Cloning and Characterization of Cholesterol 25-Hydroxylase (ch25h) From a Marine Teleost, Chinese Tongue Sole (Cynoglossus semilaevis), and Its Gene Expressions in Response to Dietary Arachidonic Acid

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Our previous studies have shown that ARA regulated the gonadal steroidogenesis and the synthesis of gonadotrophin-releasing hormone (GnRH) in the brain in a gender-dependent manner in Chinese tongue sole, a marine teleost with typical sex dimorphism. As a following up-study, the current study aimed to clone and characterize a gene responding to dietary ARA differently between males and females, cholesterol 25-hydroxylase (ch25h), which has important roles in cholesterol and lipid metabolism of mammals, but is rarely investigated in fish. The full-length cDNA of Chinese tongue sole ch25h was cloned and characterized, and its transcription in the gonads, brain and liver of both males and females in response to dietary ARA levels (0.34, 2.53, and 9.63% of TFA) was investigated. The Chinese tongue sole Ch25h, which putatively is Ch25h subtype B, shared moderate identity to its known orthologs of other teleost and lower identity to human Ch25h. It has high transcription levels in the gonads, followed by skin and muscle, but low levels in the intestine, spleen, and kidney. High ARA levels significantly increased the ch25h transcription in the gonads and brain of male fish, but did not affect the transcription in the females. However, the effect of dietary ARA on ch25h transcription in the liver showed an opposite gender-difference pattern to those in the gonads and brain. To our knowledge, this is the first study in marine teleost investigating the nutritional regulation of ch25h expression.

Keywords: Cynoglossus semilaevis, Ch25h, cloning, characterization, gene expression, arachidonic acid

INTRODUCTION

The membrane-associated enzyme cholesterol 25-hydroxylase (Ch25h) catalyzes the formation of 25-hydroxycholesterol (25HC, oxysterol) from cholesterol, and thereby plays important roles in cholesterol and lipid metabolism (Lund et al., 1998; Horton et al., 2002; Joseph et al., 2002). It has been reported that 25HC is a co-repressor that blocks sterol regulatory element binding protein...
(SREBP) processing and ultimately leads to inhibition of gene transcription (Lund et al., 1998). 25HC also acts as a ligand of liver X receptor (LXR). The regulation of LXR/SREBP signaling pathway by 25HC reduces cholesterol synthesis and increases its efflux and elimination (Janowski et al., 1996; Accad and Farese, 1998; Radhakrishnan et al., 2007). The regulation of LXR/SREBP signaling pathway by 25HC also affects lipid metabolism in other ways depending on the roles of SREBP and LXR in lipid metabolism (Shimano, 2001; Oosterveer et al., 2010; DeBoise-Boyd and Ye, 2018). In addition to the well-known metabolic role of oxysterols, some publications have also reported the function of 25HC in immune regulation and virus resistance (Yi et al., 2012; Shrivastava-Ranjan et al., 2016; Cagno et al., 2017; Doms et al., 2018; Shawli et al., 2019; Zhang et al., 2019).

Compared to mammals, the functions of Ch25h or 25HC in fish have been poorly understood. Only in zebrafish, the interferon independent antiviral role of 25HC was validated (Pereiro et al., 2017). In Chinese tongue sole, a recent study of ours with feeding trial followed by transcriptomic analysis has shown that ch25h transcription in the brain was significantly affected by dietary arachidonic acid (ARA), which plays important roles in fish reproduction (Izquierdo et al., 2001; Norberg et al., 2017), and this effect was different between male and female fish (Xu et al., 2019). Our previous studies have also shown that dietary ARA differentially regulates the gonadal steroidogenesis in Chinese tongue sole depending on fish gender (Xu et al., 2017a). Chinese tongue sole have typical characteristics of sex dimorphism. The different response of cholesterol metabolism to dietary ARA in male and female Chinese tongue sole seems interesting and is worth further investigation. As a following up study, the present study aimed to clone and characterize the full-length mRNA of Chinese tongue sole ch25h, as well as to investigate its transcription in response to dietary ARA in different tissues of both male and female fish. The results will contribute to the general knowledge of Ch25h physiology in fish.

MATERIALS AND METHODS

Feeding Trial

Three experimental diets containing different levels of ARA were used in the feeding trial (Table 1). In the control diet (Diet C), tristearin was used as the main supplemented lipid source. Diets with low (Diet ARA-L) and high (Diet ARA-H) ARA level were prepared by replacing tristearin in diet C with ARA-enriched oil. The ARA content in the three experimental diets was 0.34, 2.53, and 9.63% of total fatty acids (TFA), respectively. Table 2. Constant levels of n-3 LC-PUFA enriched oil and soya lecithin were supplemented to all the diets to meet the requirement. The experimental diets were prepared following the routine procedures in our laboratory (Xu et al., 2016).

A 10-week feeding trial was conducted to investigate the effects of dietary ARA on the ch25h gene expressions in Chinese tongue sole. Chinese tongue sole hatched in the last autumn was used in the feeding trial. The fish have been fed a commercial diet before the experiment. Fifteen male fish with an average initial body weight of 20.3 g and eight female fish with an average initial body weight of 72.0 g were reared in each polyethylene tank (200 L). At the beginning of the feeding trial, the fish were fed the control diet for 7 days to acclimate to the experimental conditions. The feeding trial was conducted in a flow-through seawater system in Huanghai Aquaculture Co., Ltd., (Haiyang, China). Each diet was randomly assigned to triplicate tanks. Fish were hand-fed to apparent satiation twice daily (9:00 and 17:00). The tanks were cleaned daily by siphoning out residual feed and feces.

At the end of the feeding trial (late autumn), after being anesthetized with eugenol (1:10,000), the developmental status of fish gonads was determined before sampling. Most male fish were mature. The maturity of male fish was confirmed by the release of milt when handled. However, unfortunately, visual observation and microscopic examination of oocyte morphology showed that most female fish were immature, and the ovaries had not developed at all. Five mature male fish and five immature female fish per tank were dissected, and whole brain, gonad

| Table 1 | Formulation and composition of the experimental diets (g kg⁻¹ dry matter)a,b.
| Ingredients | C | ARA-L | ARA-H |
| --- | --- | --- | --- |
| Fish meal | 400.0 | 400.0 | 400.0 |
| Soybean meal | 150.0 | 150.0 | 150.0 |
| Casein | 60.0 | 60.0 | 60.0 |
| Corn gluten meal | 50.0 | 50.0 | 50.0 |
| Wheat meal | 222.2 | 222.2 | 222.2 |
| Vitamin premix | 2.0 | 2.0 | 2.0 |
| Mineral premix | 5.0 | 5.0 | 5.0 |
| L-ascorbyl-2-phosphate | 5.0 | 5.0 | 5.0 |
| Choline chloride | 5.0 | 5.0 | 5.0 |
| Monocalcium phosphate | 10.0 | 10.0 | 10.0 |
| Ethoxyquin | 0.5 | 0.5 | 0.5 |
| Betaine | 3.0 | 3.0 | 3.0 |
| Calcium propionate | 0.5 | 0.5 | 0.5 |
| Soya lecithin | 20.0 | 2.00 | 2.00 |
| n-3 LC-PUFA enriched oil | 15.0 | 1.50 | 1.50 |
| ARA enriched oil | 0.0 | 6.1 | 25.7 |
| Tristearin | 51.8 | 45.7 | 26.1 |
| Proximate composition | | | |
| Dry matter | 926.2 | 912.5 | 915.5 |
| Crude protein | 496.1 | 496.1 | 495.6 |
| Crude lipid | 136.4 | 127.6 | 123.5 |
| Ash | 90.6 | 101.8 | 101.8 |

aAll the ingredients were purchased from Qingdao Great Seven Co., Ltd. bVitamin premix (mg kg⁻¹ diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B6, 0.1 mg; vitamin K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin, 1 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocophorol, 120 mg; wheat middling, 60 mg; soya lecithin were supplemented to all the diets to meet the requirement. The experimental diets were prepared following the routine procedures in our laboratory (Xu et al., 2016).
were performed on peqSTAR (PEQLAB, Erlangen, Germany). All PCR amplifications were performed on peqSTAR (PEQLAB, Erlangen, Germany). The primers were synthesized by TsingKe Biological Technology Co., Ltd., (Qingdao, China). PCR amplifications were performed on peqSTAR (PEQLAB, Erlangen, Germany). The primers were synthesized by TsingKe Biological Technology Co., Ltd., (Qingdao, China). PCR amplifications were performed on peqSTAR (PEQLAB, Erlangen, Germany). All PCR products were run on a 1.5% agarose gel, and then purified by Zymoclean Gel DNA Recovery Kit (ZMYO RESEARCH, Irvine, CA, United States). PCR products were cloned into pEASY-T1 simple cloning vector (TransGen, Beijing, China) and sequenced in TsingKe (Qingdao, China). Other details of the PCR amplification were similar to our previous studies (Xu et al., 2014).

### Table 2: Fatty acids composition of experimental diets (% total fatty acids).

| Fatty acids | C | ARA-L | ARA-H |
|-------------|---|-------|-------|
| C14:0       | 2.61 | 2.42 | 2.19  |
| C16:0       | 37.83 | 34.83 | 25.60 |
| C18:0       | 19.35 | 18.58 | 13.39 |
| C20:0       | 0.30 | 0.36 | 0.45  |
| ΣSFA        | 60.09 | 56.19 | 41.63 |
| C16:1n-7    | 1.71 | 1.63 | 1.70  |
| C18:1n-9    | 4.90 | 5.45 | 7.12  |
| C18:1n-7    | 1.08 | 1.10 | 1.20  |
| ΣMUFA       | 7.69 | 8.19 | 10.02 |
| C18:2n-6    | 10.51 | 11.17 | 13.65 |
| C20:4n-6    | 0.34 | 2.53 | 9.63  |
| Σn-6 PUFA   | 10.86 | 13.70 | 23.28 |
| C18:3n-3    | 1.25 | 1.26 | 1.27  |
| C18:4n-3    | 0.70 | 0.67 | 0.67  |
| C20:5n-3    | 5.36 | 5.46 | 5.45  |
| C22:5n-3    | 1.35 | 1.35 | 1.49  |
| C22:6n-3    | 9.00 | 9.17 | 9.53  |
| Σn-3/Σn-6   | 17.65 | 17.92 | 18.40 |
| ARA/EPA     | 0.06 | 0.10 | 0.79  |

SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; n-6 PUFA, n-6 poly-unsaturated fatty acids; n-3 PUFA, n-3 poly-unsaturated fatty acid.

and liver samples were collected. All the tissue samples were immediately frozen with liquid nitrogen, and stored at −86°C before analysis. All sampling protocols, as well as fish rearing practices, were reviewed and approved by the Animal Care and Use Committee of Yellow Sea Fisheries Research Institute.

### RNA Extraction and cDNA Synthesis

Total RNA in livers was extracted using RNAiso Plus [TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China] and then electrophoresed on 1.5% agarose gel to test the quality and integrity. The concentration was determined with Colibri Microvolume Spectrometer (Titertek-Berthold, Germany). The RNA was then reversely transcribed with PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa) according to the user manual.

### Cloning and Sequencing of ch25h

The complete CDS of ch25h can be obtained from GenBank (Accession No.: XM_008315046.3). The predicted sequence from GenBank was validated with specific PCR and sequencing of the product. The full-length cDNA of ch25h was cloned with rapid amplification of cDNA ends (RACE). Specific primers for ch25h were designed based on the known ch25h sequence to clone the 5′- and 3′-end, respectively. The SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA, United States) was used to perform the RACE cloning, and the 3′- and 5′-end cDNA templates were synthesized according to the user’s manual. The primers were synthesized by TsingKe Biological Technology, Co., Ltd., (Qingdao, China). PCR amplifications were performed on peqSTAR (PEQLAB, Erlangen, Germany). All PCR products were run on a 1.5% agarose gel, and then purified by Zymoclean Gel DNA Recovery Kit (ZMYO RESEARCH, Irvine, CA, United States). PCR products were cloned into pEASY-T1 simple cloning vector (TransGen, Beijing, China) and sequenced in TsingKe (Qingdao, China). Other details of the PCR amplification were similar to our previous studies (Xu et al., 2014).

### Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR) Analysis

Real-time fluorescent quantitative PCR (qRT-PCR) was used to assay the relatively quantitative mRNA expression of ch25h in different tissues of Chinese tongue sole (10 3-year-old fish used, six males and four females, all at phase II of gonadal development), as well as the gene expression in fish from different dietary groups. β-actin (GenBank Accession No. KP033459.1) and β-2 microglobulin (β2M, GenBank Accession No. FJ965563.1) were used as the reference genes (Vandesompele et al., 2002). Specific primers for target genes and reference genes were designed using Primer 5.0 (Table 3) and synthesized by TsingKe Biological Technology Co., Ltd., (Qingdao, China). The amplification efficiency for all primers, which was estimated by standard curves based on a 6-step 4-fold dilution series of target template, was within 95~105%, and the coefficients of linear regression (R²) were more than 0.99. SYBR® Premix Ex Taq TM [TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China] and a quantitative thermal cycler (Roche LightCycler 96, Basel, Switzerland) were used for the real-time qPCR. The reaction system consists of 2 µl cDNA template, 10 µl SYBR® Premix Ex TaqTM (2×), 0.8 µl forward primer (10 µM), 0.8 µl reverse primer (10 µM), and 6.4 µl sterilized water. The program was as follows: 95°C for 5 min followed by 40 cycles of “95°C for 5 s, 55°C for 20 s, 72°C for 10 s.” Melting curve analysis (1.85°C increment/min from 58°C to 95°C) was performed after the amplification phase for confirmation of a sole product. Each sample was run in triplicate. The mRNA expression levels were calculated with qRT-PCR method: 2−ΔΔCT (Livak and Schmittgen, 2001).

### Table 3: Sequences of the primers used in this work.

| Primer | Sequence (5′-3′) | Type |
|--------|-----------------|------|
| ch25h-F0 | TGAACCTCGAGAAGACGACATGGT | CDS validation |
| ch25h-R0 | TGTCCCTCAGACACTTCTGCTCT | 3′ Race (outer) |
| ch25h-F1 | AAGGACACCTGTGCCTGACTGCA | 3′ Race (inner) |
| ch25h-F2 | ACCACACTGAAATCTCATTGAT | 3′ Race (inner) |
| ch25h-R1 | CCAGTTTTGACAGATGAGGGAAG | 5′ Race (outer) |
| ch25h-R2 | CTCGGAGGCTGGACCTTGTGTCG | 5′ Race (inner) |
| ch25h-F3 | CTCAGCGACACAACGAGGCT | qPCR |
| ch25h-R3 | GGAAGGAAGAAGTTTCTCACAAT | qPCR |
| β-actin-F | GGTACATCTTCTTCCACACCACA | qPCR |
| β-actin-R | GGGACAGAGACTTATCCAA | qPCR |
| β2M-F | AGCTGCTGTGCTTCTTGAT | qPCR |
| β2M-R | CCAACCTTCTTGCGCATG | qPCR |
**Statistical Analysis**

Similarity searches of the sequenced cDNA of ch25hs were done by blastn\(^1\). The multiple-sequence alignments of amino acids were performed using BioEdit. The deduced amino acid sequences were analyzed with DNAm and ExPASy Compute pI/MW\(^2\). SMART program\(^3\) and PROSITE program\(^4\) were used to predict the functional sites or domains in the amino acid sequence. Phylogenetic analyses based on amino acid sequences were carried out using the neighbor-joining method, and the trees were constructed using MEGA 4.1.

All gene expression data were subjected to one-way analysis of variance in SPSS 16.0 for Windows. Differences between means were tested by Tukey's multiple range test. The level of significance was chosen at \(P < 0.05\) and the results were presented as means ± standard error.

**RESULTS AND DISCUSSION**

**Cloning and Characterization of ch25h**

The full length of ch25h cDNA from Chinese tongue sole (uploaded under GenBank Accession No. MN646884.1) was 1468 bp, including a 5′-untranslated region (UTR) of 372 bp, a 3′-UTR of 340 bp, and an open reading frame of 756 bp encoding a polypeptide of 250 amino acids with predicted molecular weight of 28.7 KDa and theoretical isoelectric point of 8.22 (Figure 1). The deduced protein sequence possessed a characteristic fatty acid hydroxylase domain, containing three transmembrane regions (20–42, 74–91, and 106–128) and clusters of histidine residues that are essential for catalytic activity (Figure 1). Unlike most other sterol hydroxylases, Ch25h is not a cytochrome P450, but rather it is a member of a small family of enzymes that utilize diiron cofactors to catalyze the hydroxylation of hydrophobic substrates (Lund et al., 1998). The deduced Chinese tongue sole Ch25h has three potential phosphorylation sites (T-7, S-18, and Y-23), but no predicted signal peptide sequence.

**Multiple Sequences Alignment and Phylogenetic Analysis**

The multi-sequence alignment (Figure 1) revealed that the Chinese tongue sole Ch25h shared moderate identity to its known orthologs of other teleost (63–76% for the listed species in Figure 1) and lower identity to human Ch25h (54.6%).

The phylogenetic analysis showed that Chinese tongue sole Ch25h clusters closer to Ch25h subtype B of zebrafish, compared to other subtypes of zebrafish Ch25h, Ch25hA, Ch25hC1, Ch25hC2, and Ch25hD (Figure 2). The zebrafish Ch25hB showed synteny conservation with its human homolog, highlighting that this gene copy is probably the original gene of the Ch25h teleost repertoire (Pereiro et al., 2017). In contrast to fish, the mammalian genomes only possess one copy of the CH25H gene.

Chinese tongue sole Ch25h clusters to Ch25h of other fish species such as Nile tilapia, large yellow croaker, and gilthead seabream, distant from Ch25h of frog, and further distant from Ch25h of mammals (Figure 2). However, Chinese tongue sole Ch25h clusters closer to human Ch25h than to other Ch25h subtypes of zebrafish. This indicated that Ch25h subtypes may have diverged from a common progenitor before the fish/mammalian divergence.

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\(^{1}\)www.ncbi.nlm.nih.gov/blast/

\(^{2}\)http://web.expasy.org/compute_pi/

\(^{3}\)http://smart.embl-heidelberg.de/

\(^{4}\)http://kr.expasy.org/prosite/
Tissue Distribution of ch25h in Chinese Tongue Sole

In mice, expression of ch25h is high in the lung, heart, and kidney (Lund et al., 1998). Another study in mice showed that ch25h is highly expressed in the liver and peritoneal macrophages (Liu et al., 2018). In Chinese tongue sole, however, ch25h is highly expressed in the gonad, followed by skin and muscle, but is lowly expressed in the intestine, spleen, and kidney (Figures 3, 4).

There is minor difference in tissue expression pattern of ch25h between male and female Chinese tongue sole. It seemed that the females have relatively higher ch25h expression in the brain compared to males.

High expression of ch25h in the gonads indicated that Ch25h may have important roles in reproductive physiology of Chinese tongue sole. Recent data obtained in bovine sperm showed that sperm capacitation is associated with the formation of oxysterols (Brouwers et al., 2011). Another recent study with human semen also supports a role for 25HC in sperm function (Zerbinati et al., 2017). Results of that study showed that CH25H was detected in human spermatozoa at the neck and the post acrosomal area, and that the 25HC concentration positively correlated with spermatozoa number (Zerbinati et al., 2017).

High expression of ch25h in both testis and ovary of Chinese tongue sole indicated that Ch25h may have important functions in genesis of both sperm and egg.

Unexpectedly, the liver of Chinese tongue sole only has a moderate level of ch25h transcription, lower than skin and muscle, irrespective of fish gender. Liver is a very important organ for lipid and cholesterol metabolism. The present results indicate that Ch25h or 25HC may have special functions in skin and muscle of flatfish, but this speculation needs to be validated by future studies.

The Chinese Tongue Sole ch25h mRNA Expression in Response to Dietary ARA

In the present study, we analyzed the ch25h mRNA expression in the gonad, liver and brain of Chinese tongue sole fed diets with different ARA levels. We analyzed the ch25h mRNA expression in the gonads for two reasons: (1) As mentioned above, ch25h is highly expressed in the gonads; (2) Reproductive physiology is a focus of this study and its previous studies. A very interesting result of this study was that ch25h responded to dietary ARA differently between the testes and ovaries. The relative mRNA expression of ch25h in the testes significantly (P < 0.05) increased with increasing dietary ARA levels, but the transcription in the ovaries was not affected by dietary ARA (Figure 5).
This result was similar to the \( ch25h \) transcription in the brain in response to dietary ARA (Figure 6). The brain \( ch25h \) expression was significantly (\( P < 0.05 \)) higher in male fish fed the high-ARA diet compared to fish fed the low-ARA diet or the control diet. However, the brain \( ch25h \) transcription in female fish was not affected by dietary ARA. This result validated the previous transcriptomic results which indicated the gender-difference in brain \( ch25h \) transcription in response to dietary ARA and inspired the current study.

The differential regulation of gonadal steroidogenesis and brain GnRH synthesis by dietary ARA between male and female Chinese tongue sole has been shown in our previous studies (Xu et al., 2017a, 2019). Those results showed that dietary ARA supplementation stimulated the testosterone and GnRH production in males, but reduced the estradiol production in the females. ARA supplementation significantly reduced the mRNA expression of aromatase in ovaries but significantly increased the gene expression of \( 3\beta \)-hydroxysteroid dehydrogenase (\( 3\beta \)-HSD) in testes. Moreover, this gender-dependent differential regulation was in accord with the different ARA abundance in testes and ovaries (Xu et al., 2017a,b).

In the present study, it is interesting to validate another target gene, \( ch25h \), for the differential ARA action between male and female Chinese tongue sole. The present results indicated that \( ch25h \) may have more active function in male fish. Considering the roles of \( ch25h \) in sperm mentioned previously (Brouwers et al., 2011; Zerbinati et al., 2017), the more positive roles of ARA in male Chinese tongue sole may stimulate the gene expression of \( ch25h \) in male fish by accelerating the overall reproductive performance. Also, mammal studies showed that Leydig cells can directly metabolized 25HC enzymatically produced by testicular macrophages to testosterone (Lukyanenko et al., 2001, 2002). Moreover, liver X receptors (LXR\( \alpha \) and LXR\( \beta \)), receptors of oxysterols, have been shown to present crucial activities in reproductive organs of male animals such as testis and epididymis, as well as prostate [well-reviewed by El-Hajaji et al. (2011)]. 25HC, the product of Ch25h, may function in the testis and brain of male animals in LXR-dependent mechanisms (Liu et al., 2018).

In spite of the potential different functions of Ch25h between male and female animals, another important factor contributing to the current gender-difference in Ch25h transcription in Chinese tongue sole was the asynchronous gonadal development. In the present study, most of the male fish was mature but the females were immature. However, whether this asynchronous gonadal development resulted in gender-difference in Ch25h transcription or vice versa cannot be concluded based on the current information. In addition, considering that both ARA and Ch25h are involved in immunity (McDonald and Russell, 2010; Xu et al., 2010; Li et al., 2012; Cyster et al., 2014; Shahkar et al., 2016; Adam et al., 2017; Nayak et al., 2017; Wu et al., 2018), Ch25h-mediated immune response could be another process differently responding to dietary ARA between male and female Chinese tongue sole.

In the liver of Chinese tongue sole, a more interesting result was that the response of \( ch25h \) transcription to dietary ARA was obviously different from those in the gonad and brain.
The ch25h expression in the liver of male fish was not significantly affected by dietary ARA (Figure 7). However, the hepatic ch25h expression in female fish was significantly ($P < 0.05$) higher in the low-ARA group compared to the control group, while the high-ARA group had an intermediate expression level (Figure 7). This result indicated that Ch25h may function differently between liver and gonad/brain of Chinese tongue sole. Liver plays a crucial role in maintaining cholesterol homeostasis by regulating absorption and synthesis (Goldstein and Brown, 1990; Goldstein and DeBose-Boyd, 2006; Lu et al., 2017). Due to the lipid nature of the ligands (oxysterol), the physiological roles of LXRs have been extensively detailed in the homeostasis of cholesterol in the gut-liver axis (D’Errico and Moschetta, 2008). However, no information is available about the difference between males and females in Ch25h-mediated hepatic cholesterol metabolism, neither in mammals nor in fish. Gender-difference in correlation between cholesterol level and liver inflammation (Comhair et al., 2011) or response of hepatic cholesterol level to dietary nutrients (Kishida et al., 2006) has been reported in rodents, but no results can be used to explain the present results. In fish, studies in freshwater fish Notopterus notopterus have shown that the hepatic cholesterol content changes differently between males and females during the gonadal development and reproductive process (Shankar and Kulkarni, 2005, 2007). Therefore, a possible explanation of the current result could be the different reproductive phases the male and female Chinese tongue sole stayed when sampled.

CONCLUSION

In conclusion, as a following-up study of previous studies which showed the differential function of ARA between male and female Chinese tongue sole, the current study cloned and characterized a gene responding to ARA in a gender-dependent manner, ch25h. This gene was highly expressed in gonads, followed by skin and muscle. Its mRNA expression in the gonad and brain of male fish was significantly increased by high dietary ARA levels, but the transcription in female fish was not affected by dietary ARA. The ch25h expression in the liver in response to dietary ARA showed an opposite gender-difference pattern to those in the gonad and brain. The interaction among Ch25h, ARA, and sex dimorphism of Chinese tongue sole warrants further studies.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the GenBank, Accession No. MN646884.1.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Care and Use Committee of Yellow Sea Fisheries Research Institute.

AUTHOR CONTRIBUTIONS

HX and ML designed the study and wrote the manuscript. BS and YW conducted the feeding trial. LJ and ZL did the qPCR studies. HX and ML designed the study and wrote the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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