Genetics and Molecular Research

Cloning, characterization, expression, and feeding response of thyrotropin receptor in largemouth bass (*Micropterus salmoides*)

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Genet. Mol. Res. 15 (3): gmr.15038658
Received March 24, 2016
Accepted May 6, 2016
Published July 29, 2016
DOI http://dx.doi.org/10.4238/gmr.15038658

ABSTRACT. Thyrotropin receptor (TSHR) is a G-protein-coupled receptor that regulates the synthesis, storage, and secretion of thyroid hormones in the thyroid tissue. The aims of the present study were to characterize the full-length TSHR cDNA in largemouth bass (*Micropterus salmoides*), and to determine the TSHR gene transcription levels in different tissues. In addition, the response of TSHR transcription levels to daily feeding in thyroid tissue was investigated. The results showed that the full-length cDNA sequence was 2743 bp with an open reading frame of 2340 bp encoding a 779-amino acid peptide. BLAST analysis indicated that the amino acid sequence displayed 58.4-90.2% identity and 5.6-125.8 divergence, compared with other known fish species.

The most abundant TSHR transcription levels were found in the spleen, head kidney, and kidney. Feeding did not affect the transcription level of TSHR in thyroid tissue over the course of the day. Thus, the current study suggests that there was no relationship between daily nutritional status and TSHR transcription level in the thyroid tissue of largemouth bass. The spleen, head kidney, and kidney exhibited the most abundant TSHR transcription levels.

Key words: *Micropterus salmoides*; Thyrotropin receptor; Feeding; Thyroid hormones; Gene transcription

INTRODUCTION

Thyroid hormones, including thyroxine (T4) and triiodothyronine (T3), are essential for vertebrate development, growth, and metabolism. In the teleost fish, the brain-hypothalamus-pituitary-thyroid axis (HPT) regulates the synthesis and release of thyroid hormones (Blanton and Specker, 2007), and thyrotropin (TSH) plays an important role in the HPT regulation process. The output of TSH is controlled by neurochemicals from the hypothalamus. After release, TSH binds to the TSH receptor (TSHR), which is a G-protein-coupled receptor that belongs to the glycoprotein hormone receptor subfamily. Receptors in this subfamily are distinguished by a very large extracellular domain containing multiple leucine-rich repeat motifs (Kumar et al., 2000). Once bound by TSH, the TSHR initiates a dual intracellular signaling cascade, including adenylate cyclase, protein kinase A, phospholipase C, and calcium pathways, which is necessary for the synthesis, storage, and secretion of T4 in the thyroid follicles (Ji et al., 1998; Vischer and Bogerd, 2003). The primarily T4 released from the thyroid follicles is then converted to T3 in most tissues. Hence, TSHR plays a vital role in the HPT regulation process.

A paucity of information is available concerning the characteristics of TSHR and its function in the HPT axis in teleost fish. The complete cDNA sequence of TSHR has been cloned in some fishes including *Oncorhynchus rhodurus* (Oba et al., 2000), *Morone saxatilis* (Kumar et al., 2000), *Clarias gariepinus* (Vischer and Bogerd, 2003), *Ictalurus punctatus* (Goto-Kazeto et al., 2003), *Dicentrarchus labrax* (Rocha et al., 2007), and *Solea senegalensis* (Ponce et al., 2010). These sequences have allowed us a partial understanding of the molecular structure and functions of TSHR in teleost fish. Nevertheless, we are far from a complete understanding.

The HPT plays an important role in fish development and reproduction, and a large number of experiments have been conducted to further our understanding (Blanton and Specker, 2007). In addition, the HPT regulation system interacts with the nutrition status of animal. The influence of the nutritional status on fish thyroid function was studied by Valente et al. (2003). The authors revealed that satiation, restricted feeding, and fasting all affected the thyroid hormones (Valente et al., 2003). Restricted feeding and fasting may decrease the sensitivity of the thyroid tissue to TSH and hepatic deiodinase activity (Le Bail and Rœuf, 1997). However, no study has corroborated the effects of nutritional status on TSHR gene expression pattern in thyroid tissue in teleost fish.

Largemouth bass (*Micropterus salmoides*) is a freshwater fish that is increasingly cultured in China, mainly in the southern parts. The production reached 351,772 tons in 2014 (Ministry of Agriculture in China, 2015). Although attention has been paid to the sustainable

development of largemouth bass in China, the incomplete knowledge of nutrition physiology still limits the extension of largemouth bass culture. The current study aimed to characterize the full-length TSHR cDNA in largemouth bass. Moreover, a gene expression study was carried out to analyze the tissue-specific responses of the TSHR gene to daily feeding.

MATERIAL AND METHODS

Fish and tissue collection

The largemouth bass used in this study were purchased from a fish farm in Huzhou, Zhejiang, China. Fish with an average weight of 190 ± 8.5 g (N = 5) were reared in a fiber-glass tank (300 L) equipped with recirculation system. Each tank was aerated by an air stone connected to an aquarium pump. The tanks were covered with a transparent lid and the light conditions were 24 h continuous light. The average water temperature was 26°C, and the average dissolved oxygen level at the outlet was 5.9 mg/L. The operation of fish in current study was under the protocols from Ministry of Agriculture in China, which complied with international animal welfare guidelines and laws (FAO, 2004). Two weeks of acclimation was applied. The fish were fed a commercial feed twice daily until satiation during this period. The feed was produced by Jiasheng Feed, and the crude protein and crude fat contents were 42 and 3.0%, respectively.

After that, five fish were euthanized with MS-222 (0.2 g/L in freshwater) and sacrificed by head beat. The jaw tissue between the first and third gill, which contains the thyroid follicle, was collected for molecular cloning and transcription level determination after dissection. In addition, liver, spleen, stomach, intestine, kidney, head kidney, heart, and muscle tissues were sampled for transcription level determination. The collected tissues were immediately extracted for total RNA.

In order to determine the effects of nutritional status on TSHR gene expression patterns in thyroid tissue, a fish feeding trial was conducted. After the fish arrived to our laboratory, they were acclimated for three weeks. They were then randomly distributed into two fiber-glass tanks (300 L) with 40 fish in each tank. One tank was set as the fed group (weight: 202.6 ± 5.2 g; length: 25.42 ± 0.49 cm) and the other tank was set as the fasted group (weight: 198.1 ± 6.9 g; length: 25.07 ± 0.43 cm). The water temperature was 21°C, and the average dissolved oxygen level at the outlet was 6.6 mg/L. The fish were acclimated for two weeks in their respective tanks before the assay, and were fed a commercial feed twice daily until satiation during this period. The fasting was applied for 48 h prior to treatment period for both groups. The fed group was given one meal during the treatment period, whereas the fasted group did not receive any feed. The feed intake accounted for 1.39% of body weight. Sampling of fed group was conducted at the following time points: 1 h before feeding (-1 h), right after feeding (0 h), and 1, 2, 4, 6, 12, and 24 h post feeding. Five fish were sampled at each time point. The same sampling time points were used in fasted group. At each sampling time, five fish were gently and carefully caught and extracted from the tank, in order to minimize the stress to the other fish in the same tank. The fish were euthanized with MS-222 (0.2 g/L in freshwater) followed by sacrifice and dissection. The tissue containing thyroid follicle was collected for real-time polymerase chain reaction (PCR) analysis, following the same method as mentioned above. The collected tissues were immediately extracted for total RNA.

Molecular cloning

First, the total RNA was isolated from the surface of the ventral aorta and surrounding tissues, which contain thyroid follicles, using TRIzol® reagent (Life Technologies Corporation, Shanghai, China). The isolation of total RNA was conducted in accordance with the manufacturer specifications. Total RNA was treated with DNase (Life Technologies Corporation) and then reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Shanghai, China) with oligo $(dT)_{18}$ primer. The TSHR cDNA sequences from *S. senegalensis* (accession No. FN677495.1), *D. labrax* (accession No. DQ386646.1), *M. saxatilis* (accession No. AF239761.1), *Oreochromis niloticus* (accession No. XM_005449554.1), *Haplochromis burtoni* (accession No. XM_005917077.1), and *Maylandia zebra* (accession No. XM_004542242.1) were aligned using the Bioedit program v. 6.0 (Elkins, 2013). Highly-conserved regions were selected for designing degenerate primers, TSHR-F1 and TSHR-R1 (Table 1). The primers utilized in the current study were designed by using Primer Premier v. 6.0 (Álvarez-Fernández, 2013), and are listed in Table 1. The PCR of amplifying the reverse-transcribed first-strand TSHR cDNA was performed using the following cycling conditions: one cycle at 94°C for 2 min followed by 30 cycles at 94°C for 30 s, 59°C for 30 s, and 72°C for 45 s; and a final cycle at 72°C for 10 min. The amplified PCR products were purified using a GenClean agarose gel DNA recovery kit (Shanghai Generay Biotech Co. Ltd., Shanghai, China) according to the manufacturer instructions. The purified products were subsequently sent to Invitrogen (Shanghai, China) for Sanger sequencing.

Table 1. Primers used in the study of *Micropterus salmoides*.

Primer	Nucleotide sequence (5'-3')	Application	Product length (bp)
TSHR-F1	GTGTGGTTYGTKAGYYTGCTGGC	Partial cDNA	721
TSHR-R1	TAGAGRAAYGGRTTGGCACAGG	Partial cDNA	
TSHR-R2	GACAAGATGTAGACCTGAGCCAC	5'-RACE	1493
TSHR-R3	AAGACCCAGCCACCTAGCATCA	5'-RACE	
TSHR-F2	ACTGGTTGGGGTGAGCAGTTAC	3'-RACE	630
TSHR-F3	TAACCCTCACTACCGCTCTGGA	3'-RACE	
TSHR-F4	AGCATACCACCTCTCCAG	Real-time PCR	126
TSHR-R4	TACTTCAACCACGCCATAG	Real-time PCR	
GAPDH-F	GGCTTTCCGTGTTCCAACTC	Real-time PCR	154
GAPDH-R	GACAACCTGGTCCTCCGTGTATC	Real-time PCR	
UPM	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT		
NUP	AAGCAGTGGTATCAACGCAGAGT		

The first-strand cDNA used for 5'- and 3'-RACE reactions were generated with 5'-RACE CDS primer and 3'-RACE CDS primer using SMARTer™ RACE cDNA amplification kit (Clontech Laboratories, Inc., USA). For 5'-RACE, primer pairs UPM/TSHR-R2 and NPU/TSHR-R3 were used in a nested PCR amplification with the following conditions: one cycle at 94°C for 1 min followed by 35 cycles at 98°C for 10 s, 60°C for 15 s, 68°C for 1 min, and a final extension at 72°C for 10 min. The primer pairs UPM/TSHR-F2 and NPU/TSHR-F3 were used for 3'-RACE in a nested PCR amplification with the following conditions: one cycle at 94°C for 2 min followed by 35 cycles at 98°C for 10 s, 60°C for 30 s, 72°C for 1 min; and a final cycle at 72°C for 10 min. After purification, the RACE products were cloned into T-vector pMD20 using DNA Ligation Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The resulting plasmids that were confirmed by restriction digestion with different enzymes and PCR amplification of the target fragment were sent for sequencing of the complete TSHR cDNA sequence. The partial sequences obtained from RACE were

assembled using the SeqMan software v. 5.01 (Staden et al., 2002). The obtained full-length cDNA sequence was translated by ExPASy (http://web.expasy.org/translate/). The predicted amino acid sequence was compared with those from other fish species including *C. gariepinus* (accession No. AAN01360.1), *I. punctatus* (accession No. AY533543), *M. saxatilis* (accession No. AAF80596.1), *S. senegalensis* (accession No. CBK38913.1), and *O. rhodurus* (accession No. JC7389 and BAB07801.1), using the MegAlign software v. 5.01 from DNASTAR. (Staden et al., 2002). The signal peptide was predicted by SignalP (http://www.cbs.dtu.dk/services/SignalP/), whereas transmembrane helix prediction was performed using TMHMM (http://www.cbs.dtu.dk/services/TMHMM/). N-linked glycosylation sites were predicted using NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc/). Generic phosphorylation sites and secondary structure were predicted using NetPhos (http://www.cbs.dtu.dk/services/NetPhos/) and PSIPRED v. 3.3 (http://bioinf.cs.ucl.ac.uk/psipred/), respectively. Phylogenetic analysis of the TSHR amino acid sequences among different fish species was performed using the neighbor-joining method in MEGA v. 6.0 (Tamura et al., 2007). Concomitant, percent identity, and divergence of amino acid sequences among different fish species were calculated by MegAlign v. 5.01 (Staden et al., 2002). The conservative domains of the deduced amino acid sequence were analyzed using the simple modular architecture research tool at EMBL (http://smart.embl-heidelberg.de).

Real-time PCR analysis

Total RNA from the different tissues of five fish were extracted using TRIzol® Reagent as described above. From each tissue, 2 mg total RNA were reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) with oligo $(dT)_{18}$ primer. TSHR gene transcript abundance in the different tissues was quantified by real-time PCR using SYBR® Premix EX TaqTM II (TaKaRa Biotechnology Co., Ltd.), with GAPDH set as the internal control. Real-time PCR was performed by MyiQ2 Two Color Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) using the primers listed in Table 1. The conditions used for real-time PCR were: one cycle at 94°C for 2 min followed by 45 cycles at 95°C for 10 s, 59.5°C for 30 s; and a final 71 cycles at 60°C for 30 s. After the final amplification, the specificity of the PCR was investigated using the amplicon melting temperature, calculated from the dissociation curve obtained by increasing the temperature from 60° to 95°C at a rate of 0.3°C/s (De Martinis et al., 2007).

Calculations and statistics

The data generated by real-time PCR was analyzed using the ΔΔCT method (Stephenson, 2010). The transcription level of TSHR in different tissues was calculated as expression relative to the thyroid tissue transcription level. The results from the food intake trials were analyzed using a GLM procedure in SAS (SAS Institute Inc., 1999). A two-way ANOVA was performed to investigate the transcription differences of the TSHR genes at different time points and between the fasted and fed groups. Significant differences ($P < 0.05$) among groups were ranked using the Duncan procedure in SAS.

RESULTS

cDNA cloning and characterization of TSHR

The complete TSHR cDNA sequence contained a 54-bp 5'-untranslated region and a 349-bp 3'-untranslated region. We identified an open reading frame of 2340 bp encoding a 779-amino acid peptide with a predicted molecular weight of 87.58 kDa (Figure 1). The nucleotide sequence of TSHR has been submitted to GenBank with the accession No. KP334115. The first 22 amino acids (MQVITCALFTLVTLPISTVSEA) were predicted to constitute the signal peptide at the amino terminus. The deduced amino acid peptide of TSHR in largemouth bass was compared to that of known TSHR in other fish species (Figure 1). The TSHR amino acid peptide contained five potential N-linked glycosylation sites and twenty conserved cysteine residues. Thirty-seven phosphorylation sites were predicted (22 serine, 9 threonine, and 6 tyrosine). Seven transmembrane domains (431-453, 460-482, 504-526, 547-569, 589-611, 636-658, and 668-690), four outside membrane domains (1-430, 483-503, 570-588, and 659-667), and four inside membrane domains (454-459, 527-546, 612-635, and 691-779) were identified. The predicted secondary structure of TSHR amino acid included 18 helices (2-13, 71-72, 119-122, 152-158, 250-253, 266-276, 296-301, 431-439, 457-483, 487-496, 505-528, 545-563, 590-614, 627-652, 646-658, 683-689, 692-704, and 708-713) and 15 strands (30-31, 39-41, 56-59, 80-84, 91-92, 105-108, 179-182, 190-192, 406-407, 426-430, 443-450, 529-534, 615-618, 667-676, and 751-732).

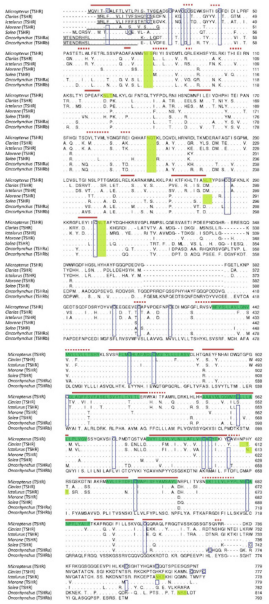

Figure 1. Comparison of the deduced amino acid sequence of TSHR among different fish species (*Micropterus salmoides*, *Clarias gariepinus*, *Ictalurus punctatus*, *Morone saxatilis*, and *Oncorhynchus rhodurus*). Dots represent residues that are identical to *Micropterus salmoides*. The putative signal peptide is underlined and the predicted transmembrane domains are shaded in green. Potential N-linked glycosylation sites are marked in lemon yellow and cysteines are boxed in blue. The positions of α-helices and β-sheets are upper lined with red solid and dashed lines, respectively.

Three conservative domains of TSHR were identified, including two 91-amino acid leucine-rich repeat (LRR_5) domains, and a 248-amino acid 7 transmembrane receptor (7tm_1) domain. The homology analysis, carried out using the BLAST server at the NLM NIH (http://blast.ncbi.nlm.nih.gov/Blast.cgi), revealed high similarities among the three identified conservative domains in largemouth bass and other species. The 7tm_1 domain was 89% identical to *D. labrax*, 97% to *M. saxatilis*, 94% to *O. rhodurus*, 96% to *S. senegalensis*, 93% to *Oncorhynchus* sp, and 90% to *Boleophthalmus pectinirostris*. The first LRR_5 was 93% similar to *M. saxatilis* and *D. labrax*, 87% to *S. senegalensis*, 81% to *Oncorhynchus* sp, and 82% to *O. rhodurus*. The second LRR_5 was 92% identical to *M. saxatilis* and 91% to *S. senegalensis*. The complete amino acid sequence showed 58.4-90.2% identity and 5.6-125.8 divergence with some fish species (Table 2). A phylogenetic tree composed of fourteen representative species was constructed using the neighbor-joining method (Figure 2). The clades and branch lengths showed an overall consistency with the known evolution of these species.

Table 2. Percent identity and divergence of TSHR amino acid sequence among different species.

		Identity (%)						
		1	2	3	4	5	6	7
Divergence	1	-	58.4	61.7	90.2	83.8	76.4	76.4
	2	47.9	-	80.0	58.6	57.9	59.2	59.5
	3	43.0	17.3	-	61.7	60.4	62.3	61.3
	4	5.6	48.3	43.4	-	84.3	77.4	75.1
	5	12.8	49.5	44.6	12.4	-	74.0	71.6
	6	125.8	163.7	159.9	125.6	136.7	-	85.3
	7	23.1	47.8	46.1	24.4	29.7	105.0	-

1: *Micropterus salmoides*; 2: *Clarias gariepinus* (AAN01360.1); 3: *Ictalurus punctatus* (AY533543); 4: *Morone saxatilis* (AAF80596.1); 5: *Solea senegalensis* (CBK38913.1); 6: *Oncorhynchus rhodurus* (JC7389); 7: *Oncorhynchus rhodurus* (BAB07801.1).

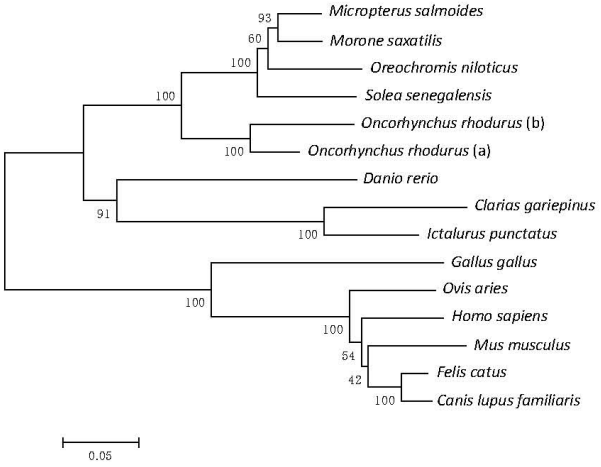

Figure 2. Phylogenetic tree based on the deduced amino acid sequences of 14 different species. The value at each branching displayed as bootstrap information. The value on the scale displayed as genetic distance.

TSHR transcription level in largemouth bass

The transcription of TSHR was detected in all collected tissues in the present study (Figure 3). The most abundant transcription levels were found in the kidney, head kidney, and spleen. In contrast, low transcript levels were found in the thyroid tissue, liver, stomach, intestine, heart, and muscle. The transcription level of TSHR in thyroid tissue was significantly higher at 2 and 4 h after feeding, compared to all other times (Figure 4). It was significantly lower after 6 h than at -1, 0, 2, 4, and 24 h. However, at any sampling time, the transcription level did not show significantly differences between the fasted and fed groups.

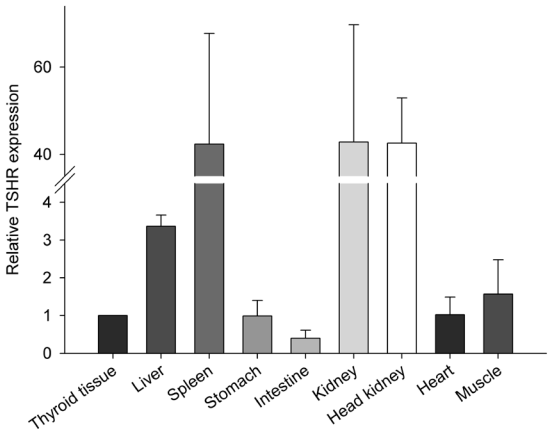

Figure 3. Expression of TSHR mRNA levels across different tissues measured by real-time PCR using the ΔΔCT method. Vertical bars represent the mean ± SE (N = 5). The expression values were calculated relative to the expression in thyroid tissue (Thyroid tissue = 1).

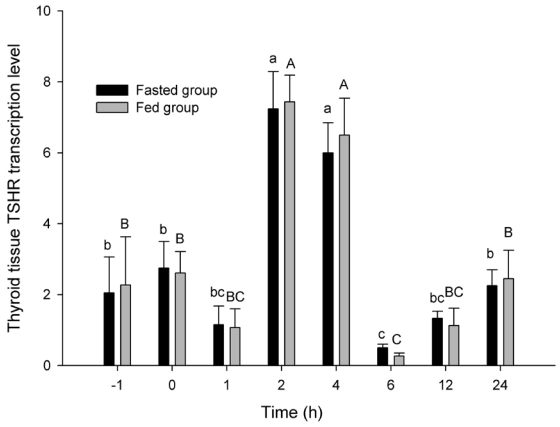

Figure 4. Transcription of TSHR in thyroid tissue of largemouth bass (*Micropterus salmoides*) in response to feed intake. Lower case and upper case superscripted letters indicate significant differences among the different time points within the fasted and fed groups, respectively.

DISCUSSION

Like other members of the glycoprotein hormone receptor family (Farid and Szkudlinski 2004), the TSHR in largemouth bass had a large amino-terminal extracellular domain. This was connected to seven transmembrane domains, followed by the intracellular carboxy-terminal domain. The seven transmembrane domains accommodate small ligands between the helices and extracellular loops (Hsu and Hsueh, 2000).

Cysteine residues in the extracellular domain are responsible for intramolecular disulfide bridges. These facilitate tertiary folding and are important for the linkage between the extracellular and transmembrane domains (Kursawe and Paschke, 2007). As most glycoprotein hormone receptors, the amino-terminal cysteines (residues 27, 31, 33, and 43) in TSHR form two disulfide bonds that influence the cell-surface expression. However, instead of binding TSH, the amino-terminal cysteine cluster was considered to be involved in the epitope for the binding of thyroid-stimulating autoantibodies (Chen et al., 2001).

Glycosylation is a posttranslational modification that determines correct folding and secretion of TSHR (Kursawe and Paschke, 2007). Five potential N-linked glycosylation sites were identified in the TSHR extracellular domain. With the exception of one site (residue 224-226), the sites were found to be identical to those identified in *M. saxatilis* (Kumar et al., 2000) and *S. senegalensis* (Ponce et al., 2010). In largemouth bass, three of the identified sites, corresponded to NIS (residues 77-79), NGT (residues 198-200), and NLT (residues 302-304) of TSHR, confirming that these are highly conserved in teleosts. The first conserved glycosylation site is unique to TSHRs, whereas the other two are also found in other members of the glycoprotein hormone receptor family (Vischer and Bogerd, 2003). It has been reported that four glycosylation sites are necessary for expression and biologic activity in human TSHR, regardless of location. In contrast, the number of potential N-linked glycosylation sites in the extracellular domain of TSHR was found to be three for *C. gariepinus* (Vischer and Bogerd, 2003), *I. punctatus* (Goto-Kazeto et al., 2003), and *O. rhodurus* (Oba et al., 2000). The relationship between the functionality and the three potential N-linked glycosylation sites in teleosts is still not clear.

The TSHR transcripts both in thyroid tissue and in extra-thyroidal organs were detected in the present study. The abundant transcription was detected in the spleen, head kidney, and kidney. The spleen and head kidney are organs related to immune function in fish. This has been done also in other species, including *C. gariepinus* (Vischer and Bogerd, 2003), *I. punctatus* (Goto-Kazeto et al., 2009), *S. senegalensis* (Ponce et al., 2010), *M. saxatilis* (Kumar et al., 2000), and *D. labrax* (Rocha et al., 2007). In previous studies, TSHR expression was measured in the gonads, brain, muscle, cerebellum, pituitary, liver, kidney, head kidney, stomach, intestine, etc. In general, a high extra-thyroidal transcription is reported for the brain, cerebellum, pituitary, gonads, and ventral aorta, whereas lower transcription is typically found for the other tissues and organs. It is known that the TSH binds to TSHR and that the TSHR then initiates procedures for the synthesis, storage, and secretion of thyroid hormones in the thyroid follicles (Ji et al., 1998; Vischer and Bogerd, 2003). Maugars et al. (2014) concluded that TSHb3 together with TSHRb was distributed in non-thyroidal tissues due to autocrine/paracrine. However, the functions of TSHb3 and TSHRb are still not completely understood.

The present study detected abundant transcription in the spleen, head kidney, and kidney. The spleen and head kidney are organs related to immune function in fish. Thus, these results may indicate that TSH may be involved in the immune response via TSHR. The

response of the immune system and cells to thyroid hormones has been reported previously (Quesada-García et al., 2014). Other studies have suggested that the immune cells, including monocytes, lymphocytes, macrophages, and subset of naïve peripheral T cells used TSH as a mediator for communication during the earliest stages of antigen immune response (Wang and Klein, 2001). TSH has also been found to exhibit a variety of immune-regulating cytokine-like activities (Klein, 2003). However, the role of TSHR in the immune response process is still not fully understood. The transcription level of TSHR in thyroid tissue was lower than that in liver, spleen, kidney, head kidney, heart, and muscle. This may be because the thyroid follicles in fish consist of individual follicles located between the gill arch and the bulbus arteriosus, rather than a compact glandular structure (Porazzi et al., 2009). Therefore, the thyroid tissue sample used for real-time PCR may have consisted of a mix of other tissues, resulting in an altered TSHR transcription level.

The TSHR transcription level in the thyroid tissue did not show significant differences between the fasted and fed groups. This indicates that feeding did not affect the transcription level of TSHR over the course of the day. Le Bail and Rœuf (1997) found no relationships between appetite, time of meal, or level of food ration and T3 over the course of the day. This is corroborated by the TSHR expression patterns observed in the present study. The variance in transcription level of TSHR at different sampling times may be due to the daily life cycle. Ebbesson et al. (2008) also found variance in daily thyroid hormone levels in plasma of parr and smolt *Salmo salar*.

In conclusion, the cDNA coding for a functional TSHR in *M. salmoides* was cloned in the present study. The TSHR transcription level both in thyroid tissue and in extra-thyroidal organs suggested that TSHR may play a role in the immune response, in addition to regulating thyroid function. Daily feeding did not affect the transcripts of TSHR in thyroid tissue.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by grants from the Zhejiang Provincial Natural Science Foundation (#LY14C190009), the Ningbo Natural Science Foundation (#2014A610193), the Ningbo Science and Technology Bureau (#2012B82016), and the Science and Technology Department of Zhejiang Province (#2012C12907 and #2013C32054). The authors would like to thank Dr. Karl D. Shearer for assistance with the English language check.

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