The Transcriptional Factor PPARαb Positively Regulates Elovl5 Elongase in Golden Pompano Trachinotus ovatus (Linnaeus 1758)

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INTRODUCTION

The nuclear peroxisome proliferator-activated receptors (PPARs) regulate the transcription of elongases of very long-chain fatty acids (Elovls), which are involved in polyunsaturated fatty acid (PUFA) biosynthesis in mammals. In the present study, we first characterized the function of Elovl5 elongase in Trachinotus ovatus. The functional study showed that ToElovl5 displayed high elongation activity toward C18 and C20 PUFA. To investigate whether PPARαb was a regulator of Elovl5, we also reported the sequence of T. ovatus PPARαb (ToPPARαb). The open reading frame (ORF) sequence encoded 469 amino acids possessing four typical characteristic domains, including an N-terminal hypervariable region, a DNA-binding domain (DBD), a flexible hinge domain and a ligand-binding domain (LBD). Thirdly, promoter activity experiments showed that the region from PGL3-basic-Elovl5-5 (−146 bp to +459 bp) was defined as the core promoter by progressive deletion mutation of Elovl5. Moreover, PPARαb overexpression led to a clear time-dependent enhancement of ToElovl5 promoter expression in HEK 293T cells. Fourth, the agonist of PPARαb prominently increased PPARαb and Elovl5 expression, while PPARαb depletion by RNAi or an inhibitor was correlated with a significant reduction of Elovl5 transcription in T. ovatus caudal fin cells (TOCF). In conclusion, the present study provides the first evidence of the positive regulation of Elovl5 transcription by PPARαb and contributes to a better understanding of the transcriptional mechanism of PPARαb in fish.

Keywords: Trachinotus ovatus, promoter activity, transcription factors, PPARα, Elovl5
In mammals, PPARα is activated by fatty acids or their derivatives and plays pleiotropic roles in lipid metabolism, such as stimulating the expression of genes related to peroxisomal and mitochondrial fatty acid oxidation and LC-PUFA biosynthesis (Desvergne et al., 2006). PPARα agonists (WY14643) affect fatty acid elongation pathways, thereby increasing Elovl5 expression in adult Rattus norvegicus (Wang et al., 2005). Moreover, in PPARα-defective mice, PPARα was required for the WY14643-mediated induction of Elovl5 and Elovl6 (Wang et al., 2006). Cold-induced Elovl3 mRNA levels were under the control of PPARα in Mus musculus (Jakobsson et al., 2005). Nevertheless, the role of PPARα in the expression of Elovl5 is less understood in fish. Furthermore, PPARα stimulates the expression of target genes directly through binding to PPAR response elements (PPREs) in the promoter regions of target genes. Dong et al. (2017) indicated that PPARα bound to the Fads2 promoter region and upregulated the transcription of Fads2 in fish. PPARα has been implicated as a trans-acting factor that promotes insulin-induced gene (Insig2α) expression, consequently suppressing sterol-regulatory element binding protein 1c (SREBP-1c) processing during fasting (Lee et al., 2017).

The rate-limiting condensation step is catalyzed by Elovl5 in the elongation of fatty acids in LC-PUFA biosynthesis (Nugteren, 1965; Jakobsson et al., 2006). Elovl5 has been verified and functionally characterized as a critical enzyme in the elongation step of LC-PUFA biosynthesis (Castro et al., 2016; Li et al., 2017; Lin et al., 2018). Elovl5 could effectively elongate C18, C20, and C22 PUFAs and has been isolated from various teleost species (Bell and Tocher, 2009; Monroig et al., 2012; Xie et al., 2016).

In fish, Elovl5 was isolated, and in PUFA biosynthesis, it was consistent with that in mammals and invertebrates (Monroig et al., 2012; Gregory and James, 2014; Kabeya et al., 2015; Li et al., 2016), suggesting a conserved function of Elovl5 in metazoans. Teleost fish, particularly marine fish, are unique and rich sources of omega-3 (n-3) LC-PUFAs in the human diet (Tocher, 2015). The golden pompano Trachinotus ovatus (Linnaeus 1758), Carangidae, and Perciformes are broadly cultivated in the Asia-Pacific region and considered important aquaculture species in China (Sun et al., 2014; Zhen et al., 2014). Furthermore, high levels of LC-PUFA content were detected in T. ovatus muscle (Zhang et al., 2010). Hence, to investigate whether T. ovatus PPARα (ToPPARα) would be a mediator of ToElovl5, the sequence characterization, tissue distribution and transcriptional regulation of ToPPARα were determined. The present study of ToPPARα presents a potential molecular pathway of LC-PUFA biosynthesis mechanisms.

### MATERIALS AND METHODSS

#### Ethics Statement

All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of fishery Sciences (No. SCSFR196-253) and performed according to the regulations and guidelines established by this committee.

### Gene Cloning and Bioinformatics

The Elovl5 and PPARα predicted sequence were obtained from genomic data for T. ovatus (Accession No. PRJEB22654 under ENA, Sequence Read Archive under BioProject PRJNA406847). To determine the accuracy of the encoding sequence of Elovl5 and PPARα, gene-specific primers were designed (Supplementary Table S1) based on the putative sequence. Total RNA (1 µg) was extracted from T. ovatus liver (Trizol reagent, Invitrogen, United States) and was reverse transcribed into cDNA by random hexamer primers (Cloned AMV First-Strand cDNA Synthesis Kit, Invitrogen, United States). The 3’ of the transcript was cloned from liver cDNA using specific primers with the SMART™ RACE cDNA amplification kit (Clontech, Mountain View, CA, United States). PCR was conducted as previously described (Zhu et al., 2014).

Amino acid sequence of ToPPARα was used as queries to search for the homologous genes in NCBI database1. All available PPARα genes and mature peptides were downloaded from Ensembl2 and Genome Browser3. The gene structure was predicted by the SANTA CRUZ Genome Browser (see footnote 3), and signal peptides were detected with SignalP software4. Molecular weight and theoretical isoelectric point were calculated by Compute pl/Mw software5. A three-dimensional (3D) model of the ToPPARα amino acid sequence was developed by the SWISS-MODEL Protein Modelling Server. To better understand the relationship of PPARα in metazoans, all PPARα amino acid sequences were aligned by ClustalW26. Artificially arranged the ambiguously aligned sequences, and then a maximum likelihood (ML) phylogenetic tree (LG + G model, bootstrap 1000) of PPARα putative proteins was constructed by MEGA 6 software (Tamura et al., 2013).

### Heterologous Expression of the ToElovl5 Elongase ORFs in Yeast

PCR fragment corresponding to the ORF of the Elovl5 elongase was amplified from T. ovatus liver cDNA using primers that included HindIII and XhoI restriction sites (Supplementary Table S1). Subsequently, the DNA fragment was digested with the relevant restriction endonucleases (New England BioLabs, Herts, United Kingdom) and ligated into a coincident restricted pYES2 yeast expression vector (Invitrogen, Paisley, United Kingdom). The recombinant plasmid (pYES2-Elovl5) was then used to transform Saccharomyces cerevisiae competent cells (S.c. EasyComp Transformation Kit, Invitrogen). Transformation and selection of yeast with recombinant plasmids, and yeast culture were prepared according to previously described methods (Li et al., 2017). Fatty acids are: 18:3n-3 (α-linolenic acid), 18:3n-6 (γ-linolenic acid), 18:4n-3 (stearidonic acid), 20:4n-6 (arachidonic acid, ARA) and 20:5n-3 (eicosapentaenoic acid, EPA) were used as substrates for detecting the elongase activity.

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1. [http://blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi)
2. [http://asia.ensembl.org/](http://asia.ensembl.org/)
3. [http://genome.ucsc.edu/cgi-bin/hgBlat](http://genome.ucsc.edu/cgi-bin/hgBlat)
4. [http://www.cbs.dtu.dk/services/SignalP/](http://www.cbs.dtu.dk/services/SignalP/)
5. [http://www.expasy.org/protparam/](http://www.expasy.org/protparam/)
6. [http://www.ebi.ac.uk/Tools/msa/clustalw2/](http://www.ebi.ac.uk/Tools/msa/clustalw2/)
Moreover, the ORF of ToPPAR\textsuperscript{−}\textsuperscript{+}Kpn reporter plasmid (Promega, United States). Five recombinant including restriction sites for Nhe\textsuperscript{−}\textsuperscript{−} were amplified by specific primers (Supplementary Table S1) and subcloned into the Kpn\textsuperscript{−}\textsuperscript{−} and Xho\textsuperscript{+}\textsuperscript{+} restriction sites of the pGL3-basic luciferase reporter plasmid (Promega, United States). Five recombinant plasmids, denoted pGL3-basic-Elovl5-1 (–382 to +89), pGL3-basic-Elovl5-2 (–793 to +89), pGL3-basic-Elovl5-3 (–1262 to +89), pGL3-basic-Elovl5-4 (–146 to +265) and pGL3-basic-Elovl5-5 (–146 to +459), were constructed (Figure 5). Moreover, the ORF of ToPPAR\textsubscript{b} was amplified with primers including restriction sites for Nhel and HindIII, respectively. The DNA fragment was digested with the corresponding restriction endonucleases (Takara, Japan) and ligated into a pCDNA3.1 vector (Invitrogen, United States).

Plasmid Construction, Cell Culture, and Dual-Luciferase Reporter Assays
Total DNA was extracted from T. ovatus muscle using a Genomic DNA Isolation Kit (Invitrogen, United States). To investigate the role of PPAR\textsubscript{b} in the transcriptional regulation of ToElovl5, five different promoter regions of ToElovl5 were amplified by specific primers (Supplementary Table S1) and subcloned into the Kpn\textsuperscript{−}\textsuperscript{−} and Xho\textsuperscript{+}\textsuperscript{+} restriction sites of the pGL3-basic luciferase reporter plasmid (Promega, United States). Five recombinant plasmids, denoted pGL3-basic-Elovl5-1 (–382 to +89), pGL3-basic-Elovl5-2 (–793 to +89), pGL3-basic-Elovl5-3 (–1262 to +89), pGL3-basic-Elovl5-4 (–146 to +265) and pGL3-basic-Elovl5-5 (–146 to +459), were constructed (Figure 5). Moreover, the ORF of ToPPAR\textsubscript{b} was amplified with primers including restriction sites for Nhel and HindIII, respectively. The DNA fragment was digested with the corresponding restriction endonucleases (Takara, Japan) and ligated into a pCDNA3.1 vector (Invitrogen, United States).

The Renilla luciferase plasmid pRL-TK (Promega, United States) was used as an internal control. Plasmids for transfection were prepared using the TransGen Plasmid Mini DNA Isolation Kit (Invitrogen, United States). To investigate the knockdown of ToElovl5, human embryonic kidney (HEK 293T) and T. ovatus caudal fin (TOCF) cell culture and transfection experiments were performed according to Li et al. (2017) and Wei et al. (2018), respectively.

PPAR\textsubscript{b} Overexpression and Knockdown
RNA interference (siRNA) of PPAR\textsubscript{b} (PPAR\textsubscript{b}-si) and corresponding negative controls (si-NC) were purchased from Genecreate (Wuhan, China). Lipofectamine RNAiMAX transfection reagent (Invitrogen, United States) was used for transfection in TOCF cells. The PPAR\textsubscript{b} siRNA sequence is listed in Supplementary Table S1. Additionally, the agonist and inhibitor of PPAR\textsubscript{a} were used to clarify the role of the transcription factor in the regulation of ToElovl5 elongases. WY-14643 (0.1, 1, and 4 \mu mol/L, Sigma, United States) was used as a PPAR\textsubscript{b} agonist, whereas GW6471 (0.1, 1, and 4 \mu mol/L, Sigma, United States) was used as a PPAR\textsubscript{b} inhibitor. Total RNA was extracted from TOCF cells as described above. The experiment was performed according to Li et al. (2017).

Quantitative Real-Time PCR
The tissue distributions of PPAR\textsubscript{b} mRNA levels were described by quantitative real-time polymerase chain reaction (qRT-PCR) using adult T. ovatus tissues (n = 6), including small intestine, liver, white muscle, brain, spleen, fin, gill, head-kidney, stomach, blood, and male (n = 3) and female gonad (n = 3) cDNA, as templates. Then, total RNA was isolated from 12 tissues as described above. The PrimeScript\textsuperscript{®} RT reagent Kit with gDNA Eraser (Takara, Japan) was used to synthesize cDNA from total RNA (1 \mu g). Specific primers and the housekeeping gene EF-1\textalpha (elongation factor 1, alpha) are displayed in Supplementary Table S1. The qRT-PCR was performed as previously described (Zhang et al., 2018). Relative expression was evaluated by the 2\textsuperscript{−}\Delta\DeltaCT method (Livak and Schmittgen, 2001).

Statistical Analysis
Statistical analysis was performed using SPSS 19.0 software (IBM, United States). The data from different tissues and groups were analyzed by the Duncan test using one-way ANOVA. Data are shown as the means ± SD, and p < 0.05 indicates statistical significance.

RESULTS
Sequence Characterization of ToElovl5 and ToPPAR\textsubscript{b}
The genomic sequence of ToElovl5 elongase is 6,617 bp, including seven exons and six introns, while the full-length cDNA sequence is 3,764 bp, containing 185 bp of 5' untranslated region (5'-UTR), a 885 bp ORF encoding a polypeptide of 294 amino acids and a 2,694 bp 3'-UTR including a polyA signal sequence (GenBank accession number: KY860144; Supplementary Figure S1). Furthermore, similar to other teleost Elovl5 proteins, ToElovl5 deduced proteins possess three highly conserved domains (CD1-3), including the histidine box motif (HXXHH) (CD2), conserved in the elongase family (Xie et al., 2016). KXXXX motif was regarded as putative endoplasmic reticulum (ER) retention signal in Elovl5 carboxyl terminal (C-terminal). Five putative transmembrane-spanning regions, including hydrophobic amino acid (aa) stretches were predicted by comparison with other vertebrate Elovl proteins.

The genomic sequence of ToPPAR\textsubscript{b} is a 13,262 bp sequence, including six exons and five introns, containing a 1,407 bp ORF encoding a polypeptide of 469 amino acids (GenBank accession number: MH321826; Supplementary Figure S2) with a predicted molecular weight of 52,644 kDa and theoretical isoelectric point of 5.48. Furthermore, similar to other teleost PPAR\textsubscript{b} proteins, ToPPAR\textsubscript{b} deduced proteins possess four domains containing an N-terminal hypervariable region (A/B), conserved DNA-binding domain (DBD) (C), flexible hinge domain (D) and ligand-binding domain (LBD) (E/F) (Figure 1B). The twelve \alpha-helices (H) and four parts of the \beta-sheet (S) were predicted by comparison with other vertebrate PPAR\textsubscript{a} proteins, and two zinc finger domains (Amino acid residues located in the C\textsuperscript{103}-C\textsuperscript{123} and C\textsuperscript{140}-C\textsuperscript{157}) were in the DBD.

Functional Characterization of the ToElovl5 Elongase
The role of the ToElovl5 elongase in LC-PUFA biosynthesis was investigated by growing transgenic yeast expressing the ToElovl5
cDNA in the presence of potential PUFA substrates. The results of heterologous expression showed that ToElovl5 possessed high conversion activity toward C20 PUFA, especially 20:5n-3 (86.6 %) and 20:4n-6 (84.8 %), followed by C18 substrates containing 18:3n-6 (67.4 %), 18:4n-3 (58.3 %), and 18:3n-3 (49.7 %) (Figure 2 and Table 1).
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FIGURE 2 | Functional characterization of the putative Elovl5 in transgenic yeast. Fatty acid methyl esters (FAMEs) were extracted from yeast transformed with the pYES2-Elovl5 and grown in the presence of PUFA substrates 18:3n-3 (A), 18:3n-6 (C), 18:4n-3 (E), 20:4n-6 (G), and 20:5n-3 (I). Based in retention times, additional peaks (marked with a triangular sign) were identified as 20:3n-3 (B), 20:3n-6 (D), 20:4n-3 (F), 22:4n-6 (H), and 22:5n-3 (J). Peaks 1–4 represent the main endogenous FAs of T. ovatus, namely C16:0, C16:1 isomers, C18:0 and C18:1n-9, respectively. Raw data are presented in Supplementary Data Sheets 1–12.

TABLE 1 | Conversion rates of pYES2-Elovl5 transformed yeast grown in presence of 18:3n-3, 18:3n-6, 18:4n-3, 20:4n-6, and 20:5n-3 substrates.

| FA substrate | Product | Conversion (%) | Activity |
|--------------|---------|----------------|----------|
| 18:3n-3      | 20:3n-3 | 49.7%          | C18→C20  |
| 18:3n-6      | 20:3n-6 | 67.4%          | C18→C20  |
| 18:4n-3      | 20:4n-3 | 58.3%          | C18→C20  |
| 20:4n-6      | 22:4n-6 | 84.8%          | C20→C22  |
| 20:5n-3      | 22:5n-3 | 86.6%          | C20→C22  |

Conversions are expressed as a percentage of total FA substrate converted to elongated products.

ToPPARαb Structural Analyses

In general, the 3D structure of ToPPARαb was highly similar to that of the Danio rerio and Homo sapiens homologs (Figure 3) (Liang et al., 2016; Ning et al., 2016). Moreover, the genomic structural features of PPARαb were further examined in metazoans. The phylogenetic relationship of PPARα in T. ovatus and other representative species was constructed (Figure 4A). The distribution and lengths of the exons and introns of each PPARα gene are also shown in Supplementary Table S2. All PPARαa and PPARαb sequences had seven exons and six introns in fish, except for Gasterosteus aculeatus PPARαa, which possessed eight exons and seven introns, while D. rerio PPARαa possessed six exons. Furthermore, the sizes of homologous intron sequences are different, while the exonic sequences showed nearly no diversity. Moreover, ToPPARαb was grouped together with Oreochromis niloticus, which was also in the order Perciformes. The homology with ToPPARα, from close to distant, was other Osteichthyes, Amphibia, Aves, Mammalia, and Invertebrates. This result corresponded with the findings of conventional taxonomy.

Tissue Expression of ToPPARαb

The tissue expression pattern of ToPPARαb was analyzed by qRT-PCR. The PPARαb gene was extensively expressed in twelve tissues (Figure 4B). The transcription of ToPPARαb was tissue specific, and this gene was highly expressed in small intestine and head-kidney, followed by white muscle, stomach, gonads and brain ($P < 0.05$), with lower expression in the spleen, fin and blood ($P < 0.05$).
**FIGURE 4** | The structure and tissue expression of the ToPPARαb gene. (A) Genome structure analysis of PPARα genes according to the phylogenetic relationship. Lengths of exons and introns of each PPARα gene are displayed proportionally. Different color boxes and lines represent exons and introns, respectively. The identical color boxes represent homologous sequences. (B) Gene transcription of ToPPARαb in various tissues. The twelve tissues are small intestine (In), head-kidney (K), white muscle (Wm), stomach (St), female gonad (Fg), male gonad (Mg), brain (Br), liver (Li), gill (Gi), spleen (Sp), fin (Fi), and blood (Bl). The data from different tissues were analyzed by the Duncan test using one-way ANOVA. Data are shown as the means ± SD. Different letters indicate significant differences (p < 0.05).

**PPARαb Positively Promotes ToElovl5 Expression**

A total of 1,721 bp of the 5′ flanking sequence of the Elovl5 gene was cloned and defined as the candidate promoter. To determine the promoter activity of ToElovl5 with the transcription factor PPARαb in HEK 293T cells, a series of progressive deletion constructs were made (Figure 5A). Compared with the activity of the promoter candidate (Elovl5-4), a deletion of fragment from −146 bp to +459 bp (Elovl5-5) increased promoter activity with PPARαb. The expression levels of Elovl5-5 were 6.8-fold greater than those of Elovl5-4 with PPARαb (Figure 5A), suggesting that the core promoter region was located at +265 bp to +459 bp, which contained the PPARαb binding sites. To further confirm the interaction of ToPPARαb with ToElovl5, the influence of ToPPARαb overexpression on ToElovl5 transcription was determined. PPARαb overexpression increased the promoter activity of ToElovl5-5 at all tested time points in heterologous HEK 293T cells, and the maximum difference occurred at 24 h posttransfection, which was detected as 6.2-fold higher in PPARαb-overexpressing cells than that in the controls (Figure 5B). These results indicated that constitutively expressed PPARαb positively regulated ToElovl5 expression in HEK 293T cells.

**ToPPARαb Knockdown Decreased ToElovl5 Transcription in TOCF Cells**

In addition to the above results in HEK 293T cells, the function of PPARαb on Elovl5 was further confirmed in TOCF cells (Figures 6A,B). In the RNAi experiment, the mRNA expression of ToPPARαb was drastically reduced in a time-dependent manner, except at 0 h, suggesting the effective knockdown of ToPPARαb expression. When ToPPARαb mRNA was depleted, ToElovl5 transcription was significantly repressed compared with the control at the corresponding time points. This result
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FIGURE 6 | ToPPARαb (A) and ToElovl5 (B) mRNA expression levels by qRT-PCR after the transfection of either control RNA (control) or siRNA (RNAi). TOCF cells were stimulated with 0.1, 1, and 4 mM of PPARαb agonist (WY-14643) (C) and inhibitor (GW6471) (D) for 24 h, and the expression levels of ToPPARαb and ToElovl5 were significantly increased and decreased, respectively, in a concentration-dependent manner. All values are expressed as the means ± SD (n = 3). Bars on the same group with different letters are statistically significant from one another (p < 0.05).

demonstrated a positive regulatory role for ToPPARαb on ToElovl5 mRNA expression in the native T. ovatus host.

The Expression of Elovl5 Was Monitored by the Specific Inhibition and Activation of PPARαb

After stimulation for 24 h, the mRNA expression of ToPPARαb was drastically increased by a PPARαb activator (WY-14643) and memorably decreased by an inhibition (GW6471) in a concentration-dependent manner (Figures 6C,D). Moreover, both ToPPARαb and ToElovl5 showed the same expression trend. The mRNA levels of Elovl5 (P < 0.05) dramatically increased with the addition of the PPARαb activator (Figure 6C), nevertheless the expression of Elovl5 was suppressed after addition of the PPARαb inhibitor (Figure 6D) in a concentration-dependent manner. These results demonstrated that ToPPARαb played a positive regulatory role in ToElovl5 transcription in T. ovatus.

DISCUSSION

Trachinotus ovatus is widely cultured because of its great commercial value in China. Recently, a study investigating the LC-PUFA content in T. ovatus muscle showed that high retention of LC-PUFA occurred in muscle (Zhang et al., 2010). Elongases play core roles in the biosynthesis of LC-PUFA in fish (Castro et al., 2016). Consequently, a better understanding of the potential regulating mechanisms for the transcription of Elovl5 elongase would conduce to improve the endogenous LC-PUFA synthetic ability of the T. ovatus.

Similar to other teleost Elovl5 proteins, the isolated T. ovatus Elovl5 possessed all the features of the elongase family including a histidine box (HXXHH), canonical C-terminal ER retrieval signal (KXRXX), and transmembrane domains, supporting its role in LC-PUFA biosynthesis (Jakobsson et al., 2006; Monroig et al., 2012; Xie et al., 2016). The ToElovl5 could efficiently elongate C18 (18:3n-3, 18:3n-6, and 18:4n-3) and C20 (20:4n-6 and 20:5n-3) substrates to C20 and C22 PUFA, respectively, consistent with previously reported specificities in mammal (Leonard et al., 2000) and teleost (Hastings et al., 2005; Zheng et al., 2009; Gregory et al., 2010; Mohd-Yusof et al., 2010; Morais et al., 2011; Castro et al., 2016), clearly demonstrating that vertebrate Elovl5 universally had extensive substrate specificity. Furthermore, the Siganus canaliculatus Elovl5 had a predilection for n-3 over n-6 PUFA substrates, which was similar to that in most species studied previously, containing both freshwater and marine fish (Mohd-Yusof et al., 2010; Morais et al., 2011).
Additionally, previous studies found that LC-PUFA and their metabolites can regulate transcription of lipid metabolism related genes through modulation of transcription factors including, among others, PPARs (Sampath and Ntambi, 2005). Thus far, three major types of PPARs have been identified, namely, PPARα/β/γ. PPARα is the major PPAR subtype found in hepatocytes and is involved in the regulation of lipid and carbohydrate metabolism genes. Three PPARs function by dimerization with the retinoid X receptor (RXR) and binding to a prescribed DNA sequence, termed the PPAR response element (PPRE) (Desvergne and Wahli, 1999). Similar to PPARα in other species, the ToPPARα amino acid sequence revealed four representative domains. The DBD domain, the most conserved domain in PPARs, comprises two zinc finger-like motifs folded in a circular structure that identifies the DNA target sequence AGGNCA, and the binding of the PPAR/RXR heterodimer to the PPRE regulates the target gene (Ijpenberg et al., 1997). Analysis of the ToElovl5 promoter region revealed the presence of typical binding sites of PPARα and Elovl5, and putative binding sites of between ToPPARα and the ToElovl5 promoter region need further verification. Nevertheless, the regulatory mechanism of ToElovl5 is complex. PPARα is one of the important factors for the increased expression of ToElovl5 in T. ovatus.

Based on the tissue expression profile of ToPPARα, high mRNA levels were detected in metabolically active adipose tissues containing fatty acids, such as intestine, kidney, muscle, stomach, gonads and brain. A similar pattern of expression was determined in several other marine fish species, such as Liza haematocheila, O. niloticus, and Lateolabrax japonicus, which also showed limited LC-PUFA biosynthesis capacity (Dong et al., 2015; Ning et al., 2016; Yang et al., 2017). Since these tissues are major metabolic sites for LC-PUFA (Agbaga et al., 2010), it was reasonable that the ToPPARα gene showed relatively high expression.

Numerous studies have shown that PPARα was necessary for the clofibrate stimulation of peroxisomal and microsomal enzymes, such as acyl-CoA oxidase (AOX) (Berthou et al., 1995), the rate-limiting enzyme for fatty acid β-oxidation (Brandt et al., 1998), SREBP-1c (Yoshikawa et al., 2003) and fatty acid transport proteins and translocases in the liver (Frohnert et al., 1999). Moreover, PPARs are ligand-activated transcription factors that regulate gene expression in the PUFAs biosynthesis pathway (Sampath and Ntambi, 2005). In the present study, the positive regulatory role of ToPPARα in ToElovl5 transcription in T. ovatus was characterized. The results of the luciferase reporter assay, as well as RNAi analysis, clearly demonstrated that ToElovl5 expression was regulated by PPARα in T. ovatus (Figures 5, 6A,B). These results provided the first evidence of the involvement of PPARα in the expression of the rate-limiting enzyme Elovl5. ToElovl5 transcription indicated increasing profiles in either native TOCF cells or heterologous HEK 293T cells. These results were reasonable due to the stress caused by the disturbed biological environment during in vitro TOCF cell culture or Elovl5 promoter expression in the heterologous host (Liu et al., 2018).

To further determine the transcription mechanism of ToPPARα in T. ovatus, the mRNA levels of ToPPARα and ToElovl5 were detected. The transcription of ToPPARα and ToElovl5 was prominently increased or decreased in a concentration-dependent manner of activator or inhibition, respectively (Figures 6C,D). This observation was consistent with the results of studies implemented in mammals (Wang et al., 2005, 2006), suggesting that ToPPARα could up-regulate ToElovl5 in fish. The results of the in vitro experiment in the present study confirmed the above findings by over-expression and suppression of ToPPARα. These results verified the direct stimulatory role of PPARα on Elovl5 and suggested that such regulatory mechanisms operated differently compared to mammals.

In general, structural complexity was caused by intron gain or loss, which is a core evolutionary mechanism in most gene families (Yu et al., 2018). An exon–intron structure analysis of the ToPPARα gene indicated that all PPARα genes had six exons, while PPARαβ had seven exons in fish, except G. aculeatus PPARαβ, which possessed eight exons, and D. rerio PPARαβ, which possessed six exons. These findings might represent introns gained or lost during evolution and may also suggest that the metazoan PPARα genes consisted of highly conserved numbers of exons and introns. The results of the phylogenetic analysis were consistent with the findings of conventional taxonomy, suggesting that ToPPARα exhibited a closer genetic relationship with Perciformes, such as O. niloticus PPARα.

In summary, we demonstrated clear associations between PPARα and the ToElovl5 promoter, as well as the positive regulatory functions of PPARα in ToElovl5 transcription in T. ovatus. Moreover, the proposed synthesis pathway of LC-PUFA in T. ovatus (Supplementary Figure S3). The present study provided the first evidence of a positive regulator of ToElovl5 transcription. It would be interesting to further clarify the interactions between PPARα and the proposed cooperative companions to better comprehend the mechanisms underlying the PPARα-mediated regulation of ToElovl5 transcription. Furthermore, the specific mechanism of PPARα in regulating ToElovl5 by directly binding or being assisted by other proteins still needs further investigation.

**AUTHOR CONTRIBUTIONS**

K-CZ, S-GJ, and D-CZ designed the research and wrote the paper. LS, C-PZ, and K-CZ performed the research. H-YG and NZ analyzed the data. B-SL and LG contributed reagents, materials, and analysis tools.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01340/full#supplementary-material

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The handling Editor declared a shared affiliation, though no other collaboration, with several of the authors C-PZ, LS at time of review.

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