from yellow catfish *P. fulvidraco*, explored their mRNA abundance across the tissues and their mRNA changes to dietary carbohydrate levels. Structural analysis indicated that the nine members shared conserved functional domains to the orthologues of the mammalian SOCS members, such as SRC homology 2 and the SOCS domains. Their mRNAs were constitutively expressed in various tissues but changed among the tissues. Their mRNA expression in response to dietary carbohydrate levels were explored in the liver, muscle, intestine, testis and ovary. Dietary carbohydrate addition showed significant effects on the mRNA levels of the nine SOCS members. Moreover, their mRNA expressions in response to dietary carbohydrate levels were also tissue-dependent. These indicated that SOCS members potentially mediated the utilization of dietary carbohydrate in yellow catfish.

## 1. Introduction

Cytokines are secreted proteins. They can activate cell surface receptor complexes and accordingly mediate numerous important biological processes [1]. These complexes can interact with some members of the Janus kinase (JAK) family, which lead to the phosphorylation of the specific regions of signal transducer and activator of transcription (STAT) proteins [2]. The phosphorylated STATs form dimmers, which results in nuclear migration and regulates the expression of genes [3]. Excessive cytokine signaling will disorder cellular functions and destroy cellular homeostasis. In order to prevent excessive cytokine signals, organisms have evolved an effficient mechanism where the cytokine signals are negatively regulated by numerous proteins. Among these proteins, the suppressors of cytokine signaling (SOCS) family are the key inhibitors of cytokine receptor signals for feedback regulation (Alexander 2002; [4]. The proteins/genes from SOCS family was first reported in mammals, and consisted of at least eight distinct members, such as SOCS1-7 and CIS (cytokine-inducible SH2-containing protein) [5,6]. They share conservative structure, such as a N-terminal region, a central Src homology 2 (SH2) domain and a C-terminal SOCS box [7]. However, although SOCS proteins have structural similarities, emerging evidences indicate that different SOCS family members possess distinct roles. For example, studies pointed out that mice without SOCS-1 or SOCS-3 had different phenotypes [3,8], meaning that SOCS-1 and SOCS-3 had different roles in signaling outside the cytokine pathways. Thus, their functions of SOCS members seem differentiated during evolution.

Despite wide research on SOCS family in mammals, studies in fish are very scarce. To our knowledge, cDNA sequences of some SOCS family members have been obtained in fish, such as SOCS1, 2 and 3 genes in *Tetraodon nigroviridis*, *Danio rerio*, *Oryzias latipes, Takifugu rubripes* and *Gasterosteus aculeatus* [1,9,10], SOCS1, 2, 3, 6, 7 and CISH in rainbow trout *Oncorhynchus mykiss* [11,12], SOCS1, 3b, 5b, 7 and CISH in channel catfish *Ictalurus punctatus* [13,14], SOCS3 gene in common carp *Cyprinus carpio* [15], SOCS1, 2 and 3 in tilapia *Oreochromis niloticus* [16], SOCS1 and 3 in yellow perch *Perca flavescens* [17], SOCS3b, 5, 6 and CISH in *Paralichthys olivaceus* [18], and SOCS1, 2 and CISH in *Salmo salar* [19]. About their mRNA tissue expression [11], reported that mRNAs of the trout SOCS1, 2 and 3 genes were constitutively expressed in eight tissues: skin, the gills, muscle, spleen, liver, brain, intestine and head kidney. Several studies also reported the mRNA expressions of other SOCS members [12,17]. However, up-to-date, not all these SOCS family members have been obtained in a single fish species.

On the other hand, although the SOCS were originally described in

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**Abbreviations**

| | |
|---|---|
| 18S rRNA | 18S ribosome RNA |
| b2m | beta-2-microglobulin |
| CISH | cytokine-inducible SH2-containing protein |
| EF-1α | elongation factors alpha |
| ELFA | translation elongation factor |
| ESS | extended SH2-subdomain |
| GAPDH | glyceraldehyde-3-phosphate dehydrogenase |
| GH | growth hormone |
| Hprt | hypoxanthine-guanine phosphoribosyltransferase |
| JAK | Janus kinase |
| KIR | kinase inhibitory region |
| MS-222 | tricaine methane sulphonate |
| NJ | neighbor joining |
| ORF | open reading fame |
| qPCR | real-time fluorescence quantitative PCR |
| RACE | rapid-amplification of cDNA ends |
| RPL7 | ribosomal phosphoprotein large 7 |
| RT-PCR | reverse transcription-PCR |
| SEM | standard error of mean |
| SOCS | suppressor of cytokine signaling |
| STAT | signal transducer and activator of transcription |
| TBP | TATA-Box binding protein |
| TUBA | tubulina |
| UBCE | ubiquitin conjugating enzyme |

cytokine signaling pathways) SOCS2 has been characterized as a negative regulator of growth hormone (GH) signaling in mice [20]. Furthermore, studies from Ref. [21] indicated that they belonged to the insulin signaling circuitry and that insulin increased SOCS3 mRNA abundances in 3T3-L1 adipocytes. Insulin also upregulated SOCS3 expression in the muscle, liver and white adipose tissue [22]; however, they did not find out the effects of insulin on either SOCS2 or CIS mRNA abundances, suggesting that the insulin regulation of SOCS/CIS genes was tissue-specific [21]. In the hIR cell lines, insulin stimulates the expression of SOCS1, SOCS2, SOCS3 and CIS [4,8]. It remains well known that insulin is a kind of important hormone for the regulation of blood glucose homeostasis. Thus, it is reasonable to speculate that SOCS members mediated the control of glucose metabolism. Therefore, it is very meaningful and imperative to explore dietary carbohydrate-induced changes of mRNA abundances of these genes.

Here, we report the molecular identification of nine SOCS genes in the economically important freshwater teleost fish, yellow catfish *Pelteobagrus fulvidraco*, and their expression and modulation by dietary carbohydrate addition. Our study expands our understanding into the function of SOCS genes, and offer new knowledge for the relationship between SOCS and glucose metabolism in teleosts.

## 2. Materials and methods

### 2.1. Cloning and mRNA tissue expression of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH cDNA

#### 2.1.1. Experimental animals

For cloning the SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH genes, mixed sex ratio (1:1) of yellow catfish (23.5 ± 3.3 g, mean ± standard error of mean (SEM)) were purchased from a fish farm in Wuhan, China. Heart (H), muscle (M), liver (L), brain (B), kidney (K), fat (F), intestine (I), spleen (S), testis (T), ovary (O) and gill (G) were removed on ice, frozen in liquid $N_2$ and kept at −80 °C for the following analysis (n = 3 replicates and 4 fish were sampled for each replicate).

#### 2.1.2. The full-length cDNA cloning and analysis of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH

cDNA cloning of nine SOCS members followed the protocols described in Ref. [23]. The Primer Premier 5.0 software package was used to design gene specific primers (Supplementary Table 1) and the cDNA fragment of the SOCS genes was amplified. Nested 3′ and 5′ RACE PCR was used to obtain the 3′ and 5’ end sequences of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH, based on the protocols described in Ref. [23].

#### 2.1.3. Molecular characterization and phylogenetic analysis

Molecular characterization, phylogenetic analysis and the generation of the phylogenetic trees were based on the methods described in Refs. [23,24].

**Table 1**
The information for full-length cDNA sequences of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH from *P. fulvidraco*.

| | Accession No. | 5′-UTR (bp) | ORF (bp) | 3′-UTR (bp) | Full length (bp) | No. of amino acids |
|---|---|---|---|---|---|---|
| CISH | MH410164 | 575 | 675 | 1683 | 2934 | 225 |
| SOCS1 | MH410161 | 208 | 561 | 254 | 1023 | 186 |
| SOCS2 | MH410162 | 124 | 735 | 766 | 1625 | 245 |
| SOCS3 | MH497390 | 462 | 672 | 1228 | 2361 | 224 |
| SOCS3b | MH497391 | 624 | 606 | 1371 | 2524 | 201 |
| SOCS5 | MH497392.1 | 338 | 1572 | 824 | 2734 | 525 |
| SOCS5b | MH497393.1 | 186 | 1680 | 1305 | 3171 | 560 |
| SOCS6 | MH410163.1 | 1898 | 1584 | 355 | 3837 | 528 |
| SOCS7 | MH497394 | 58 | 1740 | 574 | 2371 | 580 |

**Abbreviations:** CISH, cytokine-inducible SH2-containing protein; SOCS, suppressor of cytokine signaling.

**Table 2**
Amino acid sequence identities of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH between *P. fulvidraco* and other species (%).

| Genes | *Ictalurus punctatus* | *Danio rerio* | *Xiphophorus maculatus* | *Mus musculus* | *Homo sapiens* |
|---|---|---|---|---|---|
| CISH | 92.0 | 63.2 | 53.6 | 47.8 | 49.1 |
| SOCS1 | 78.0 | 58.6 | 52.2 | 46.2 | 46.8 |
| SOCS2 | 73.8 | 43.1 | 41.8 | 43.9 | 43.9 |
| SOCS3 | 62.7 | 87.1 | 47.1 | 60.9 | 61.9 |
| SOCS3b | 54,5 | 56.7 | 53.5 | 53.2 | 52.7 |
| SOCS5 | 96.9 | 89.1 | 84.6 | 78.1 | 77.8 |
| SOCS5b | 47.8 | 80.4 | 64.1 | 66.9 | 66.9 |
| SOCS6 | 91.5 | 71.5 | 65.6 | 56.2 | 60.2 |
| SOCS7 | 89.9 | 55.3 | 54.0 | 53.1 | 52.3 |

**Notes:** Accession numbers as follows (the order is Ictalurus punctatus, Danio rerio, Xiphophorus maculatus, Mus musculus and Homo sapiens): CISH (XP_017335537.1, NP_001070085.1, XP_005807377.1, BAA06713.1, BAA92328.1); SOCS1 (XP_017344362.1, NP_001003467.1, XP_005809212.1, NP_001258532.1, NP_003736.1); SOCS2 (XP_017342895.1, NP_001108022.1, XP_005812748.1, NP_001162127.1, NP_001257399.1); SOCS3 (ADO29063.1, NP_998469.1, XP_005794680.1, NP_031733.1, NP_003946.3); SOCS3b (JT461874, ABC75031.1, XP_005794680.1, NP_031733.1, NP_003946.3); SOCS5 (AHH39895.1, XP_005156658.1, XP_005795221.1, NP_062628.2, NP_054730.1); SOCS5b (AHH40830.1, CBY83941.1, XP_005795221.1, NP_062628.2, NP_054730.1); SOCS6 (XP_017309195.1, XP_687041.2, XP_005797936.1, NP_061291.2, NP_004223.2); SOCS7 (XP_017310026.1, XP_009304138.1, XP_014328896.1, NP_619598.1, NP_055413.1).
**Abbreviations:** CISH, cytokine-inducible SH2-containing protein; SOCS, suppressor of cytokine signaling.

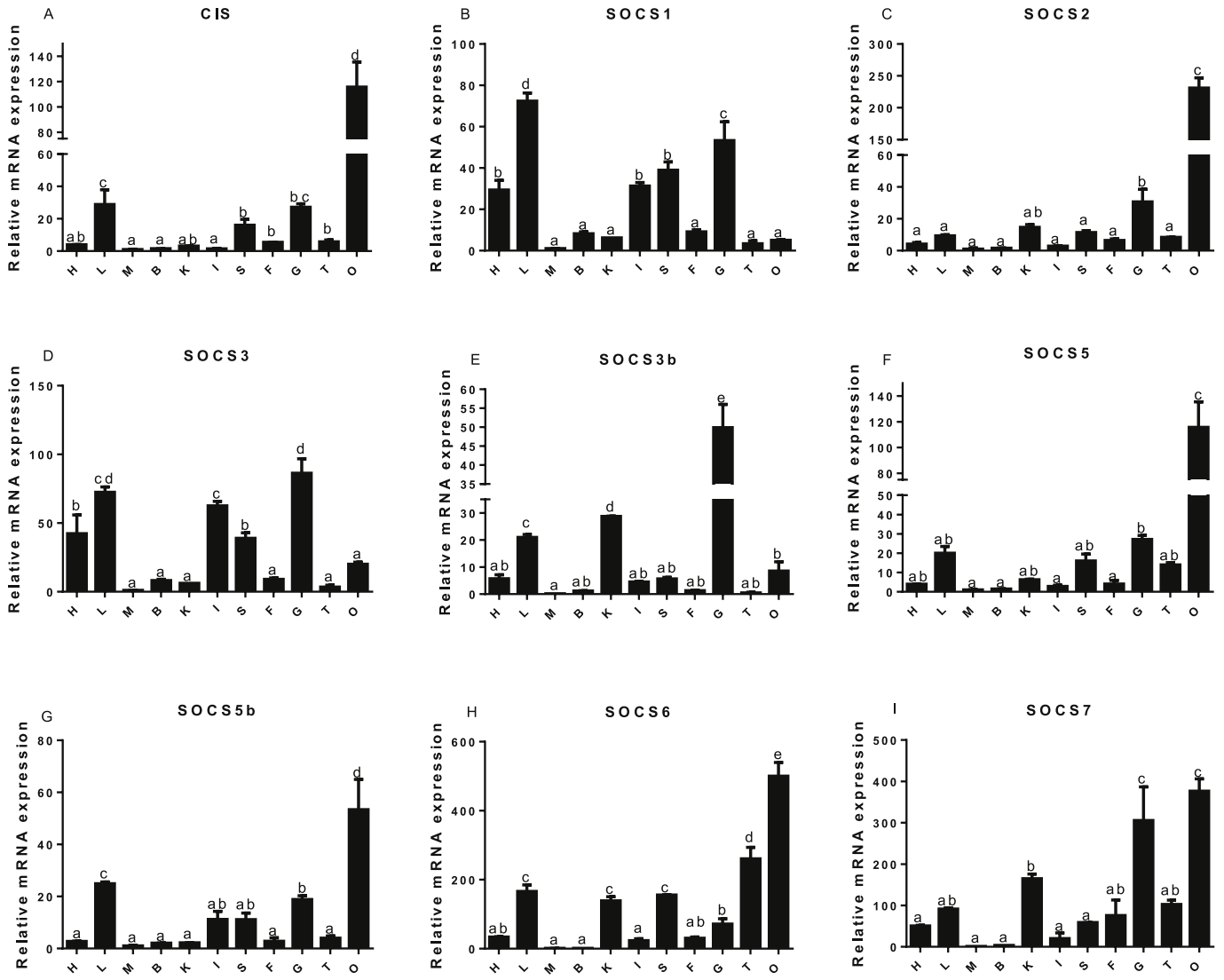


**Fig. 1.** Relative expression levels of the SOCS genes in heart (H), liver (L), muscle (M), brain (B), kidney (K), intestine (I), spleen (S), fat (F), gill (G), testis (T), and ovary (O) of *P. fulvidraco*. Data (mean ± SEM, n = 3) were expressed relative to expression of housekeeping gene (β-actin and ELFA (M = 0.6245)). Bars that share different letters indicate significant differences among the tissues($P < 0.05$).

### 2.2. Transcriptional responses of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH to dietary carbohydrate levels

#### 2.2.1. Feed formulation, feeding management and sampling

Feed formulation and feeding management were in agreement with those described in our recent publication [24]. Briefly, dietary carbohydrate levels for three experimental diets were 17.2% (low), 22.8% (middle) and 30.2% (high). Each experiment diet was assigned to three tanks (300 -L in water volume) and each tank has 30 uniform-sized fish (mean initial weight: 4.1 ± 0.01 g). There are nine tanks in the experiment. All of the fish were fed to apparent satiation twice daily for 10 weeks. At the end of the feeding experiment, all fish were fasted for 24 h. Then three fish per aquarium were euthanized with MS-222, and their liver, muscle, intestine, testis and ovary were sampled, and quickly placed in liquid $N_2$, and kept at −80 °C for the mRNA expression analysis of genes.

#### 2.2.2. Quantitative real-time PCR (qPCR)

Analyses on genes mRNAs levels were examined by real-time fluorescence quantitative PCR (qPCR) method, as described in our recent publications [23,25]. Primers were shown in Supplementary Table 2. When normalizing to the geometric mean of the best combination of two genes as suggested by geNorm, the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels. Prior to the analysis, 11 housekeeping genes (hypoxanthine-guanine phosphoribosyltransferase (hprt), beta-2-microglobulin (b2m), TATA-Box binding protein (TBP), tubulina (TUBA), ubiquitin conjugating enzyme(UBCE), translation elongation factor (ELFA), ribosomal phosphoprotein large 7 (RPL7), β-actin, 18S ribosome RNA (18S rRNA), elongation factors alpha (EF-1α) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to check the stability of mRNA expression. Based on these analysis, two most stable control genes were ELFA and TBP (M = 0.1286) in the liver, hprt and GAPDH (M = 0.5696) in intestine, ELFA and TBP (M = 0.0484) in muscle, β-actin and TBP (M = 0.1636) in ovary, RPL7 and UBCE (M = 0.1046) in testis.

### 2.3. Statistical analysis

All of these data were presented as means ± SEM. Prior to the analysis, Kolmogornov-Smirnov test was used to test the normality of distribution. Barlett's test was used to analyze the homogeneity of variances among the treatments. Then, they were subjected to one-way ANOVA and Tukey's multiple range test. The significant levels were set at $P < 0.05$. The SPSS 19.0 for Windows was used for the statistical

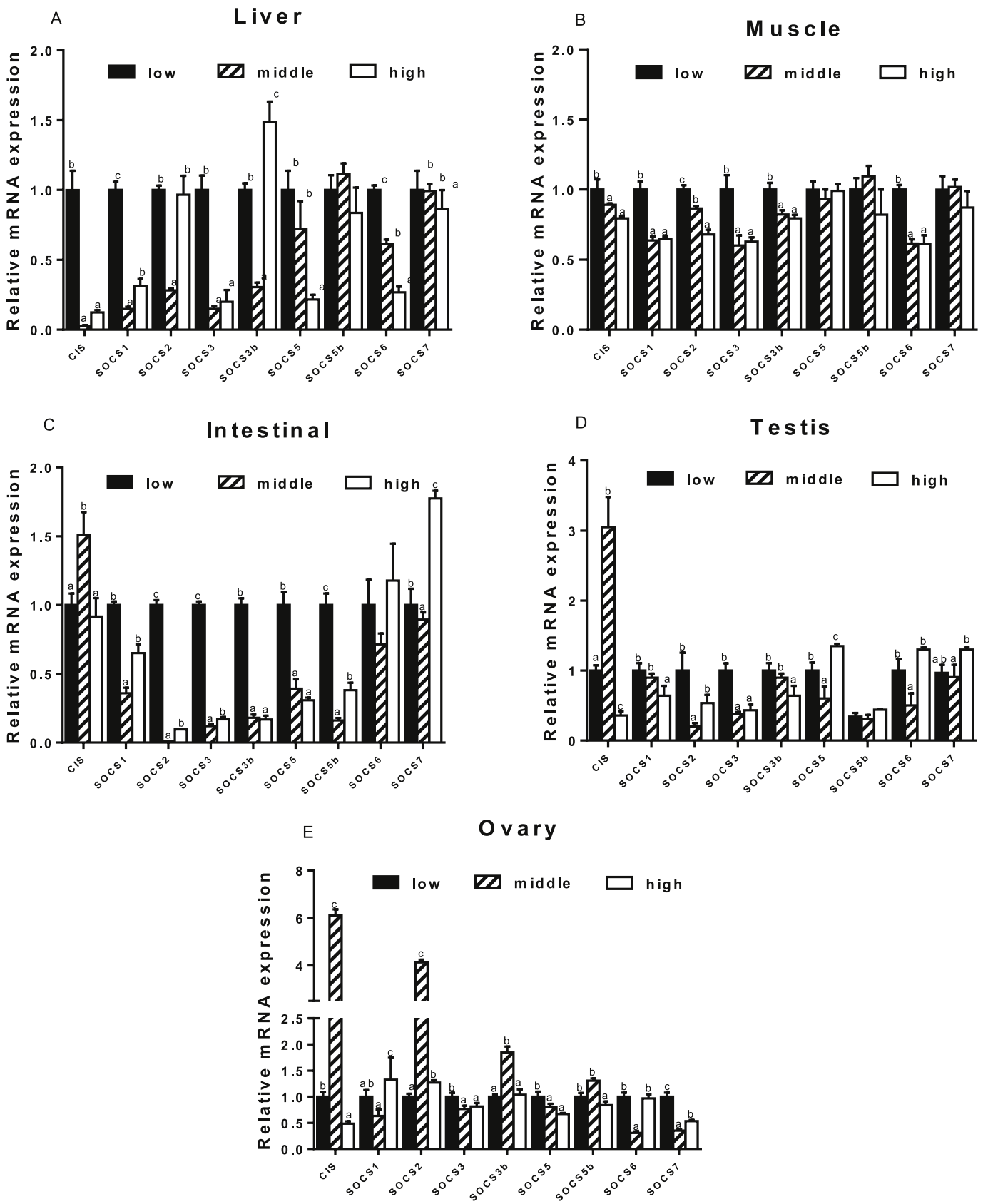


**Fig. 2.** Effect of dietary carbohydrate levels on liver (A), muscle (B), intestine (C), testis (D), and ovary (E) mRNA expression of the SOCS genes in *P. fulvidraco*. Data (mean ± SEM, n = 3) were expressed relative to expression of housekeeping gene (liver: β-actin and ELFA (M = 0.1286); muscle: ELFA and TBP (M = 0.0484); intestine: hprt and GAPDH (M = 0.5696); testis: RPL7 and UBCE (M = 0.1046); ovary: β-actin and TBP (M = 0.1636)). Bars that share different letters within the same tissue indicate significant differences among groups ($P < 0.05$).

analysis.

## 3. Results

### 3.1. Molecular characterization of the full-length cDNA sequences of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH

We successfully obtained the full-length cDNA sequences of CISH and SOCS1, 2, 3, 3b, 5, 5b, 6, 7 genes, which were 2934 bp, 1023 bp, 1625 bp, 2361 bp, 2524 bp, 2734 bp, 3171 bp, 3837 bp and 2371 bp in length, respectively. Their cDNA sequences included an ORF of 675 bp, 561 bp, 735 bp, 672 bp, 606 bp, 1572 bp, 1680 bp, 1584 bp and 1740 bp, respectively, encoding the protein of 225, 186, 245, 224, 201, 525, 560, 528 and 580 amino acids (AA) (Table 1). Yellow catfish SOCS proteins shared a similarity with the amphibian and mammalian SOCS proteins, exhibiting 47.8–92.0%, 46.2–78.0%, 41.8–73.8%, 47.1–87.1%, 39.4–68.7%, 77.8–96.9%, 61.4–66.1%, 56.2–91.5% and 52.3–89.9% AA sequence identities, respectively (Table 2). AA sequence alignments of SOCS family in mammals, amphibians, and fish showed that these SOCS proteins were well conserved in the SOCS domains and SRC homology 2 (SH2) (Supplementary Figs. 1-7). The phylogenetic analysis indicated that all teleost SOCS1, 2, 3, 3b, 5, 6, 7 and CISH formed an independent cluster, while mammalian and amphibian SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH formed another cluster (Supplementary Fig. 8 and Fig. 9).

### 3.2. The mRNA tissue expressions of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH

CISH mRNAs were predominantly expressed in ovary, then in liver, gill, spleen and low in other analyzed tissues (Fig. 1A). The mRNA levels of SOCS1 were predominant in liver, then in the gill, spleen, intestine, heart and lowest in other analyzed tissues (Fig. 1B). The expression of SOCS2 mRNAs were highest in ovary, then in the gill, kidney, spleen, liver and the lowest in other tissues (Fig. 1C). The mRNA abundances of SOCS3 were highest in gill, followed by liver, intestine, heart, spleen and low in other analyzed tissues (Fig. 1D). The gill possessed the highest SOCS3b mRNA levels, and then kidney, liver and ovary. Other analyzed tissues possessed the lowest SOCS3b mRNA abundances (Fig. 1E). The ovary had the highest SOCS5 mRNA levels, then gill, liver and spleen, and other tested tissues had the lowest SOCS5 mRNA expression (Fig. 1F). SOCS5b mRNAs were predominant in ovary, followed by liver, gill, spleen, intestine and lowest in other tested tissues (Fig. 1G). The mRNA abundances of SOCS6 were dominant in ovary, followed by testis, liver, kidney, spleen and the lowest in other analyzed tissues (Fig. 1H). The gill and ovary had the highest SOCS7 mRNA expression, then kidney, liver and testis and other analyzed tissues had the lowest mRNA expression of SOCS7 (Fig. 1I).

### 3.3. Transcriptional responses of SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH to dietary carbohydrate levels

In the liver, mRNA abundances of CIS and SOCS3 were dominant for low dietary carbohydrate levels and had no significant difference between other two treatments. mRNA expression of SOCS1, SOCS2 and SOCS3b was the lowest for fish fed middle dietary carbohydrate levels. mRNA expression of SOCS5, SOCS6 and SOCS7 declined with increasing dietary carbohydrate levels, but SOCS5b mRNA levels showed no significant differences among three groups (Fig. 2A).

In the muscle, dietary carbohydrate addition reduced the mRNA levels of CIS, SOCS2, SOCS3b. The highest mRNA expression of SOCS1, SOCS3 and SOCS6 was observed in fish fed low dietary carbohydrate. The differences in SOCS5, SOCS5b and SOCS7 mRNA abundances were not statistically significant among three groups (Fig. 2B).

In the intestine, fish fed middle dietary carbohydrate had the highest CIS mRNA expression, but the differences were not statistically significant between other two groups. mRNA abundances of SOCS1, SOCS2, SOCS3, SOCS5b and SOCS7 were the lowest in fish fed middle carbohydrate levels of the diets. mRNA levels of SOCS3b and SOCS5 declined with increasing dietary carbohydrate levels. SOCS6 mRNA expression from three treatments showed no significant differences (Fig. 2C).

In the testis, CIS mRNAs was the highest for the group of middle dietary carbohydrate levels and lowest for the group of high carbohydrate diets. Dietary carbohydrate levels reduced the mRNA levels of SOCS1 and 3b. Among three groups, mRNA abundances of SOCS2, SOCS5, SOCS6 and SOCS7 were lowest in the group of middle carbohydrate diets. SOCS5b mRNA levels showed no significant differences among three carbohydrate groups (Fig. 2D).

In the ovary, among three groups, the mRNA abundances of CIS, SOCS2, SOCS3b and SOCS 5 were the highest, and mRNA levels of SOCS1, SOCS6 and SOCS7 were the lowest in fish fed middle dietary carbohydrate. SOCS3 mRNA abundances were the highest for fish fed low dietary carbohydrate levels. dietary carbohydrate levels reduced mRNA expression of SOCS 5 (Fig. 2E).

## 4. Discussion

For the first time, the present study characterized the full-length cDNA sequences of nine SOCS family members, and found that these yellow catfish SOCS proteins showed conserved domains of the protein family, such as the SH2 and SOCS-box domains, in agreement with several studies [14–17,26]. Studies pointed out that these domains of SOCS proteins played important roles the cytokine inhibitory actions [17,26,27]. The extended SH2-subdomain (ESS) and kinase inhibitory region (KIR) domain were also found in yellow catfish SOCS1, SOCS3 and SOCS3b, in agreement with other reports [11,16,28]. In mammals, studies suggested that the ESS domain was a necessary domain for binding to phosphopeptides, and the KIR domain for binding to the JAK2 domain [28]. On the other hand, Wang and Secombes [11] pointed out that the KIR and ESS regions were highly conserved among vertebrates but more divergent among the fish. This kind of divergence probably reflect their discrepancy in regulatory manner, as suggested by Liu et al. [16]. SOCS3 protein shared the conservative PEST (proline-, glutamic acid-, serine- and threonine-rich) domain that helped to increase protein turnover in mammals [29], and such motif was also present in SOCS3 in yellow catfish. In contrast, several studies reported no PEST motif in SOCS3 in carp [15] and yellow perch [17]. Yao et al. [14] reported 12 SOCS genes in channel catfish. The phylogenetic tree analysis suggested that all of these SOCS family members were divided into two subfamilies, named type I (SOCS4-SOCS7) and type II (SOCS1-SOCS3 and CIS), in agreement with Jin et al. [1].

Our study found that mRNAs of nine SOCS members were constitutively present in the tested tissues, but variable at the tissues, similar to fish species [11–13,15,16]. These indicated that SOCS proteins had different biological role among various tissues. On the other hand, it seemed that mRNA tissue expression profiles of SOCS family members were fish species-dependent. For example, in yellow catfish, our study indicated that CISH mRNA abundances were predominat in ovary, then in liver, gill, spleen and lowest in other tissues. Jin et al. [1] reported that CISH was present in various tissues except for intestine and spleen. Our study indicated that mRNA abundances of SOCS1 were highest in liver, followed by gill, spleen, intestine, heart and lowest in other tissues of yellow catfish. However, Liu et al. [16] pointed out that the expression levels of NtSOCS1 were higher in gills, compared to muscle and liver in Nile tilapia. Wang and Secombes [11] reported that SOCS1 was highly expressed in the head kidney, intestine, spleen, skin and gills of rainbow trout. In the present study, SOCS2 mRNA abundances were highest in ovary, followed by gill, kidney, spleen, liver and lowest in other tissues. In contrast, Wang and Secombes [11] reported that compared to other tissues examined, mRNA levels of SOCS2 gene were the lowest in the liver tissue of rainbow trout. Fish species-dependent mRNA tissue expression was also observed in SOCS3 [11,15–17],

SOCS6 and 7 [1,12]. SOCS5b has been historically called SOCS9 [30] and its expression has been studied in a few fish species, such as channel catfish [14]. To my best knowledge, for the first time, we reported mRNA expression profiles of SOCS3b among various tissues in fish.

At present, several studies indicated that SOCS genes and protein mediated carbohydrate-induced response to stress and hormone signals. For examples, Farrell [31] reported that SOCS proteins mediated pathogenesis of the metabolic syndrome by regulating insulin and cytokine signals in obese diabetic mice. Ghanim et al. [32] pointed out that high carbohydrate meal intake influenced SOCS3 expession and induced oxidative and inflammatory stress. The intake of 75 g glucose up-regulated SOCS3 expression and induced the increase in NF-kB binding [33]. Since studies pointed out that the members of SOCS family mediated insulin signals [21,22,34], this stimulated us to analyze its expression patterns to dietary carbohydrate levels. However, at present no reports have explored the effects of dietary carbohydrate levels on the expression of SOCS genes in fish. The present study indicated that dietary carbohydrate addition affected mRNA expression of SOCS members, indicating that these members might mediate the control of carbohydrate metabolism. A role for SOCS1 in the control of glucose homeostasis has been proposed by Kawazoe et al. [35]. They reported that insulin sensitivity was increased in SOCS-1 deficient mice. Li et al. [36] found that SOCS6 overexpression significantly improved glucose clearance in mice, indicating that SOCS6 regulated glucose metabolism. Venieratos et al. [37] pointed out that in the absence of serum, 5 or 25 mmol/l glucose up-regulated SOCS-1 mRNA levels by 36–42% in cells compared to these in 1 mmol/l glucose. They also pointed out that increasing glucose concentration in the medium up-regulated SOCS-1 protein levels by 60–135% compared to cells in 1 mmol/l glucose without the serum [37]. These work could have important physiological significance, since it provides evidence for the important roles of SOCS family member in the regulation of carbohydrate metabolism. Meantime, the mRNA levels of SOCS genes in responses to dietary carbohydrate levels were tissue-dependent (as shown in liver, muscle, intestine, testis and ovary), which might indicate tissue-specific physiological roles for dietary carbohydrate utilization and metabolism. In addition, for the same tested tissues, different SOCS members differentially responded to dietary carbohydrate levels, indicating that their functions have differentiated during the evolution. Thus, SOCS gene duplication in vertebrate contributes to the evolution of gene networks and accordingly complex regulation for the gene expression can be built, as suggested by Jin et al. [1].

## 5. Conclusion

We identified the full-length cDNA sequences of nine SOCS family members: SOCS1, 2, 3, 3b, 5, 5b, 6, 7 and CISH, investigated their expression profiles in various tissues of yellow catfish *P. fulvidraco*. We demonstrate for the first time that dietary carbohydrate addition affected mRNA expression of SOCS genes in tissue-dependent manners. Our study suggest that the regulation of these SOCS members could be a potential strategy for improving carbohydrate utilization and metabolism.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2018.12.037.

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