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RESEARCH PAPER

Peroxisome Proliferator-Activated Receptor Alpha (PPARα) in Redlip Mullet, *Liza haematocheila* : Molecular Cloning, mRNA Tissue Expression, and Response to Dietary Lipid Levels

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Abstract

The full-length cDNA of proliferator-activated receptor alpha (PPARα) was abtained from the liver of redlip mullet, *Liza haematocheila*. The PPARα cDNA (GenBank no: KJ848472) was 2409 bp including a 1437 bp open reading frame, which encoded 478 amino acids with four signature domains, i.e., the hypervariable region in N-terminus, DNA-binding domain (DBD), flexible hinge domain and ligand-binding domain (LBD). The mRNA expression level of PPARα was detected in all tissues tested. Highest expression occurred in liver, followed by brain, stomach, skin, spleen and visceral fat, but the expression was weak in heart and muscle. Then, a 60-day feeding trial was conducted to study, the effects of dietary lipid levels (2.0%, 4.8%, 7.5%, 9.8%, 12.0% and 14.6 %) on the mRNA expression of PPARα in mullet. PPARα mRNA expression in liver increased significantly ($P<0.05$) with the increasing dietary lipid levels. These results indicated that PPARα was tissue-differential expressed gene and played a pivotal role in regulating the lipid metabolism mainly in liver. Results of this study will benefit the further researches on the relationships between PPARα gene and fat metabolism of redlip mullet.

Keywords: *PPARα*, cloning, expression, lipid, *Liza haematocheila*.

Introduction

The redlip mullet (*Liza haematocheila*), belonging to Mugiliformes, Mugilidae, is widely spread throughout tropical and temperate seas as well as brackish waters and is cultured intensively in several countries (Hossain & Furuichi, 2000). In China, it is mainly distributed in the Bohai Sea, Yellow Sea, East China Sea and South China Sea. Redlip mullet is an important species in mariculture in China and now a popular polyculture species for its scavenging and economic effects (Bin & Xian, 2005). In China, the formula feeds of mullet breeding are produced mainly based on the requirements for other omnivory fish due to the limited information about nutritional needs. However, farmers often harvest abdominal hypertrophic (mainly occurred in liver) mullets without the popular long linear body, and the reason is still unclear (Huang, Xiao, Hu, Zhao, & Liu, 2014). The excessive accumulation of visceral fat, especially liver fat may be caused by lipid metabolism disorder. Its occurrence and development are closely related to lipid metabolism key factors. It is well known that dietary lipid plays a key role in the growth of fish and the unreasonable lipid level could cause the physiological disorders of lipid metabolism (Xu, Qin, Yan, Zhu, & Luo, 2011), so we suspect that the unreasonable lipid level might lead to the massive fat deposition by regulating some key factors of lipid metabolism in fish body, especially in liver.

Similar to mammals, peroxisome proliferators act in fish by binding to peroxisome proliferator-activated receptors (*PPARs*) that heterodimerize with the retinoid X receptor (RXR). This could result in binding to specific peroxisome proliferator response elements (PPRE) in the promoter regions of numerous target genes (Liu, Moon, Metcalfe, Lee, & Trudeau, 2005). Three kinds of *PPAR* isotypes *PPARα, PPARβ and PPARγ* have been identified in fish. Every isotype is a product of a separate gene and has a distinct tissue distribution (Leaver *et al.*, 2005). *PPARα* has been identified as the key regulator of the genes involved in peroxisomal, mitochondrial, and microsomal fatty acids (FA) oxidation systems in liver. The induction of some of the critical enzymes of β-oxidation systems in liver by peroxisome proliferators is controlled in the transcriptional level by the *PPARα* (Reddy, 2001). The inhabitation of the *PPARα*

expression can cause the decreasing expression in proteins or enzyme genes related to the hepatic FA metabolism, the fat deposition and inflammatory reaction in liver cells, and then result in the occurrence and development of fatty liver disease (Reddy, 2001). However, to our knowledge, there has been scarcely any literature on the effect of dietary lipid levels on fat deposition in fish body of redlip mullet. Therefore, we conducted this study to clone and characterize the full-length cDNA sequences and tissue specific expressions of *PPARα* gene in order to lay the molecular basis for further study on the mechanism of lipid metabolism of redlip mullet. Besides, as one of the basic nutrients in aquatic animal, fat is an important energy material for fish. Different fat levels are suitable for different fishes, and diets with unreasonable fat levels may cause abdominal fat accumulation in fish (Tocher, 2003). In view of this, the present study also tried to investigate the effect of different dietary lipid levels on the expression pattern of *PPARα* gene in the juvenile redlip mullet.

Materials and Methods

Feeding Trial and Sample Collection

Mullets with approximately initial weight of 300 g were provided by a breeding and cultivation aquafarm (Xiangshui, Yancheng, China). Prior to beginning the experiment, the fish were stored in 3,000-L cement pits in order to acclimatize. During that time, fish were fed with commercial diet (33.6% protein and 7.5% lipid). After 2 weeks, ten healthy fish with similar weight were randomly selected and dissected to collect skin, heart, spleen, kidney, stomach, intestine, brain, gill, liver, muscle and visceral fat, and then the samples were frozen in liquid nitrogen and immediately stored at -80 °C for the *PPARα* gene cloning and mRNA tissue expression of redlip mullet. During the process of sampling, fish were anesthetized with 0.01% MS-222 (tricaine methanesulphonate, Shang Hai Buxi Chemical Co., Ltd, China).

Meanwhile, we also raised a number of juvenile mullets which were obtained from the Chang Jiang breeding and cultivation aquafarm (Sheyang, Yancheng, China). Experiments were performed at the Laboratory of Aquatic Nutrition and Feed of Yancheng Institute of Technology. Before the start of the experiment, juvenile mullets were reared in five 3000-L cement pits to acclimatize to the experimental conditions for 2 weeks. After acclimation, 540 juvenile mullets (initial weight 9.5±0.3 g) were distributed randomly into 18 barrel-shaped tanks (diameter of tank: 70 cm, water volume: 300 L), with 30 fish stocked in each tank. Each experimental diet had three replicates. Fish were fed with six iso-nitrogenous (30.7±0.1% crude protein) and iso-energetic (22.3±0.1 MJ/kg gross energy) diets. Increasing amounts of fish oil were incorporated to provide graded lipid levels (2.0%, 4.8%, 7.5%, 9.8%, 12.0% and 14.6% on a dry matter basis). Dietary ingredients and proximate composition of the experimental diets are presented in Table 1. Fish were hand-fed to apparent satiation three times daily ($6:^{30}$-$7:^{00}$, $12:^{30}$-$13:^{00}$, $18:^{30}$-$19:^{00}$) and uneaten feed was removed 40 minutes after feeding. Experimental fish were supplied with filtered, aerated, and recirculating underground water. About 30% of the water was renewed every three days to meet acceptable water quality (ammonia nitrogen <0.03 mg/L; nitrite nitrogen <0.1 mg/L; dissolved oxygen >6.0 mg/L; pH 7.0-8.0). During the experiment, water temperature was maintained at 24±2°C. At the end of the 60-day feeding trial, fish were starved for 24 h. Five fish with similar weight per tank were dissected to collect liver, muscle and visceral fat. Then the samples were frozen in liquid nitrogen and immediately stored at -80°C for further RT-PCR analysis. In the process of sampling, fish were anesthetized with 0.01% MS-222 (tricaine methanesulphonate, Shang Hai Buxi Chemical Co., Ltd, China).

Total RNA Isolation and cDNA Synthesis

The total RNA was extracted from frozen tissue samples of redlip mullets using Trizol reagent (Takara Biotechnology Co. Ltd., Dalian, China) according to the manufacturer's protocol. The yield and quality of total RNA was checked spectrophotometrically using OD_{260} and OD_{280} measurements (ND-1000, NanoDrop Technologies, Rockland, DE). Based on the manufacturer's instructions, total RNA was treated with DNase I (Takara Biotechnology Co. Ltd.) to remove DNA and reverse transcribed to cDNA (10 μL reaction system for maximum use of 500 ng of total RNA) using a PrimeScript RT Master Mix kit (Takara Biotechnology Co. Ltd.). The reverse-transcription (RT) reactions were incubated for 15 min at 37°C, followed by 5 s at 85°C to inactivate the RT enzyme. The RT products (cDNA) were stored at -20°C for real-time PCR.

PPARα Gene Cloning and Related Study

First strand cDNA from the liver of redlip mullet was generated in a volume of 20 μL containing 2 μg total RNA, oligo (dT18), and Superscript-II reverse transcriptase (Invitrogen Life Technologies, Shanghai, China). Degenerated and specific oligonucleotide primer pairs (Table 2) were designed based on multiple alignments of the *PPARα* domains. The PCR was performed on a cycler PCR (Bio-Rad Lab., Richmond, CA, USA) for 2 min at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension step of 5 min at 72°C. The products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized with ultraviolet (UV) illumination. The gel

purified PCR products were ligated to pGEM-T easy vector (Promega, Madison, WI, USA) and transformed to E. coli DH10B competent cells. The cloned DNA fragments were sequenced by Tri-I Biotech (Taipei, Taiwan). The sequence of the *PPARα* was compared using BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cg.).

According to the partial sequences obtained, specific primers (Table 2) were designed and used in the rapid amplifycation of cDNA ends (RACE) reactions (First Choice™ RLMRACE Kit, Ambion, Applied Biosystems Business, Austin, TX, USA) to amplify both 5′- and 3′-ends of *PPARα*. PCR conditions for the RACE reactions were as follows: 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, with a final extension step of 5 min at 72°C. RACE products were purified using the QIAquick™ Gel Extraction kit (Qiagen USA, Valencia, CA) and assays were performed using the same protocol described above.

Sequence Analysis

The deduced amino acid sequence was carried out by DNAstar. Similarity searching of amino acid sequences was performed by blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The multiple sequence alignments were done by CLUSTALW 1.7 program (Thompson *et al.*, 1997). The domains of *L. haematocheila PPARα* were analyzed by domain

Table 1. Ingredients and proximate composition of experimental diets

	Dietary lipid levels (%)					
Ingredients (%)	2.0	4.8	7.5	9.8	12.0	14.6
Fish meal	16.0	16.0	16.0	16.0	16.0	16.0
Soybean meal	24.0	24.0	24.0	24.0	24.0	24.0
Cottonseed meal	5.0	5.0	5.0	5.0	5.0	5.0
Rapeseed meal	12.0	12.0	12.0	12.0	12.0	12.0
Wheat flour	10.0	10.0	10.0	10.0	10.0	10.0
Corn starch[1]	28.3	22.6	17.0	11.4	5.8	0.2
Microcrystallin cellulose	0.0	3.2	6.3	9.4	12.5	15.6
$Ca(H_2PO_4)_2$	2.0	2.0	2.0	2.0	2.0	2.0
Fish oil	0	2.5	5.0	7.5	10.0	12.5
Salt (NaCl)	0.3	0.3	0.3	0.3	0.3	0.3
Aquatic Econazole	0.2	0.2	0.2	0.2	0.2	0.2
premix[2]	2.0	2.0	2.0	2.0	2.0	2.0
Edible adhesive	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition[3] (air dry matter basis)						
Moisture (%)	10.9	9.4	8.9	9.4	9.5	8.5
Gross energy (MJ Kg^{-1})	22.0	21.9	22.5	22.2	22.4	22.6
Crude protein (%)	30.5	30.7	30.8	30.6	30.6	30.8
Crude fat (%)	2.0	4.8	7.5	9.8	12.0	14.6
Ash (%)	7.4	7.8	8.2	8.2	8.3	8.3
Calcium (%)	1.2	1.2	1.2	1.2	1.2	1.2
Phosphorus (%)	0.9	0.9	0.9	0.9	0.9	0.9

[1]Corn starch ingredient refers to GB-T 8885-2008 standard of first rank standard.
[2]Premix provides the following vitamins and minerals (/kg): VE 60 mg; VK 5 mg; VA 15000 IU; VD3 3000 IU; VB1 15 mg; VB2 30 mg; VB6 15 mg; VB12 0.5 mg; Nicotinic acid 175 mg; Folic acid 5 mg; Inositol 1000 mg; Biotin 2.5 mg; Pantothenic acid 50 mg; Fe 25 mg; Cu 3 mg; Mn 15 mg; I 0.6 mg; Mg 0.7 g.
[3]Proximate composition were determined following the methods of the Association of Official Analytical Chemists (AOAC, 1995), and the values are mean of triplicate repeats (n = 3).

Table 2. Primer pairs used for PPARα gene rapid amplifycation of cDNA ends (RACE) and quantitative RT-PCR

Primer type		Primer sequence, sense/antisense
Degenerated primer	F1	GCHTGYGAGGGMTGCAAGGG
	R1	TCNACBGANGTGCACTGGCAGCA
5'RACE	GSP1	GATGGCATTGTGAGAC
	GSP2	ACTGGCACTTGTTTCGGTTC
	GSP3	GTCATACTCCAGCTTCAGCC
3'RACE	3F1	GTAAGCGGTGACTATGTGGAGTCCG
	3F2	GTGTTTCCAGCTGTGGCGTGTGAGG
qRT-PCR	PPARα-F1 (KJ848472.1)	AGCAAGATGGTGGAGAAGGAAGT
	PPARα-R1	CTTCATGTATGCTTCGTGGATCTG
	β-actin-F1 (EF638008.1)	TGATGAAGCCCAGAGCAAGAG
	β-actin-R1	TTGTAGAAGGTGTGATGCCAGAT

[1]PPARα = Peroxisome proliferator-activated receptors α.

searching program in NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Protein phylogenetic analysis was conducted by MEGA 6.06 using the neighbor-joining method.

Quantitative Real-time PCR

The mRNA expression of *PPARα* genes was evaluated by real-time PCR (RT-PCR). In this procedure, the β-actin was used as a house-keeping gene to normalize the expression data of the selected genes in the RT-PCR. Primers used for mRNA expression were presented in Table 2 and were synthesized by Invitrogen (Invitrogen Life Technologies, Shanghai, China). RT-PCR was carried out in optical 96-well plates on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq Kits (Takara Biotechnology Co. Ltd.). The amplification was performed in a total volume of 20 μL, containing 10 μL of SYBR Premix Ex Taq, 0.4 μL of each primer (10 μM), 0.4 μL of ROX Reference Dye II, 2 μL of cDNA and 6.8 μL of sterilized doubled-distilled water. The program was as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s and the collection of the fluorescence signal at 60 °C. After amplification, melt curve analysis was performed to validate the specificity of the reactions. All PCR analyses were performed using 3 replicates for each sample and a percentage difference greater than or equal to 5% was deemed acceptable. Relative gene expression (arbitrary units) was calculated using the $2^{-\Delta\Delta Ct}$ method, as described by (Livak and Schmittgen, 2001). The gene expressions of *PPARα* in different tissues were calculated with the *PPARα* expression in muscle as control, and the gene expressions of *PPARα* in liver, muscle and fat tissue of different dietary lipid concentrations were calculated with the *PPARα* expression in the lowest lipid concentrations as control.

Statistical Analysis

The experimental data were analyzed by the General Linear Model (GLM) procedure of the SAS statistical package (SAS 9.2, SAS Inc., Cary, NC). The replicate was considered as the experimental unit. The Duncan's new multiple range test was used to detect significant differences between individual means when the treatment effect was significant ($P<0.05$). Results were presented as means ± S.E. (standard error) for each treatment of three replicates.

Results

PPARα Gene Sequences

The full-length cDNA of *PPARα* (GenBank accession number: KJ848472) sequence in mullet was 2409 bp with a 1437 bp open reading frame (ORF), which encoded 478 amino acids with a theoretical molecular mass of 53.5 kDa and an isolectric point (PI) of 5.48 (Figure. 1). Similar to other species, the *PPARα* protein was predicted to contain 4 domains, including the hypervariable region in N-terminus (amino acids 1-90), DNA-binding domain (DBD) (amino acids 91-174), flexible hinge domain (amino acids 175-190), and ligand-binding domain (LBD) in C-terminus (amino acids 191-474) (Figure 1). Among them, DBD and LBD were important and highly conserved in mullet. There was an absolute conserved sequence including two zinc finger domains (amino acid 92-112 and amino acid 129-146) in DBD of mullet *PPARα*, which were important for sequence-specific DNA binding to the peroxisome proliferator response elements (PPRE) of target genes.

Multiple Sequences Alignment and Phylogenetic Analysis

Complete AA sequence alignment showed that the identities of amino-acid between *L. haematocheila PPARα* and *PPARα* gene of other species were from 64% to 91%, and *L. haematocheila PPARα* shared the highest identity of 91% with *Lateolabrax japonicas* and *Larimichthys crocea*, followed by 90% with *Stegastes partitus*, 66% with *Homo sapiens*, 65% with *Gallus gallus* and 64% with *Xenopus laevis* (Figure 2). These result suggested that *PPARα* was highly conserved throughout the evolutionary process (data not shown).

The phylogenetic analysis of the mature proteins showed two major different *PPARα* branches for mammals, fish, birds and amphibians (Figure 3). The *PPARα* gene of mullet first gathered with S*tegastes partitus*, then with Cichlidae, and then with carangid clustering of perciformes, and finally the *PPARα* gene of fish constituted a separate clade far from mammals, birds and amphibians, which were clustered to another branch. This result of the evolutionary relationship revealed in the phylogenetic tree was in agreement with the classic taxonomy (data not shown).

PPARα Tissue Expression

The RT-PCR results evaluating the mRNA expression pattern of *PPARα* in skin, heart, spleen, kidney, stomach, intestine, brain, gill, liver, muscle and visceral fat showed clear among-tissues variation (Figure 4). The mRNA expression of *PPARα* occurred predominantly in the liver (which was 141.85-fold of muscle and significantly higher than other tissues, $P<0.05$), followed by brain, skin, stomach and spleen (which were significantly higher than muscle, $P<0.05$), but was weak in heart and muscle.

Dietary-Series Analysis of PPARα Expression in Liver, Muscle and Visceral Fat

Figure 5 showed that, the mRNA expression of *PPARα* in liver significantly increased ($P<0.05$) with

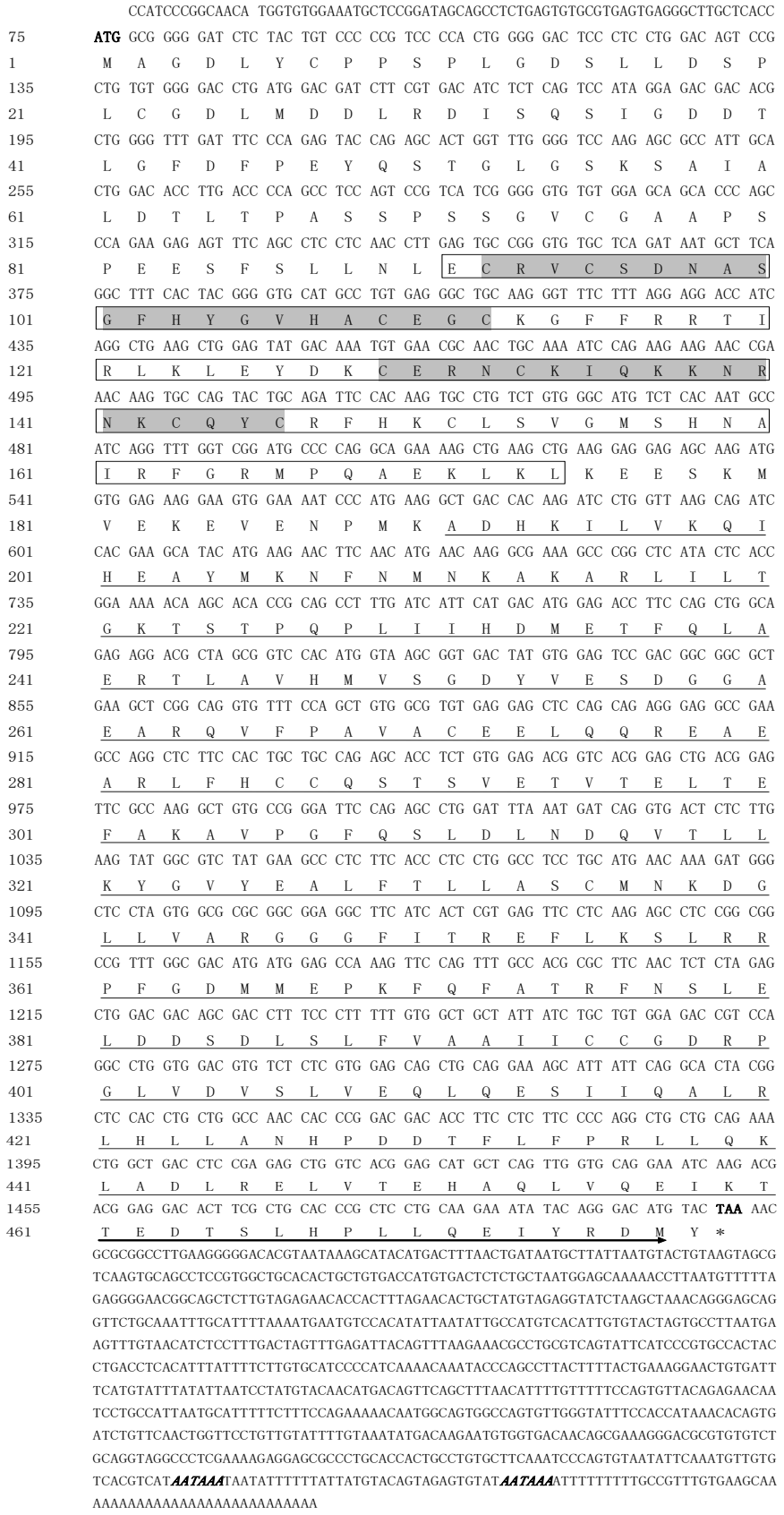

Figure 1. The nucleotide sequence of PPARα gene full-length cDNA and the deduced amino acid sequence of redlip mullet (*Liza haematocheila*). Initiation and termination codons are marked in bold font; poly A tail polyadenylation signal are marked in bold italic font. Rectangular callout boxes indicate the DNA-binding domain (DBD) and arrows indicate the ligand-binding domain (LBD). Shades of grey indicate the two zinc finger domains (Amino acid residues located in the C^{92}-$C^{112}$and C^{129}-C^{146}) in DBD. The nucleotide sequence was submitted to NCBI GenBank, accession no. KJ848472.

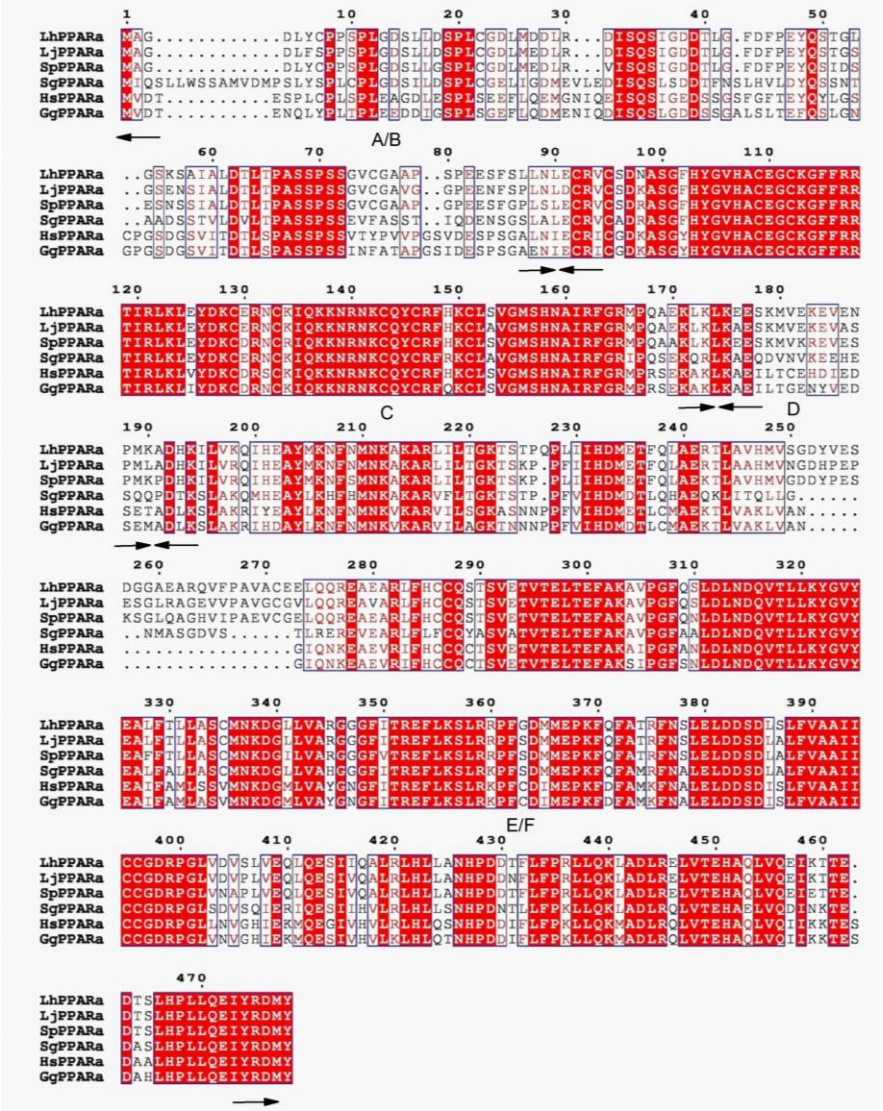

Figure 2. Multiple amino acid sequence alignment of PPARα between redlip mullet (*Liza haematocheila*) and other species. Alignment of the primary sequences of PPARα from *Liza haematocheila* (LhPPARa: AIK22388.1), *Lateolabrax japonicas* (LjPPARa: AIW63714.1), *Stegastes partitus* (SpPPARa: XP_008293086.1), *Sinocyclocheilus graham* (SgPPARa: XP_016086235.1), *Homo sapiens* (HmPPARa: NP_005027.2) and *Gallus gallus* (GgPPARa: NP_001001464.1) using ClustalW. The four domains are indicated by arrows as following hypervariable region in N-terminal (A/B), DNA-binding domain (C), flexible hinge domain (D) and ligand-binding domain (E/F), respectively.

the dietary lipid levels increased from 2.0% to 14.6%, and the highest value was obtained at dietary lipid level of 14.6%. However, there was no significant difference in the mRNA expression of *PPARα* in muscle and visceral fat among all groups respectively (P>0.05).

Discussion

In the present study, we have successfully isolated and characterized the full-length cDNA sequences of *PPARα* gene (GenBank accession number: KJ848472) from the mullet, and the sequence covered 2409 bp with an ORF of 1437 bp encoding 478 AA. Compared with other species, the complete AA sequence of *PPARα* in mullet was highly conserved in the process of evolution, suggesting that *PPARα* gene might play an important role in some of the physiological activities of animals. The *PPARα* protein of mullet was predicted to contain 4 domains, including the hypervariable region in N-terminus, DBD, flexible hinge domain and LBD in C-terminus, which further confirmed the previous studies (Green and Chambon, 1988, Zhao, Gul, Li, & Wang, 2011). The results suggested that the cloned gene was *L. haematocheila PPARα*.

PPARα gene was widely expressed in multiple tissues of mammals, birds, osteichthyes and so on, across from embryonic development to adulthood (Desvergne & Wahli, 1999, Ibabe, Bilbao, & Cajaraville, 2005, Raingeard, Cancio, & Cajaraville 2006, Zhao *et al.*, 2011). In mammals, *PPARα* gene is

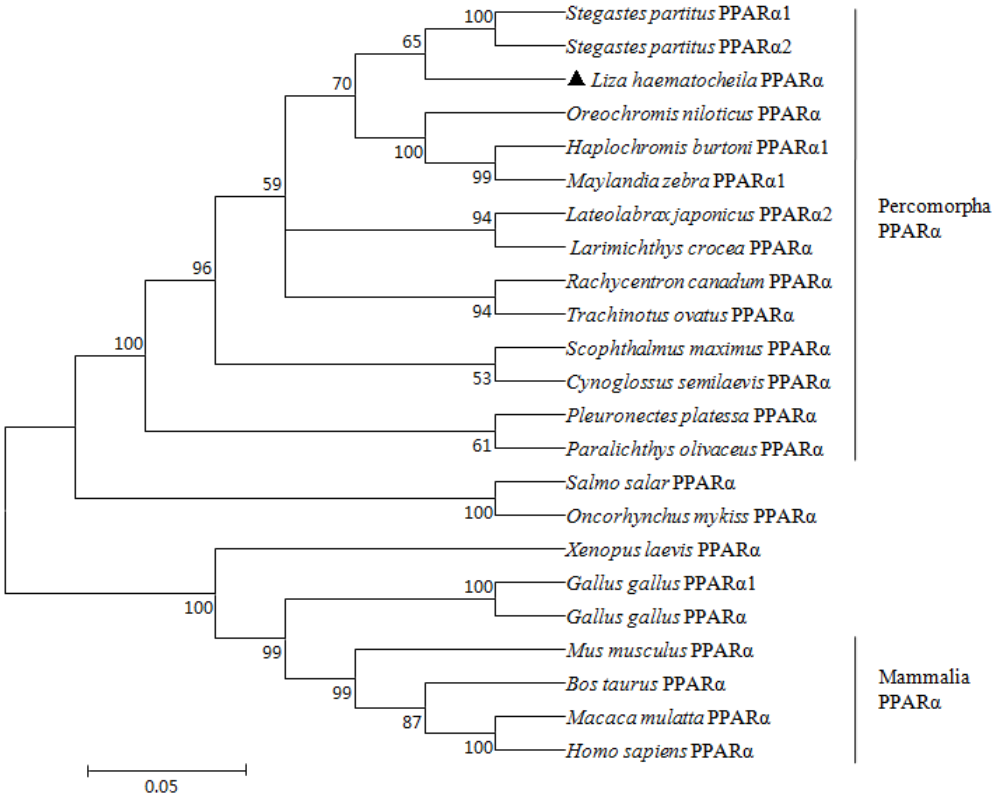

Figure 3. Phylogenetic tree depicting the evolutionary relationships between various PPARαs. An unrooted phylogenetic tree was made with Mega 6.06 software using the neighbor-joining method after alignment. The sequences were extracted from GenBank: *Liza haematocheila* (AIK22388.1), *Stegastes partitus* PPARα1 (XP_008293086.1), *Stegastes partitus* PPARα2 (XP_008293086.1), *Oreochromis niloticus* (NP_001276995.1), *Haplochromis burtoni* (XP_005915724.1), *Maylandia zebra* (XP_004560864.1), *Lateolabrax japonicas* (AIW63714.1), *Larimichthys crocea* (KKF12570.1), *Rachycentron canadum* (ABK76300.1), *Trachinotus ovatus* (ALG03140.1), *Scophthalmus maximus* (AFK08624.1), *Cynoglossus semilaevis* (XP_008314094.1), *Pleuronectes platessa* (CAD62447.1), *Paralichthys olivaceus* (ADM21468.1), *Salmo salar* (NP_001117032.1), *Oncorhynchus mykiss* (NP_001184140.1), *Xenopus laevis* (NP_001088831.1), *Gallus gallus* PPARα1 (XP_015145415.1), *Gallus gallus* PPARα (NP_001001464.1), *Mus musculus* (NP_035274.2), *Bos Taurus* (NP_001029208.1), *Macaca mulatta* (NP_001028201.1) and *Homo sapiens* (NP_005027.2)

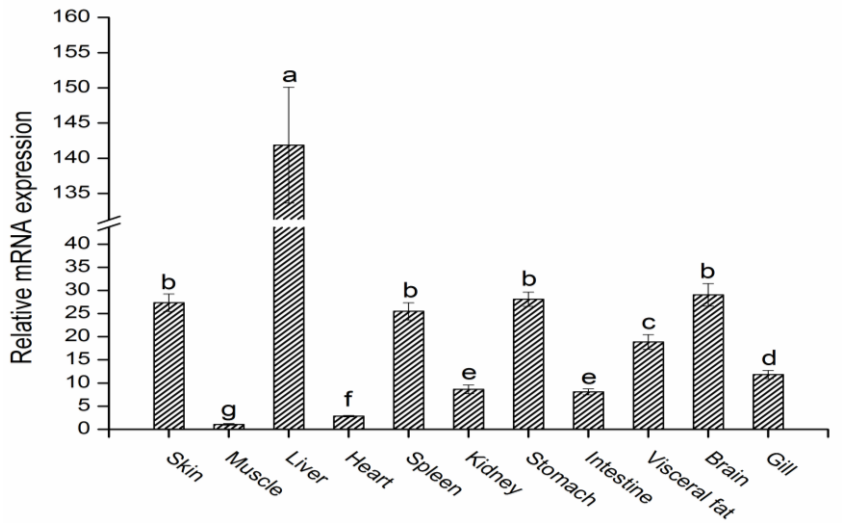

Figure 4. Relative mRNA expression of PPARα in various tissues of redlip mullet (*Liza haematocheila*). The PPARα expression was quantified by quantitative RT-PCR and was normalized to β-actin and calculated as the fold increase relative to an arbitrary fold increase of 1 for the expression level of PPARα in the muscle, which had the lowest value. The values are the means ± S.E. (standard errors, n = 3). Vertical lines indicate the standard errors. Means with different superscript letters are significantly different ($P < 0.05$).

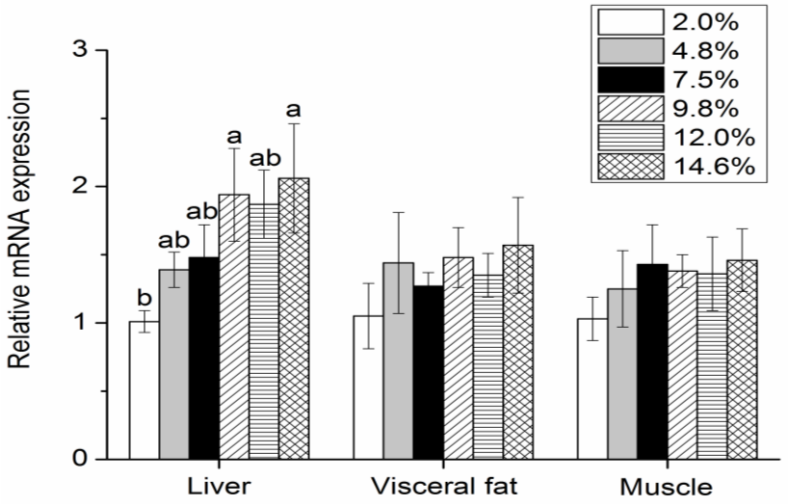

Figure 5. Relative mRNA expression of PPARα in the liver, visceral fat and muscle of juvenile redlip mullet (*Liza haematocheila*) subjected to different lipid concentrations. The PPARα expression was quantified by quantitative RT-PCR and is presented as the means ± S.E (standard errors, n=3) of relative values of expression level, after being normalized to the expression of β-actin. Vertical lines indicate the standard errors. Means with different superscript letters are significantly different ($P < 0.05$).

expressed in a variety of tissues in adult rat and mouse, with high expression in liver, kidney, heart, stomach, brown fat, and the relatively low expression in other tissues (Braissant, Foufelle, Scotto, Dauça, & Wahli, 1996; Kliewer *et al.*, 1994). For poultry, previous studies reported that the *PPARα* was expressed in heart, liver, kidney and stomach. The *PPARα* expression in liver and kidney was higher than that in other tissues, and no expression was detected in skeletal muscle (Meng *et al.*, 2005). However, Diot and Duaire (1999) found that, small amounts of mRNA of *PPARα* was expressed in chicken skeletal muscle, although it was highly expressed in liver, heart, kidney, and uropygial gland. The above studies of mammals and birds showed that, *PPARα* gene was predominantly expressed in the metabolically active tissues of FA, such as the liver, heart, kidney, etc. This distribution was consistent with the biological function of *PPARα* gene, and differential expression of different species of individual organizations might be due to its own characteristics of the species, which still needed further research.

In the study of fish, the immunohistochemical analysis of adult zebrafish tissues showed that, *PPARα* gene was mainly expressed in liver parenchymal cells, renal proximal tubule and intestinal cells (Ibabe, Grabenbauer, Baumgart, Fahimi, & Cajaraville, 2002). For gray mullet, the *PPARα* was widely expressed in brain, liver, spleen, gill, heart and gonads. The hepatic expression was the highest and muscle was the least (Ibabe *et al.*, 2004, Raingeard *et al.*, 2006). Zheng *et al*. (2015) reported that, the expression of *PPARα1* (subtype of *PPARα*) was abundant in the liver of *Pelteobagrus fulvidraco* larvae, juvenile and adult fish, and was significantly higher than other tissues. Zhao *et al*. (2011) found that, the mRNA expression of *PPARα* was detected in adipose tissue, gill, heart, liver, spleen, kidney, muscle, intestine, brain and gonad in adult and juvenile of *Megalobrama amblycephala*. But the expression was various in different growth stage. The highest expression was observed in white muscle of adult fish, followed by liver, and gonad was the lowest. The juvenile *Megalobrama amblycephala* had the highest expression in the brain, followed by intestine, and spleen to a minimum. These differences might be related to the biological function of *PPARα* in animals with special physiological stages, and the higher *PPARα* expression in the brain of juvenile mullet might be that the gene activation could play a role in the protection of brain (Inoue *et al.*, 2002). As a key regulator of lipid metabolism, *PPARα* gene regulated the FA β-oxidation pathway in mitochondria and peroxidase (Aoyama *et al.*, 1998), and the *PPARα* expression in different developmental stages might be relative with the changing energy demand or nutritional status (Cho *et al.*, 2012). In this study, the *PPARα* expression was detected in all the tissues of juvenile mullet, with the highest quantity in liver, far higher than that in other tissues, and the minimum amount of expression in muscle. The studies of fish also confirmed that, the *PPARα* gene was abundantly expressed in the FA oxidation active tissues, with higher expression in liver and generally lower expression in muscle, which was slightly different from that in mammals and birds. The reason might be that the liver was the main organ for energy storage and supply in fish and *PPARα* was closely involved in lipid metabolism.

The target genes of *PPARα* were involved in various aspects of lipid metabolism. After activated, the *PPARα* could induce gene expression related to FA intake, activation and β-oxidation, increase the

high density lipoprotein (HDL) synthesis, reduce the triglyceride level, regulate the oxidation of FA in peroxidase and mitochondria as well as the uptake and storage of lipid (Guan and Breyer, 2001, Desvergne and Wahli, 1999). The deficiency or inhabitation of the *PPARα* expression can cause the decreasing expression of genes related to the hepatic FA metabolism, the fat deposition and inflammatory reaction in liver cells, which would result in the occurrence and development of fatty liver disease (Reddy, 2001). The high *PPARα* expression in liver determined its important role in nutrient metabolism, especially in the lipid metabolism of fish. The lipid metabolism could be regulated by regulating the expression of *PPARα* in the liver, which might be an effective way of controlling the fat content of liver in fish. Many studies have reported that the excessive fat in diet could lead to unwanted fat deposition in liver or in other tissues (Stowell & Gatlin, 1992; Lee *et al*, 2002). Based on this, we speculate that the unreasonable dietary fat level may result in the abdominal obesity in mullet. Therefore, the relationship between dietary fat levels and the PPAR expression in mullet was investigated to analyze the reasons for abdominal obesity. Besides, a recent study claimed that, as a kind of physiological response to the increase of fat absorption, the mRNA expression of *PPARα* in liver enhanced with the increase of dietary fat levels (Rinella *et al*, 2008). However, in the study of fingerling blunt snout bream *Megalobrama amblycephala*, the mRNA expression of *PPARα* in liver significantly decreased by feeding high-fat (fat level of 11%, soybean oil as the fat source) diet (Li *et al*, 2015). The reason might be that the fat intake was far more than the demand of this species. The fat accumulation in the body resulted in the disorder of fat metabolism, including the blockings of the triglyceride transport and FA oxidation, and consequently the cellular and tissue damages as well as the dysfunction, which might cause the inhibition of *PPARα* expression (Choi & Ginsberg, 2011). In the present study, the mRNA expression of *PPARα* increased gradually in liver, visceral fat, muscle of juvenile mullet with the increasing dietary lipid levels. Besides, the *PPARα* expression level significantly increased in liver, suggesting that under the high levels of dietary fat, the activation of *PPARα* gene might increase the fat oxidation in order to balance the potential increase of tissue fat caused by the high-fat diet. Although far higher than the actual commercial dietary fat level (generally less than 10%) of mullet, the high-fat (12.01% and 14.59%) diet did not inhibit the *PPARα* expression, and this might result from the applying of fish oil as a source of dietary fat. It is well demonstrated that, fish oil contains large amounts of polyunsaturated fatty acids (PUFA), the PUFA and its metabolites are the active ligands of *PPARα*, which can activate the expression of *PPARα*. The activation of *PPARα* signaling pathway can resist the obesity and fatty liver induced by high-fat diet (Gao *et al*., 2015). However, the dietary fat levels and fish oil in the present study may not lead to excessive accumulation of fat in the liver of juvenile mullet, which need further study. As for the *PPARα* gene-induced beneficial effects, this complex physiological process has not yet been well understood until now, and further studies should be conducted to elucidate the underlying mechanism of dietary fat on the *PPARα* expression of mullet.

Conclusions

In summary, we cloned the full-length cDNA sequence of *PPARα* in redlip mullet, *Liza haematocheila* and understood its molecular characterization. Sequence alignment and phylogenetic analysis revealed the *PPARα* was highly conserved among various vertebrates. *PPARα* gene was differentially expressed within and among tissues, presenting highly expressed in the liver and weakly expressed in the muscle of mullets. High dietary lipid levels induced an up-regulation of *PPARα* expression, indicating that the fish feeding with high fat diet might improve the fat oxidation by activating *PPARα* gene to balance the potential increase of fat deposition in tissue caused by high fat diets. Results of this study will benefit the further researches on the relationships between PPAR genes and fat metabolism of redlip mullet.

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