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Characterization, tissue distribution, and periprandial, fasting, and refeeding changes of *pmch* mRNA in Siberian sturgeon (*Acipenser baerii*)

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Abstract

Melanin-concentrating hormone (MCH) is known to be involved in the regulation of feeding and energy balance in mammals. However, the role of MCH in feeding by fish remains largely unknown. In this study, two transcript variants of *pro-melanin-concentrating hormone* (*pmch*) named *pmcha* and *pmchb* were cloned from Siberian sturgeon (*Acipenser baerii*). PMCHa encoded a protein of 179 amino acids, and PMCHb encoded a protein of 158 amino acid, both of which showed low identity (nearly 18%–27%) when compared with those of mammals, birds and teleost fish species. Both PMCHa and PMCHb contained a conserved MCH-related mature peptide located in the C-terminal region. Quantitative real-time polymerase chain reaction analysis showed that although *pmcha* was highly expressed in the hypothalamus, both *pmcha* and *pmchb* were highly expressed in the liver and cerebellum. Feeding promoted the mRNA expression of *pmcha* and *pmchb* in the hypothalamus at +1 h after feeding, and fasting for 1 d inhibited hypothalamic expression of *pmcha* and *pmchb*, suggesting that *pmcha* and *pmchb* might act as anorexigenic genes in feeding control. In addition, feeding inhibited the expression of *pmcha* and *pmchb* at +1 h after feeding but increased their expression levels at +3 h, and fasting for 10 d inhibited the mRNA expression of *pmcha* and *pmchb* in the liver, suggesting that *pmcha* and *pmchb* might play important roles in regulating energy metabolism in the liver. Taken together, *pmcha* and *pmchb* might participate in inhibiting food intake and energy metabolism in juvenile Siberian sturgeon. These findings provide molecular insights into the feeding regulation of Siberian sturgeon and may inform the development of optimized feeding strategies in sturgeon aquaculture.

Keywords: PMCH, Fasting, Cloning, Siberian sturgeon, Gene expression

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Introduction

Feeding is the way that fish acquire nutrients from the environment which are essential for growth, development, and reproduction. The regulation of appetite in fish is governed by a sophisticated network of interacting hormones that integrate signals originating from both the central nervous system and peripheral tissues[1,2]. In the intricate hormonal cascade that modulates appetite in fish, neurohormones secreted by the brain, particularly the hypothalamic area, certain peptides such as neuropeptide Y (NPY)[3,4], ghrelin[5], and orexin[6] function as orexigenic factors in fish. Others function as anorexigenic factors, such as cocaine and amphetamine-regulated transcript (CART), cholecystokinin (CCK)[7], and leptin[8]. These appetite-related peptides are synthesized and secreted by feeding-related centers within the brain or peripheral tissues such as the liver, stomach, and intestine, thereby modulating food intake in fish.

Melanin-concentrating hormone (MCH), a 17-amino-acid cyclic peptide, encoded by the *pro-melanin-concentrating hormone* (*pmch*) gene, was originally identified in chum salmon (*Oncorhynchus keta*) and was found to be involved in the change of skin color[9]. To date, *pmch* has been identified in several mammals such as humans[10], rats[11], cattle[12], and pigs[13], as well as avian species such as chickens[14]. In fish, several studies have only identified one type of *pmch* gene in species such as ya-fish (*Schizothorax prenanti*)[15], common carp (*Cyprinus carpio*)[16], and Atlantic cod (*Gadus morhua*)[17], but more studies have identified two or more variants of *pmch* genes in species such as starry flounder (*Platichthys stellatus*)[18] and winter flounder (*Pseudopleuronectes americanus*)[19]. In mammals, it has been reported that MCH can act as orexigenic factor in appetite regulation. For example, the mRNA expression of *pmch* in the brain was inhibited by fasting in rodents[20]. Intracerebroventricular (icv) injection of MCH peptide promoted food intake of rats[21]. However, the role of *pmch* in appetite regulation in fish is controversial among species. Fasting promoted the expression of *pmch* in the hypothalamus of *Schizothorax prenanti*, starry flounder, and winter flounder, suggesting the orexigenic function of *pmch*. However, fasting inhibited the expression of *pmch* in the hypothalamus of common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), and ICV injection of MCH inhibited food intake, suggesting the anorexigenic role of *pmch*[22]. Further studies are required to explore the appetite regulation function of *pmch* in different fish species.

Siberian sturgeon (*Acipenser baerii*), a primitive nonteleost member of the ray-finned fish lineage, is extensively cultivated globally, primarily for its caviar and as a source of meat[23]. The genetic and functional characterization of *pmch* in Siberian sturgeon remains poorly understood. In this research, we cloned the Siberian sturgeon *pmch* cDNA

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sequence, providing a foundational information for further investigation into its biological functions. We examined the expression profile of *pmch* genes in the brain and peripheral tissues. Furthermore, the expression patterns of *pmch* under different feeding statuses, including feeding, fasting, and refeeding, were detected to investigate the potential involvement of *pmch* in appetite regulation within Siberian sturgeon.

Materials and methods

Animals

All Siberian sturgeon juveniles were obtained from Runzhao Fisheries (Sichuan, China). Fish were kept at indoor tanks (120 L) at the Sichuan Agricultural University farm supplied with a continuous flow of fresh water (20.4 ± 1.2 °C). Fish were provided with commercial sinking aquafeed (containing 42% protein, 6% fat, and 16% as; Unif, Zhongshan, China) at a rate of 3% of body weight once a day at 14:00, and kept under a 12-h light–12-h dark cycle for two weeks. For cloning and tissue distribution experiments, six fish (214.20 ± 29.54 g) were anesthetized with MS-222 (0.01%) and then their tissues were quickly sampled and frozen in liquid nitrogen. All animal handling procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University, and followed the guidelines on animal experiments of Sichuan Agricultural University.

RNA isolation and cDNA synthesis

Total RNA was isolated from tissues using an RNA extraction kit (Foregene, Chengdu, China) according to the manufacturer's protocol. The quality of the RNA was detected with 1% agarose gel after electrophoresis. Nanodrop 2000 was used to detect the concentration (A260/A280 > 1.8) and purity (A260/A230 > 1.8) of RNA. For this, 1 μg of RNA was reverse-transcribed into cDNA with the PrimeScript RT reagent kit containing gDNA Eraser (Takara, Dalian, China).

Molecular cloning and sequence analysis

The cloning primers of *pmcha* and *pmchb* (Table 1) were designed according to the unpublished hypothalamus transcriptome of Siberian sturgeon. Polymerase chain reaction (PCR) amplification was conducted as follows: 95 °C for 5 min; 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min (39 cycles); and 72 °C for 5 min. The PCR product was purified with the DNA purification kit (Tiangen, China) and inserted into the pMD19-T vector. The ligation products were transformed into *Escherichia coli* DH5*α* cells (TIANGEN, Beijing, China). Positive clones were selected and subjected to sequencing by Sangon (Shanghai, China). DNAMAN was used to analyze the nucleotide and deduced protein sequences. ORF Finder was used to analyze the open reading frames (ORFs). The SignalP 4.1 server was used to predict the presence of signal peptide domains. ClustalW was conducted to align the multiple sequences of PMCH amino acids (aa) (www.ebi.ac.uk/clustalw). Mega 7.0 software was used to construct the neighbor-joining phylogenetic tree.

Table 1. Primer sequences of the clone and those used for quantitative real-time PCR (qRT-PCR).

Primer name	Sequence (5'–3')	Usage
pmcha-f	GATCGGTTTCTACAAGAACTGC	Clone
pmcha-r	GCTTTGTTCATTGGAGGACTG	
pmchb-f	GATAGTTTTTACAAGAACCTCAA	
pmchb-r	CGTAACACACAGGTGAATAAC	
pmcha-qf	AACAGACACCTGCCTTAC	qRT-PCR
pmcha-qr	TCCCAGCATACATCTTAGC	
pmchb-qf	CTTCCTTATTTCTGCCAACTCG	
pmchb-qr	TAGACTCTTCCCAGCATACATC	
β-actin-qf	GTTGGTATGGGACAGAAGGACA	
β-actin-qr	CCAGTTGGTAACAATGCCGT	
ef-1α-qf	AACCTTCAACACCCCAGCC	
ef-1α-qr	CACCAGAGTCCATCACAATACC	

Periprandial experiment

To investigate the periprandial changes in the mRNA expression of *pmcha* and *pmchb*, seven groups of juvenile sturgeons (71.86 ± 20.44 g, n = 9 per group) were fed at the scheduled time (14:00, 0 h) for two weeks. Tissues samples including the hypothalamus and liver were sampled at various time points, namely 3 and 1 h prior to the scheduled feeding time (−3 h, −1 h), at the commencement of feeding time (0 h), and 1 and 3 h after feeding (+1 h, +3 h). In addition, two groups of unfed fish were sampled at 1 and 3 h after the feeding time as a control for the fed groups.

Fasting and refeeding experiment

In the fasting experiments, five groups of juvenile Siberian sturgeon (75.62 ± 15.42 g, n = 9 per group) were acclimated for two weeks and fed once daily at 14:00. Two groups of fish were fed every day; the other three groups of fish were fasted during the experiment. Six fish per group were sampled from the feeding and fasted groups on Day 1 and Day 10, and six fish in the refed group were sampled at 1 h after refeeding on Day 10. The hypothalamus and liver of Siberian sturgeon were sampled and then stored at −80 °C until RNA isolation.

Real-time quantitative PCR analysis

The quantitative PCR primers (Table 1) were based on the sequence of *pmch1* and *pmch2* through cloning. Total RNA (1 μg) was reverse-transcribed into cDNA by using the Prime Script RT reagent Kit (TaKaRa, Dalian, China) as described above. The CFX Real Time PCR Detection System (Bio-Rad, USA) was used to conduct real-time quantitative PCR (qRT-PCR). The reaction volume was 10 μL, including Taq SYBR Green qPCR Mix (5 μL, Innovagene, China), sterile distilled water (3.2 μL), 0.4 μL of each primer, and cDNA (1 μL). The reaction mixture without a template (cDNA) was used as a negative control. The qRT-PCR procedures included initial denaturation at 95 °C for 3 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing at the appropriate temperature (*pmcha*, 52 °C; *pmchb*, 53°C) for 30 s. The target genes were normalized to the houskeeping genes (geometric average of the Ct value of *β-actin* and *ef-1α*), for with the expression levels are stable. The data were analyzed using the relative Ct method[24].

Statistical analysis

The data are shown as the mean ± standard error of the mean (SEM). SPSS 23 statistical software was used to conduct the statistical analyses (IBM Inc, USA). Student's t-tests were used to compare differences between two groups in the periprandial experiments and fasting experiment. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test was conducted to compare the differences among different time points in the periprandial experiment. Here, $p < 0.05$ was considered to be a significant difference.

Results

Identification of *pmcha* and *pmchb* from Siberian sturgeon

This study cloned two transcript of *pmch* genes, namely *pmcha* and *pmchb*, in Siberian sturgeon. One fragment of 604 bp, namely *pmcha* (PQ768526), containing an ORF of 540 bp encoding 179 aa, and another cDNA fragment of *pmchb* (PQ768527) containing an ORF of 474 bp encoding 158 aa, were obtained by qRT-PCR. The amino acids of PMCHa (Fig. 1a) and PMCHb (Fig. 1b) of Siberian sturgeon contained a signal peptide at the N-terminus (27 aa), and a MCH gene-related peptide (MGRP; 12 aa, DSGRDDSKIPVG) and a mature peptide, namely MCH (19 aa, DFDMLRCMLGRVYRPCWQV) at the C-terminus.

The amino acid sequence of the PMCHa and PMCHb prepropeptides of Siberian sturgeon showed low identity when compared with PMCH sequences from mammals, birds, reptiles and other teleost fishes, as shown in Fig. 2 (18%–27%). Siberian sturgeons' pmcha amino acids showed high identity with other PMCH sequences of other sturgeons, such as *Acipenser ruthenus* (XP_033880593.1, 96%; XP_058882986.1, 76%) and *Polyodon spathula* (XP_041109963.1, 77%; XP_041113265.1, 84%), as well as PMCHb from Siberian sturgeon (77%). Siberian sturgeons' PMCHb displayed relative low identity with those of other sturgeons (61%–77%). The phylogenetic tree showed that PMCHa and PMCHb from Siberian sturgeon were included in a clade consisting of Acipenseriformes but separated from other teleost fishes (Fig. 3).

Tissue distribution of *pmcha* and *pmchb* mRNA in Siberian sturgeon

Both *pmcha* and *pmchb* mRNA were widely distributed in different brain regions and peripheral tissues in juvenile Siberian sturgeon. The mRNA of *pmcha* was highly expressed in the liver, cerebellum, and hypothalamus (Fig. 4a). In addition, *pmchb* mRNA was highly expressed in the liver and cerebellum of Siberian sturgeon (Fig. 4b).

Periprandial changes of *pmcha* and *pmchb* in Siberian sturgeon

Hypothalamic *pmcha* mRNA in the fed fish was increased in relative to that in unfed fish at +1 h after feeding but was not changed at +3 h after feeding (Fig. 5a). The expression of *pmchb* in the hypothalamus of the fed fish was promoted at +1 h after feeding and +3 h after feeding when compared with the unfed fish ($p < 0.001$, $p < 0.01$, Fig. 5b). Compared with the unfed groups, the mRNA expressions of *pmcha* and *pmchb* in the liver of Siberian sturgeon was inhibited at +1 h after feeding time ($p < 0.001$, $p < 0.01$) but increased at +3 h after feeding time ($p < 0.001$, $p < 0.01$) (Fig. 5c, d).

Fasting changes in the mRNA expression of *pmcha* and *pmchb* in Siberian sturgeon

After fasting for one day, the mRNA expression levels of both *pmcha* and *pmchb* in the hypothalamus were significantly inhibited when compared with the fed fish ($p < 0.001$, Fig. 6a; $p < 0.01$, Fig. 6b). The inhibitory effect was observed in the mRNA expressions of *pmchb* in the hypothalamus after 10 days of fasting relative to the fed groups ($p < 0.05$, Fig. 6b). Refeeding after 10 days of fasting increased the mRNA expression of *pmcha* and *pmchb* in the hypothalamus of Siberian sturgeon ($p < 0.01$, Fig. 6a; $p < 0.05$, Fig. 6b). There were no significant differences in the mRNA expression levels of *pmcha* and *pmchb* in the liver between fed fish and fasted fish on the first day. However, fasting for 10 days significantly reduced the mRNA expression of *pmcha* and *pmchb* in the liver of Siberian sturgeon ($p < 0.001$, Fig. 6c; $p < 0.05$, Fig. 6d).

Discussion

The present investigation marks the initial cloning of two transcript variants of *pmch* cDNA sequences, *pmcha* and *pmchb*, from the Siberian sturgeon, thereby expanding our understanding of the *pmch* gene repertoire in fish. The variability in the number of *pmch* genes across different fish species is intriguing, with a single *pmch* sequence identified in ya-fish[15] and common carp[16], and two *pmch* genes

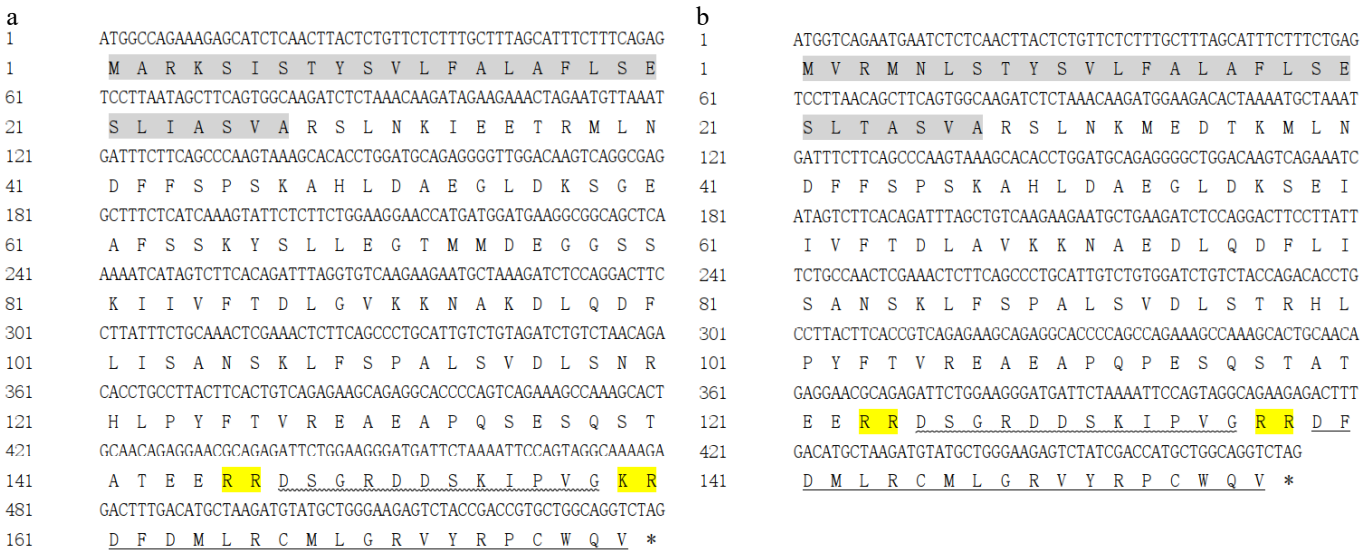

Fig. 1 Nucleotides and deduced amino acid sequences of *pmcha* and *pmchb* from Siberian sturgeon. The grey highlight indicates the signal peptide domains of *pmcha* and *pmchb*. The horizontal line represents the mature peptide region of *MCH*. The wavy line represents the *MGRP* region in PMCHa and PMCHb.

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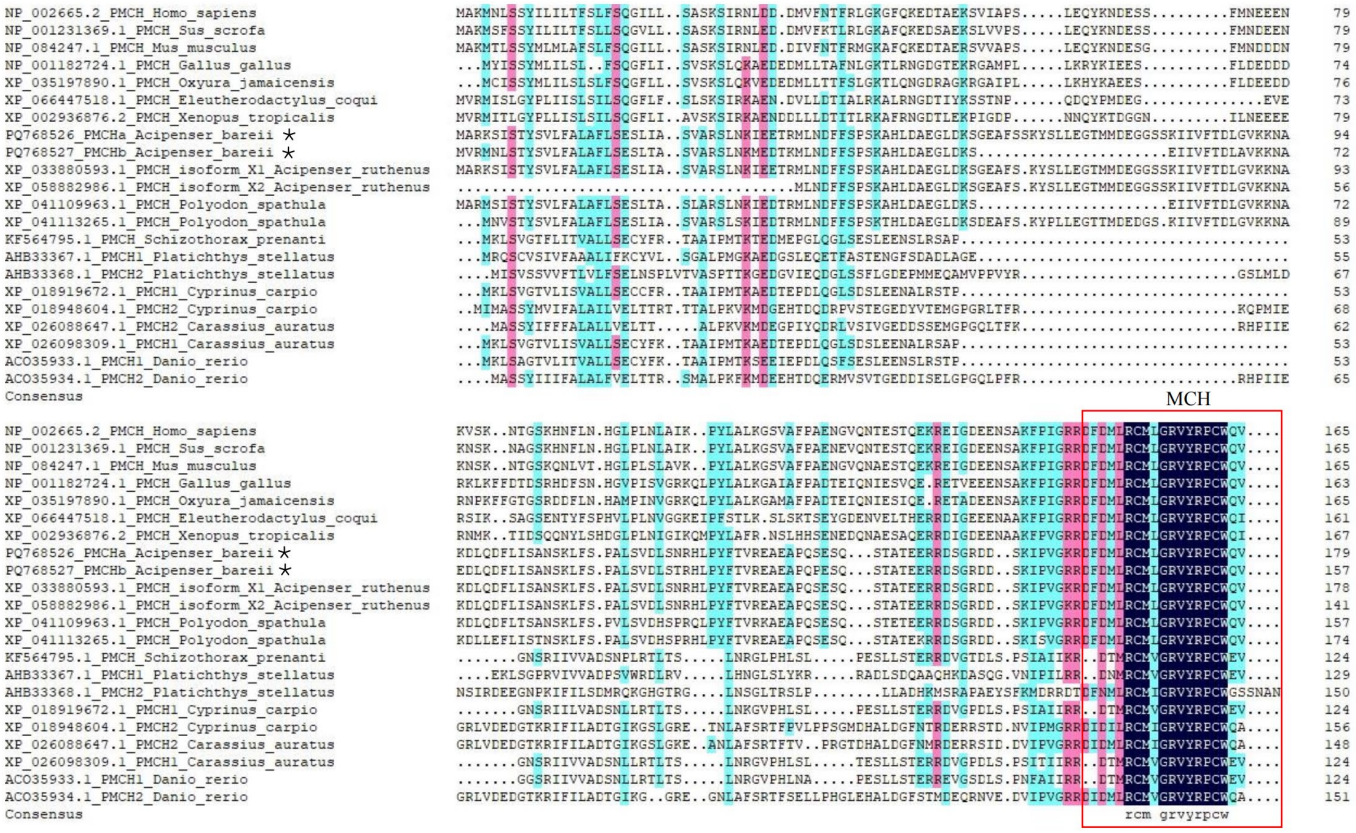

Fig. 2 The alignment of the deduced amino acid sequences of Siberian sturgeon pmch and other vertebrates. Black represents an amino acid consistency level of 100, pink represents 70, and blue represents 50.

in species such as zebrafish[25] and starry flounder[18]. Notably, the proteins PMCHa and PMCHb encoded by *pmcha* and *pmchb* in Siberian sturgeon diverged from those of other teleosts, which may reflect the unique evolutionary position of sturgeons. Indeed, the paleontological record indicates that sturgeon fossils date back to the Triassic period, underscoring their ancient lineage[26]. The teleost lineage, from which sturgeons diverged, experienced a pivotal whole-genome duplication event 320–350 million years ago[27], followed by additional whole-genome duplications in other fish lineages, such as cyprinids, 5.6–11.3 million years ago[28]. These evolutionary events likely contribute to the distinctive genetic makeup of Siberian sturgeon *pmch* genes when compared with other fish species. Furthermore, the conservation of the Siberian sturgeon's mature MCH peptide at the *C*-terminal in mammals and birds suggests that this region may be pivotal to its physiological functions.

Until now, little has been known about the distribution of *pmch* genes in fish species. The result of this study showed that *pmcha* was highly expressed in the hypothalamus of Siberian sturgeon, which is consistent with those in mammals such as humans[10] and rats[11], as well as chickens[14]. In addition, this finding is in accordance with reports in several teleosts. For example, *pmch* was highly expressed in the hypothalamus of ya-fish[15] and common carp[16], and MCH2-ir cell bodies were localized in the lateral tuberal nucleus (NLT) of the hypothalamus of zebrafish[25]. As well as the hypothalamus, *pmcha* and/or *pmchb* mRNA was highly expressed in other regions of brain, such as in the midbrain of ya-fish[15], the optic tectum/thalamus of winter flounder[19]. Interestingly, both *pmcha* and *pmchb* were highly expressed in the cerebellum of Siberian sturgeon. The cerebellum is an important structure that is involved in motor control and cognitive and emotional functions in fish[29,30]. This suggests that the high expression of *pmch* in the Siberian sturgeon cerebellum may be related to its role in adapting to environmental changes and behavioral regulation. Notably, *pmcha* and *pmchb* were highly expressed in the liver of Siberian sturgeon, a finding not previously observed in other teleosts. It could be postulated that *pmcha* and *pmchb* might play important roles in the regulation of energy metabolism in Siberian sturgeon. The distinct expression patterns of *pmcha* and *pmchb* suggest potential functional divergence between the two gene variants. Although *pmcha* shows high expression in the hypothalamus, *pmchb* does not, indicating that *pmcha* may be the primary isoform involved in central appetite regulation. In contrast, both isoforms are highly expressed in the liver and cerebellum, suggesting overlapping functions in peripheral energy metabolism and behavioral regulation. This functional divergence may have arisen from gene duplication events during evolution, allowing for subfunctionalization of the two *pmch* paralogs in sturgeon. Previous studies have reported that *pmch1* and *pmch2* were highly expressed in the pituitary of starry flounder[18], winter flounder[19], and goldfish[31]. Although this study did not detect the expression of *pmcha* and *pmchb* mRNA in the pituitary of Siberian sturgeon, a previous study observed that MCH immunoreactivity fibers were not abundant in the hypophysis of the Acipenseriform starry sturgeon (*Acipenser stellatus*)[32]. The results indicate that the tissue expression of *pmch* genes varies among species, and *pmcha* and *pmchb* might play crucial roles in the behavioral regulation and energy metabolism of Siberian sturgeon. Given that the mature MCH peptides encoded by *pmcha* and *pmchb* were 100% identical in Siberian sturgeon, their physiological functions were expected to be largely redundant at the protein level. However, in other teleosts, such as zebrafish, the two MCH paralogs have been shown to exhibit distinct tissue-specific expression patterns with little or no overlap[25], suggesting

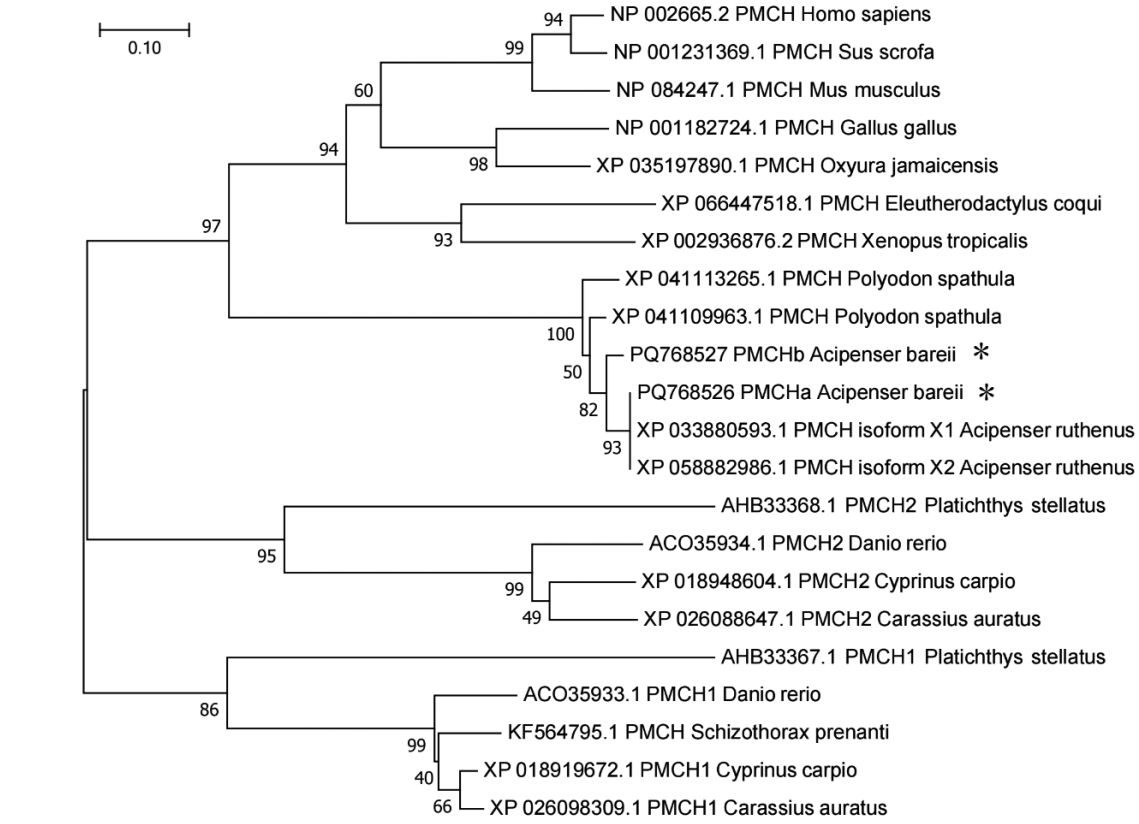

Fig. 3 The phylogenetic analysis of pmch amino acid sequences. MEGA 7.0 was used to construct the neighbor-joining phylogenetic tree and the confidence level of each branch was calculated by bootstrapping repeated 1,000 times.

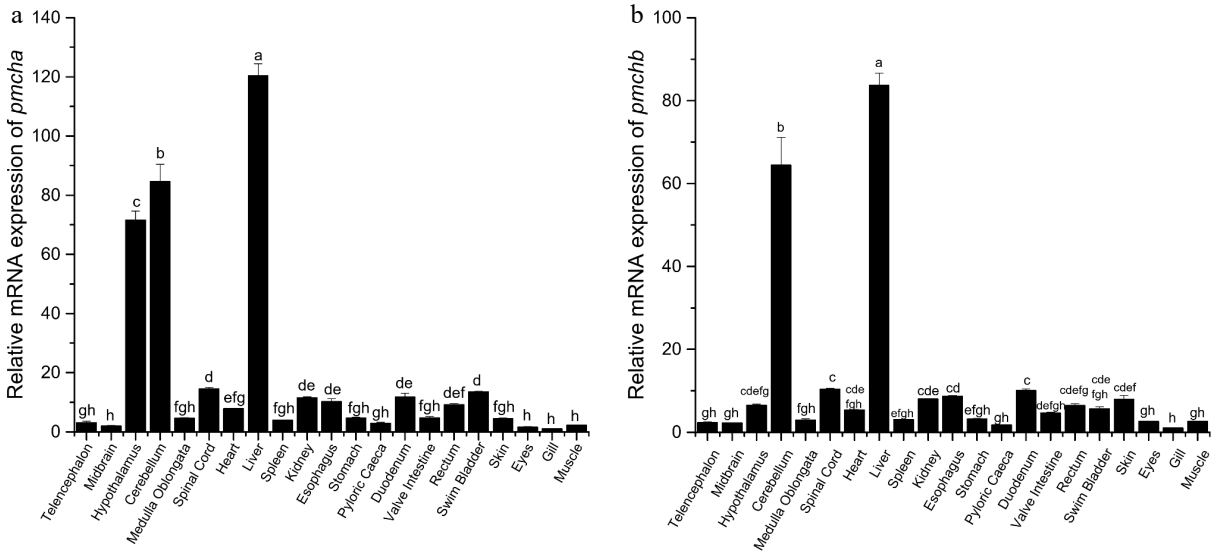

Fig. 4 Tissue distribution of (a) *pmcha* and (b) *pmchb* in Siberian sturgeon ($n = 6$). The mRNA expression of target genes was normalized to *β-actin*. Different letters indicate significant differences. $p < 0.05$ represents statistically significant.

regulatory subfunctionalization following gene duplication. The differential hypothalamic expression of *pmcha* and *pmchb* observed in this study supports this notion, implying that the two paralogs may have evolved distinct regulatory mechanisms while retaining identical coding sequences for the mature peptide. This regulatory divergence may confer a selective advantage by allowing fine-tuned, context-dependent control of MCH signaling in different tissues and physiological states, as has been proposed for other duplicated neuropeptide genes in vertebrates[33,34].

Many studies have indicated that expression levels of appetite factors could be affected by feeding status[35]. Since *pmcha* and *pmchb* mRNA was highly expressed in the hypothalamus and liver of juvenile Siberian sturgeon, which are important sites involved in feeding regulation and energy metabolism, these tissues were chosen for

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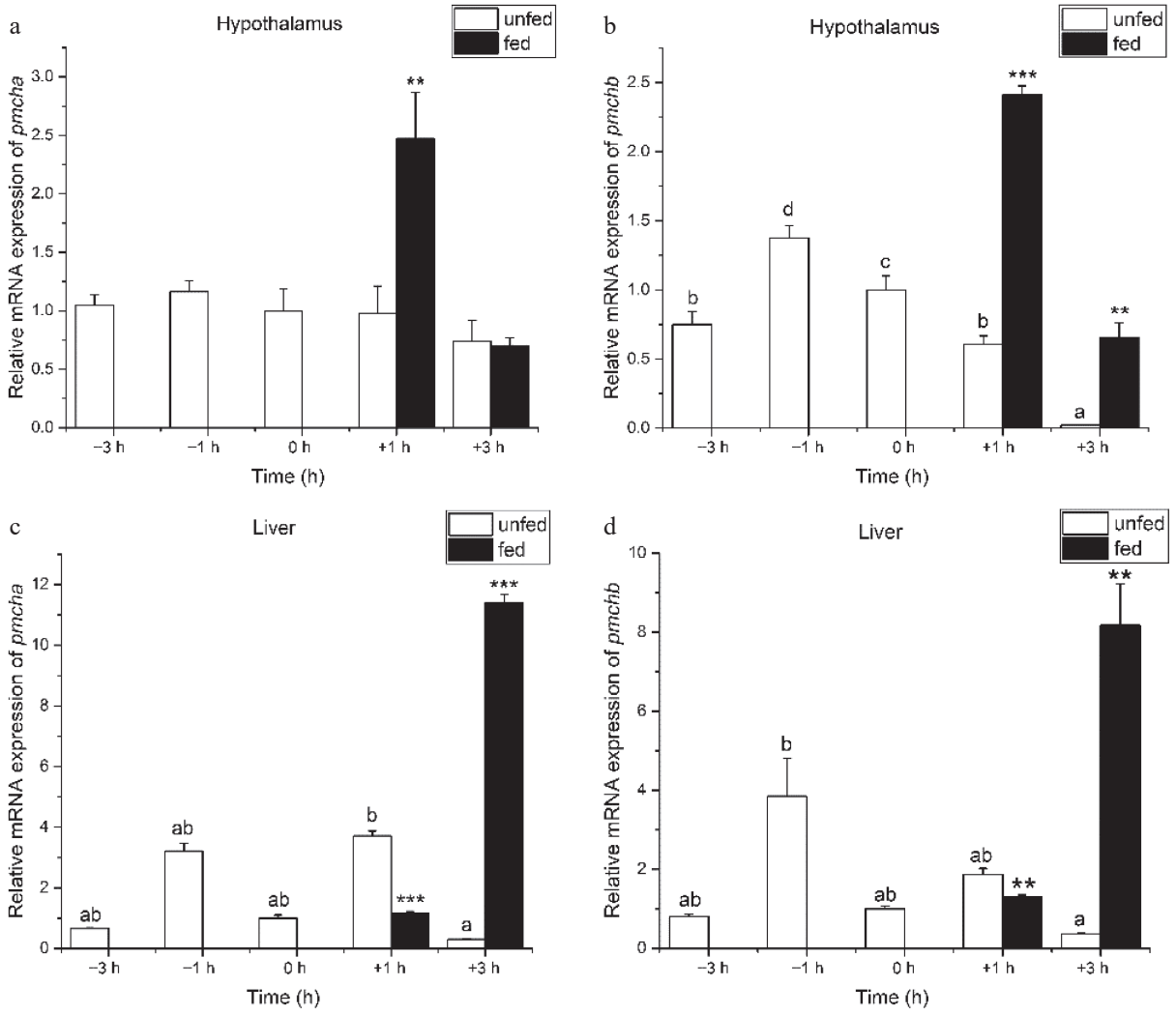

Fig. 5 Preprandial and postprandial changes in the mRNA expression of *pmcha* and *pmchb* in (a), (b) the hypothalamus and (c), (d) liver. The mRNA expression of target genes was normalized to *β-actin* and *ef-1α*. $n = 9$ fish per group. One-way ANOVA followed by Tukey's HSD test was conducted to compare the difference among different time points in the periprandial experiment. The asterisk represents significant differences between unfed fish and fed fish at the same time point (Student's *t*-test). * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$ in the comparison between the fed and unfed groups at the same time point.

periprandial and fasting experiments to explore the possible role of *pmch* in appetite regulation. In this study, mRNA expression of *pmcha* in the hypothalamus of Siberian sturgeon was promoted at 1 h after feeding, and mRNA expression of *pmchb* was promoted at 1 h and 3 h after feeding. The result suggests that *pmcha* and *pmchb* might have an anorexigenic effect in Siberian sturgeon, which is similar to the findings in common carp[16], but inconsistent with mammals and other fishes. In common carp, the level of *pmch1* mRNA in the hypothalamus was increased at 1 h and 3 h after feeding[16]. However, mRNA expression of *pmch* in the hypothalamus of *S. prenanti* decreased at 1 h and 3 h after feeding[15]. These results suggest that in Siberian sturgeon, *pmcha* and *pmchb* might act as short-term satiety indicators in the hypothalamus.

To further explore the role of *pmch* on long-term appetite regulation in Siberian sturgeon, fasting and refeeding experiments were conducted. In this study, fasting for 10 days inhibited mRNA expression of mRNA expression but did not significantly affected mRNA expression of *pmcha* in the hypothalamus of Siberian sturgeon. This result suggests that *pmcha* may undergo adaptive tolerance during chronic energy deficiency, whereas *pmchb* might serve as a more persistent sensor of long-term fasting in Siberian sturgeon. Similar observations were reported in common carp[16] and goldfish[36]. Fasting for 3–7 days inhibited *pmch* expression in the hypothalamus of common carp, and refeeding promoted its expression[16]. In goldfish, fasting inhibited MCH-like immunoreactivity in the dorsal part of the nucleus recessus lateralis (NRLd) region of the hypothalamus[36], and an icv injection of mature MCH peptide decreased food intake[22]. These results suggest that *pmchb* in the hypothalamus might exert an anorexigenic role during long-term starvation in Siberian sturgeon. However, the stimulatory effect of fasting on *pmch* expression in the hypothalamus was observed in mice, chickens[14], and several fish species such as starry flounder[18] and Atlantic cod[17]. In addition, another study in winter flounder showed that fasting did not affect mRNA expression of *pmch1* and *pmch2* in the hypothalamus[19]. These observations suggest that fasting might induce the change of *pmch* expression in the hypothalamus in a species-related manner. Furthermore, refeeding after 10 days of fasting induced a increase in the expression of both *pmcha* and *pmchb* above fed levels, suggesting an emergency anorexigenic brake to prevent overconsumption after starvation. These findings suggest distinct temporal roles for *pmcha*

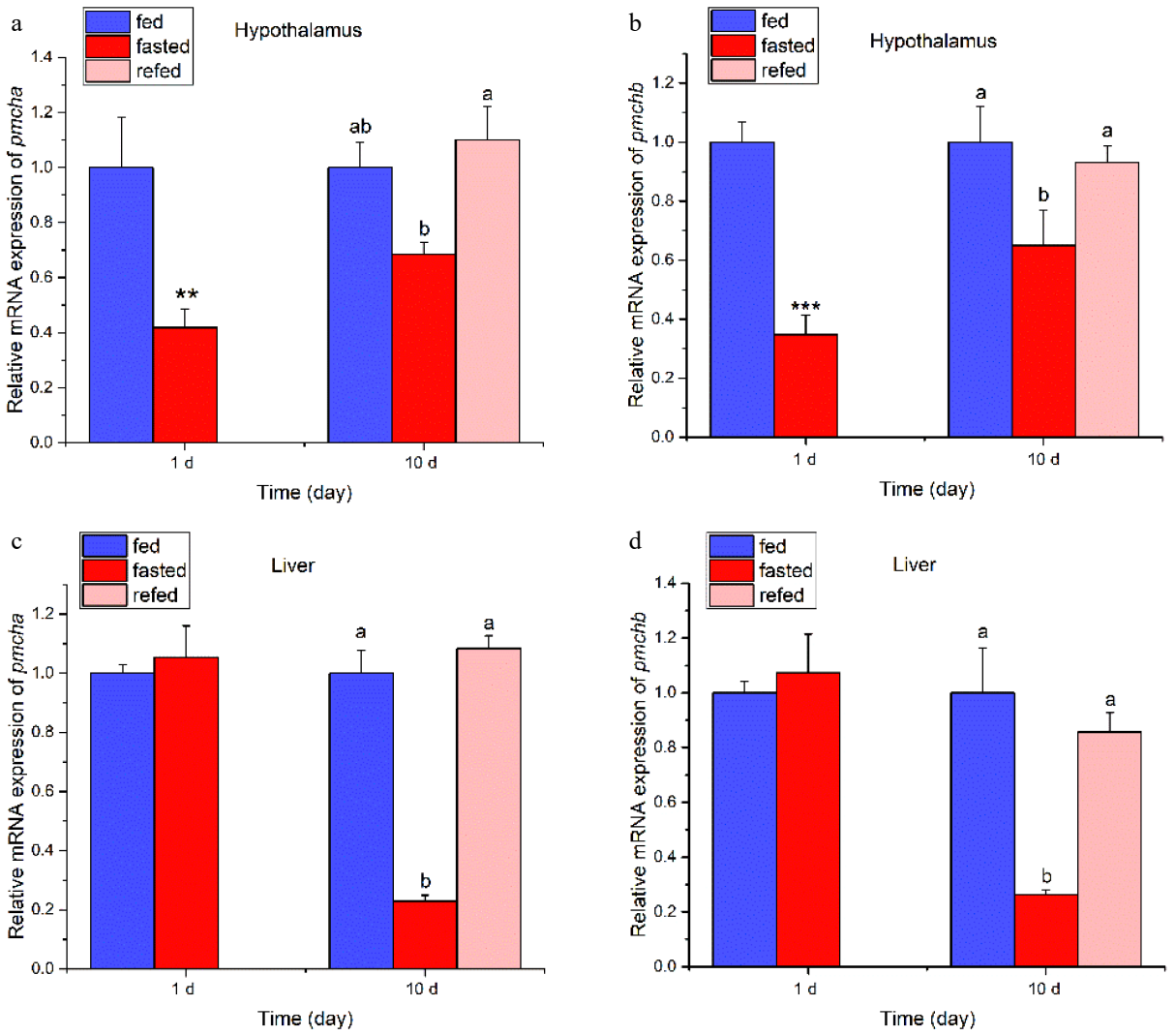

Fig. 6 Effect of fasting and refeeding on mRNA expression levels of *pmcha* and *pmchb* in the hypothalamus and liver of juvenile Siberian sturgeon. The mRNA expression levels were normalized to *β-actin* and *ef-1α*. $n = 9$ fish per group; one-way ANOVA followed by Tukey's HSD test was conducted to compare the difference among fed, fasted and refed groups. * represents significant differences between the fed group and the fasted group (Student's *t*-test), * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$. Different letters indicate significant differences among the fed group, fasted group, and refed group.

(short-term) and *pmchb* (long-term) in hypothalamic appetite regulation. The liver is the main organ for lipid storage, and starvation lead to a decrease in lipid content in the liver of sturgeons[37]. It is noteworthy that fasting for 10 days inhibited mRNA expression of *pmcha* and *pmchb* in the liver. This result suggest that *pmcha* and *pmchb* might be involved in the regulation of lipid metabolism in the liver of juvenile Siberian sturgeon during starvation periods. Although little is known about the function of *pmch* genes in the liver of fish, a previous study revealed that icv injection of MCH triggered lipid accumulation and lipid uptake in the liver of rats[38]. These results suggest that *pmcha* and *pmchb* might be involved in the regulation of appetite and also participate in the regulation of lipid metabolism in the liver of juvenile Siberian sturgeon during starvation periods.

Conclusions

This study successfully cloned two transcript isoforms of the *pmch* gene from Siberian sturgeon, namely *pmcha* and *pmchb*, encoding proteins of 179 and 158 aa, respecively. Our study revealed that the mature MCH peptide encoded by Siberian sturgeon *pmcha* and *pmchb* showed high identity with mammals' MCH, but low identity with other teleost fish species' MCH. The *pmcha* and *pmchb* mRNA were highly expressed in the liver and cerebellum, and *pmcha* was also highly expressed in the hypothalamus of Siberian sturgeon, indicating potential functional divergence between the two paralogs. The expression patterns of *pmcha* and *pmchb* in the hypothalamus and liver in response to feeding and fasting suggest their potential roles as anorexigenic factors in appetite regulation. These findings provide important insights into the regulatory mechanisms of feeding control and energy homeostasis in Siberian sturgeon. Furthermore, the results have practical implications for sturgeon aquaculture. For example, the anorexigenic role of *pmcha* and *pmchb* suggests that feeding strategies avoiding prolonged fasting could prevent the downregulation of these satiety signals, potentially improving feed efficiency. Further studies are needed to investigate the role of mature MCH peptides and MGRP peptide for feeding and energy metabolism in aquaculture of Siberian sturgeon.

Ethical statements

These animal experiments were approved by the Animal Care and Use Committee of Sichuan Agricultural University (20250628).

Author contributions

The authors confirm their contributions to the paper as follows: experimental design and supervision: Li Z, Tang N; experimental implementation: Wu H, Jiang K, Luo Y, Li J, Li Y, Xiong Y, Wang M; data analysis and visualization: Wu H, Li J; supervision: Zhang X; resources and reagents: Li Z, Tang N, Wu H; writing-original draft: Wu H; writing-review and editing: Luo Y, Tang N. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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