www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 17: 477-485 (2017) DOI: 10.4194/1303-2712-v17_3_04

RESEARCH PAPER

Peroxisome Proliferator-Activated Receptor Gamma (Pparγ) in Redlip Mullet *Liza haematocheila*: Molecular Cloning, Tissue Distribution, and Response to Dietary Lipid Levels

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Received 28 June 2016
Accepted 21 November 2016

Abstract

Redlip mullet (Liza haematocheila) PPARγ cDNA (lhPPARγ) by reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE) were isolated in this study. The full-length cDNA was 2985 bp, consisted of a 200 bp 5'UTR, 1183 bp 3'UTR and 1599 bp ORF encoding a polypeptide with 533 amino acids. lhPPARγ protein was predicted to consist of four domains, i.e. N-terminal domain, DNA-binding domain, C-terminal ligand binding domain and flexible hinge domain. Real time quantitative RT-PCR revealed that lhPPARγ mRNA was detected in all tested tissues. The highest expression level of lhPPARγ mRNA was observed in abdomen fat followed by intestine, gill, kidney, liver, skin, brain, spleen, stomach, liver, heart and muscle. These results suggested that lhPPARγ was functionally and evolutionarily conserved between redlip mullet and other vertebrates. Six iso-nitrogenous diets with different lipid level (from 2.0% to 14.6%) were fed to triplicate groups. Although fish fed diets containing 14.6% lipid showed higher lhPPARγ mRNA expression in the liver, intestine and muscle than fish fed other diets, no significant difference was observed, which indicated redlip mullet might adapt to high lipid level below 14.6%.

Keywords: Liza Haematocheila, PPARγ, cloning, cxpression, lipid.

Introduction

The peroxisome proliferator-activated receptors (PPARs) are members the nuclear hormone receptor superfamily. Three primary subtypes, α, β, and γ have been identified in teleosts, amphibians, rodents, and humans (Pavlikova, Kortner, & Arukwe, 2010; Krey *et al*., 1994; Kliewer *et al*., 1994; Sher, Yi, McBride, & Gonzalez, 1993). Although the *PPAR* subtypes are encoded by distinct single-copy genes, they all have a structure characteristic of the nuclear hormone receptors including A/B, C, D and E/F domain. N-terminal "A/B" domain has an activation function independent of the presence of ligand and is highly variable in sequence between the isoforms. The highly conserved DNA binding domain (DBD), the C domain, contains two zinc-finger-like structures with α-helical DNA binding motifs. The D or hinge region is a flexible domain follows the DBD. The C-terminal ligand binding domain (LBD), the "E/F" domain, is highly conserved. The LBD contains the activation function 2 (AF-2) dependent on the presence of bound ligand (Abbott, 2009).

PPARs regulate gene expression by binding to a specific regulatory element (peroxisome proliferator response elements, PPRE) in the promoter region or intronic sequence of target genes (Desvergne & Wahli, 1999). Genes regulated by PPARs are involved in important physiological processes including lipid homeostasis, adipogenesis, reproduction, inflammatory responses, wound healing, and carcinogenesis (Kidani & Bensinger, 2012; Shao *et al*., 2016; Bogacka, Bogacki, & Wasielak, 2013; Luo *et al*., 2015; Montagner, Wahli, & Tan, 2015). *PPARs* are activated by a broad range of fatty acids and fatty acid derivatives, and display distinct but overlapping expression and functions (Poulsen, Siersbaek, & Mandrup, 2012). Among the three *PPAR* subtypes, *PPARγ* has critical impact on lipid storage, adipogenesis, macrophage maturation, embryonic implantation and inflammation control (Abbott, 2009).

PPARs have recently been found in several fish species, including zebrafish (Ibabe Grabenbauer, Baumgart, Fahimi, & Cajaraville, 2002), medaka (Fang *et al*., 2012), salmon (Leaver *et al*., 2007), rainbow trout (Cruz-Garcia, Sánchez-Gurmaches, Monroy, Gutiérrez, & Navarro, 2015), fugu (Kondo,

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in cooperation with Japan International Cooperation Agency (JICA), Japan

Misaki, Gelman, & Watabe, 2007), tilapia (Adeogun, Ibor, Regoli, & Arukwe, 2016), sea bass (Boukouvala *et al*., 2004), orange-spotted grouper (Luo *et al*., 2015), grass carp (He *et al*., 2012). Although there were some reports on *PPARγ* related to the fish nutrition, immune and environment (Liang, Zhao, Li, & Gao, 2015; Luo *et al*., 2015; Adeogun *et al*., 2016), the regulation and function of fish *PPARγ* remain unknown. Especially, limited information is available about the expression of *PPARγ* in response to dietary different lipid levels.

The redlip mullet (*Liza haematocheila*) is an economically important fish in China due to its high survival rate, fast growth, and economic value. However, little information is available about the *PPARγ* gene and its function in response to the lipid levels, which is crucial to developing cost-effective and nutritionally balanced formulations. To achieve this goal, we cloned and characterized *PPARγ* gene (*lhPPARγ*) in redlip mullet, as well as to investigate the gene expression pattern responses to dietary different lipid levels in order to address *lhPPARγ* function in the regulation of lipid homeostasis.

Materials and Methods

Feeding Trial and Sample Collection

Juvenile redlip mullets were collected from the Chang Jiang breeding and cultivation aquaculture farm (Sheyang, Yancheng, China) and acclimatized in laboratory aquaria for over 2 weeks. Six diets with different lipid levels were formulated with iso-nitrogenous (30.7±0.1 % crude protein) and iso-energetic (22.3±0.1 MJ kg^{-1} gross energy) (Table 1). The fish meal and soybean meal were used as the main sources of protein. Levels of dietary lipid were gradually increased from 2.0% to 14.6% in the six diets. Each diet was randomly assigned to triplicate cages which cage contained 30 fish with initial weight (9.5 ± 0.3 g). Fish were fed to apparent satiation twice daily (05:00 and 17:00).

At the end of the 60-day feeding trial, fish were fasted for 24 h. Three mullet from each cage were anesthetized with MS222 and sacrificed by decapitation. Tissues used for cloning and tissue expression analysis were sampled and frozen immediately in liquid nitrogen, and stored at -80°C until RNA extraction.

RNA extraction and reverse transcription (RT)

Total RNA from different tissues was extracted with RNAiso Reagent (Takara, Dalian, China) and treated with DNase I (Takara). RNA concentration and purity were tested by spectrophotometry. RNA with an OD260/280 ratio between 1.9 and 2.2 and an OD260/230 ratio of 2 or greater was considered satisfactory and used in the following step. Then, 1 μg of total RNA was reverse transcribed with oligo (dT) and random primers in a 10 μl final volume using M-MLV reverse transcriptase (Takara) according to the manufacturer's protocol.

Table 1. Ingredients and proximate composition of experimental diets

Ingredients (%)	Dietary lipid levels (%)					
	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).

Molecular Cloning of lhPPARγ

A pair of degenerate primers (F1 and R1) was designed based on highly conserved regions of other fish *PPARγ* genes available in GenBank and synthesized by Shanghai Biosune Biotechnology Company Limited (Shanghai, China) (Table 2). PCR was performed in 25 µl reactions with liver cDNA as template. Amplification were performed as followed: an initial denaturation for 3 min at 94°C; 30 cycles of denaturation for 30 s at 94°C, annealing for 60 s at 58°C, and extension for 1 min at 72°C; a 5 min final extension at 72°C. The PCR products were run on a 1.2% agarose gel. The positive fragment were purified by purification kit (Takara), cloned into pMD18-T vector (Takara) and sequenced in BioSune (Shanghai, China).

According to the partial sequence of *lhPPARγ*, gene specific primers were designed for 5'RACE (rapid-amplification of cDNA ends) and 3'RACE (Table 2). Rapid amplification of the 5'end was performed using the 5'RACE system for rapid amplification of cDNA ends (Invitrogen, California, USA) following the manufacturer's protocol. RT was performed with a specific reverse primer GSP1 to obtain the first strand cDNA. The cDNA was amplified with the 5' universal forward primer and GSP2. Then the second round PCR products was diluted 1:100 and used as templet for the third round PCR, which was conducted with nested PCR primer GSP3 and 5' universal forward primer.

Rapid amplification of the 3' end was conducted with SMARTer™ RACE cDNA amplification kit (Clontech, California, USA) following the manufacturer's protocol. Briefly, the first and nested PCR rounds were performed using primers 3F1 and 3F2, respectively.

According to the sequence of 5' and 3'RACE, a pair of primers was designed to amplify the ORF of *lhPPARγ* (Table 2). Briefly, the annealing temperature was 58°C in 25 ul reactions. All PCR products were purified, cloned into vector, and sequenced following the procedures mentioned above.

Sequence Analysis

The deduced amino acid sequence was carried out by DNAstar. Similarity searching of amino acid sequences (or nucleotide sequences) was performed by blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The multiple sequence alignments were done by ClustalW (Larkin *et al*., 2007).A signal cleave site was predicted by SignaIP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). The domains of lhPPARγ were analyzed by domain searching program in NCBI (http://www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi). Protein phylogenetic analysis was conducted by MEGA 5.1 using the neighbor-joining method.

Real Time Quantitative RT-PCR

Real-time quantitative RT-PCR was used to assay the relatively quantitative mRNA expression of *lhPPARγ* in a range of tissues. The tissue panel included skin, muscle, liver, heart, spleen, kidney, stomach, intestine, abdomen fat, brain and gill. For analyzing *lhPPARγ* mRNA expression in response to dietary lipid levels, three tissues were selected including liver, abdomen fat and muscle.

Real time PCR was performed on the Thermal Cyclery Dice™ Real-Time System (Takara) in a final reaction volume of 20 µl containing 1× SYBR Green I mix (Takara), 8 pmol of each primer. A negative control with RNA was contained in each assay. Real time PCR program was as follows: denaturation at 95°C for 3 min, followed by 40 cycles at 95°C for 5 s, 62°C for 20 s. A dissociation step was performed to identify a single, specific melting temperature for each primer set. The amplification temperature was gradually increased at a rate of 0.2°C from 65°C to 92°C. After amplification, fluorescent data were converted to threshold cycle values (Ct). The *lhPPARγ* relative mRNA expression levels in different tissues were determined by $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001), using ß-actin as a reference gene (Shen, Jiang, Wang, & Shen, 2015).

Table 2. Information of primers used in this experiment

Primer type		Sequence (5'- 3')	Amplicon length
PCR	F1	CCACRTTDGACTACRCYTCCAT	1134 bp
	R1	GTTTCCTGAGACTCTTGAGGAA	
5'RACE	GSP1	GGTGTTCTTCCCTCTG	
	GSP2	CCACACCAGCGGAAGAGATG	
	GSP3	GGGAGACAGGGAGGAAGGGA	413 bp
3'RACE	3F1	ACGGAGTTTGCCAAGAGTATCCCAGG	
	3F2	GCACCTCTGATGAACAAAGACGGGAC	1627 bp
ORF	ORFF1	CAACACGCTGATCAGCAGAC	1649 bp
	ORFR1	GTCTTCGGCTCCTGTGGA	
qRCR	qF1	CATTCACAAGAAGTCCCGCAAC	86 bp
	qR1	AGCGAATGGCGTTGTGTGAC	
	β-actin-qF1	TGATGAAGCCCAGAGCAAGAG	111 bp
	β-actin-qR1	TTGTAGAAGGTGTGATGCCAGAT	

Note: The degenerate prime where R=A/G, Y=C/T, and D=A/G/T

Statistical Analyses

For the experimental results of the qRT-PCR, the expression level of *lhPPARγ* mRNA in liver, abdomen fat and muscle was arbitrarily defined as 1 at the lipid content of 2.0%, and all the relative ratios of gene expression levels were then calculated to derive the means and standard deviation (SD). In this experiment, qPCR data meet requirements for statistical analysis using parametric tests. Statistical analysis was performed using SPSS software. Statistical differences were estimated by one-way analysis of variance (ANOVA) followed by Duncan multiple range tests. Differences results were considered to be significant if $P<0.05$.

Results

Cloning and Sequence Analysis of lhPPARγ Gene

A conserved partial sequence of *lhPPARγ* cDNA was obtained by RT-PCR with degenerate primers. This cDNA sequence was extended by 3'RACE and 5'RACE. The full-length cDNA (GenBank accession number: KJ848473.1) was 2985 bp with a 200 bp 5'UTR, 1183 bp 3'UTR and 1599 bp ORF. The 3'UTR of *lhPPARγ* contained a putative polyadenylation signal sequence (AATAAA) 22 bp upstream of the poly (A) tail. The ORF of *lhPPARγ* encoded a polypeptide of 533 amino acids with an estimated molecular mass of 60.03 KDa and a predicted isoelectric point (pI) of 6.43.

There was no signal peptide cleavage site implied that lhPPAR might be a non-classical secretory protein. Blastp program in NCBI revealed that the lhPPARγ amino sequence exhibited high identity among fish. Three putative conserved domains was detected in the deduced primary sequence of lhPPARγ by conserved domain searching program in NCBI, which showed that lhPPARγ was also a member of the nuclear receptor superfamily. The N-terminal region (amino acids 1-102), the highly conserved DBD (amino acids 131-214) and LBD (amino acids 250-532) were shown in Figure 1.

Multiple Sequences Alignment and Phylogenetic Analysis

The multiple alignment analysis showed that the sequence identity of PPARγ was varied between *L. haematocheila* and other vertebrate (Figure 2). lhPPARγ shared the highest identity of 99.3% with *Rachycentron canadum*, followed by 88.9% with *Paralichthys olivaceus*, 82.4% with *Takifugu rubripes*, 65.1% with *Gallus gallus* and 64.8% with *Homo sapiens*. However, the DBD and LBD of PPARγ shared high levels of identity above 72%, which implied that PPARγ have been highly conserved throughout the evolutionary process.

A phylogenetic tree was constructed by ClustalX and MEGA 5.1 using the neighbor joining method (Figure 3). The phylogenetic analysis of the mature proteins revealed distinct PPARγ clades for mammals, fish, birds and amphibians respectively. Among the fishes, *L. haematocheila* PPARγ sequence was similar to other marine fish PPARγ such as *Lateolabrax japonicas* PPARγ, *Thunnus orientalis* PPARγ and *R. canadum* PPARγ. These marine fish PPARγ were clustered to Percomorpha PPARγ and divergent from zebrafish and tetraploid salmon PPARγ. This result of the evolutionary relationship revealed in the phylogenetic tree was in agreement with the classic taxonomy.

Tissue Expression of lhPPARγ mRNA

The relative expression levels of *lhPPARγ* mRNA were analyzed with real time quantitative RT-PCR normalized by *β-actin*. *lhPPARγ* mRNA was detected in all tissues including skin, muscle, liver, heart, spleen, kidney, stomach, intestine, abdomen fat, brain and gill. As shown in Figure 4, the highest expression level of *lhPPARγ* mRNA was observed in abdomen fat followed by intestine, gill, kidney, liver, skin, brain, spleen, stomach, liver, heart and muscle, and 294 times in abdomen fat than in muscle. However *lhPPARγ* mRNA was also abundantly expressed in the intestine, gill, and kidney. The expression level of *lhPPARγ* mRNA was 190 times in intestine, 161 times in gill and 113 times in kidney than that in muscle, respectively.

Expression of lhPPARγ mRNA in Response to Dietary Lipid Levels

Three tissues were selected to analyze the *lhPPARγ* mRNA expression in response to dietary lipid levels. They were the abdomen fat with the highest *lhPPARγ* mRNA expression level, the muscle with the lowest *lhPPARγ* mRNA expression level, and the liver playing an important role in lipid metabolism. Six different diets with gradually increased lipid level were formulated. Expression levels of *lhPPARγ* mRNA in the liver, intestine and muscle were presented in table 3. Fish fed diets containing 14.6% lipid showed higher *lhPPARγ* mRNA expression in the liver, intestine and muscle than fish fed other diets, however no significant difference was observed among fish fed different diets in the three tissues.

Discussion

In the preset study, the full-length sequence encoding *lhPPARγ* was cloned and characterized for the first time. Unlike mammalian possessing two copies of *PPARγ* (Abbott, 2009), marine fish and zebrafish possess only one copy of *PPARγ* although tetraploid salmon has two copies of *PPARγ*. The full-length lhPPARγ sequence encoded 533 amino acids,

gcagcaacagcagcagcaccatgagcagaaaacaccacagcgagcggacctgaccactttgacg
ctgcgctcccgtgcgcacggtgcggacgccacgatctccacggccgaccagcccacacgcggacaagt
cgagacaacttcggaaacaacaaagtgggatccgtctcttctgcgcttca acacgctgatcagcagac
ATG GTG GAC ACC CAG CAG CTC CTA GCT TGG CCT GTT GGA TTC AGT CTG AGC ACA GTG GAC
M V D T Q Q L L A W P V G F S L S T V D 20
CTG CCA GAG CTG GAC GAC AGC TCT CAC TCC CTC GAC ATG AAA CAT TTG TCC ACA TTA GAC
L P E L D D S S H S L D M K H L S T L D 40
TAC GCT TCC ATT TCC TCC TCC TCC ATC CCT TCC TCC CTG TCT CCC CCA CTT GTG TCC TCC
Y A S I S S S S I P S S L S P P L V S S 60
ATC TCT TCC GCT GGT GTG GCC TAC GAC ATC AGC CCA CCA CAG AGG GAA GAA CAC CTG ACC
I S S A G V A Y D I S P P Q R E E H L T 80
AAC ATG GAC TAC ACA AAC ATG CAC AGC TAC AGG ACA GAC CTG GAC ACA CAC AAT TCA ATC
N M D Y T N M H S Y R T D L D T H N S I 100
AAG CTG GAG CCA GAG TCC CCT CCA CAG TAC TCC GAC AGT CCG GTG TTC TCT AAG CTC CAG
K L E P E S P P Q Y S D S P V F S K L Q 120
GAC GAT ACA TCG GCA GCA GCG CTA AAC ATC GAG TGC CGT GTG TGT GGA GAC AAA GCC TCA
D D T S A A A L N I E C R V C G D K A S 140
GGG TTT CAC TAT GGC GTC CAT GCC TGT GAG GGC TGT AAG GGT TTC TTC AGG CGC ACA ATC
G F H Y G V H A C E G C K G F F R R T I 160
AGG TTA AAG TTG GTG TAC GAT CAC TGC GAT CTT CAC TGT CGC ATT CAC AAA AAG TCC CGC
R L K L V Y D H C D L H C R I H K K S R 180
AAC AAA TGC CAA TAC TGT CGC TTC CAG AAG TGC CTC AAT GTC GGC ATG TCA CAC AAC GCC
N K C Q Y C R F Q K C L N V G M S H N A 200
ATT CGC TTT GGC CGA ATG CCT CAA GCA GAG AAG GAG AAA CTG CTC GCT GAG TTT TCA TCT
I R F G R M P Q A E K E K L L A E F S S 220
GAC ATG GAG CAC ATG CAT CCA GAG GCA GCA GAT CTG AGG GCT CTG GCT CGG CAT CTT TAT
D M E H M H P E A A D L R A L A R H L Y 240
GAG GCC TAT CTG AAA TAC TTC CCC CTC ACC AAG GCC AAG GCC AGG GCC ATC CTC TCT GGG
E A Y L K Y F P L T K A K A R A I L S G 260
AAG ACC GGA GAC AAC ATG CCT TTC GTC ATC CAT GAC ATG AAG TCT CTG ATG GAA GGA GAG
K T G D N M P F V I H D M K S L M E G E 280
CAG TTT ATT AAT TGT AGG CAG ATA CCC ATG TTG GAG CAC CAG CAG CAG ATG TCC GCT GTT
Q F I N C R Q I P M L E H Q Q Q M S A V 300
ACA GCT GGA CAC GGA GGG ATC AGC GGA GGT CAT CAA GGT TCA GAC TGT GGC ATT CTG GGA
T A G H G G I S G G H Q G S D C G I L G 320
ATG ACG AGC CTC AGC GGA CAG GAA CCC ACC GAC ACC GTG GAG CTG CGA TTC TTC CAA AGC
M T S L S G Q E P T D T V E L R F F Q S 340
TGT CAG TCA CGT TCA GCG GAG GGA GTG AGG GAG GTG ACG GAG TTT GCC AAG AGT ATC CCA
C Q S R S A E G V R E V T E F A K S I P 360
GGA TTC ATC AAC CTT GAC CTC AAT GAT CAG GTA ACT TTG CTG AAG TAC GGT GTG ATC GAG
G F I N L D L N D Q V T L L K Y G V I E 380
GTC TTG ATC ATC ATG ATG GCA CCT CTG ATG AAC AAA GAC GGG ACC CTG ATC TCC TAT GGA
V L I I M M A P L M N K D G T L I S Y G 400
CAG ATT TTT ATG ACG CGG GAG TTT ATC AAG AGT CTC AGG AAA CCT TTC TGT CAA ATG ATG
Q I F M T R E F I K S L R K P F C Q M M 420
GAA CCC AAG TTT GAG TTC TCC GTC AAG TTT AGC ACG CTG GAG CTG GAT GAC AGC GAC ATG
E P K F E F S V K F S T L E L D D S D M 440
GCG CTG TTT CTG GCC GTT ATT ATC CTC AGT GGG GAC CGC CCG GGC CTG CTG AAC GTG AAG
A L F L A V I I L S G D R P G L L N V K 460
CCC ATC GAG AAG CTT CAG GAG ACG GTG CTT CAT TCT CTG GAG CTG CAG CTG AAA CTA AAC
P I E K L Q E T V L H S L E L Q L K L N 480
CAC CCG GAC TCT CTG CAG CTG TTC GCC AAG CTG CTC CAG AAG ATG ACG GAC CTG CGT CAG
H P D S L Q L F A K L L Q K M T D L R Q 500
ATA GTC ACC AAT CAC GTC CAC CTC ATC CAG CTG CTG AAG AAG ACC GAG GTG GAC ATG TGC
I V T N H V H L I Q L L K K T E V D M C 520
TTA CAC CCA CTG CTG CAG GAG GTC ATG AAG GAC TTG TAT **TAG**aaatccacaggagccgaaga
L H P L L Q E V M K D L Y * 540
caagtacagaaaagtgcgaagaagtgtaaaagagagatcaaaatgaagagatttttttagaataaaa
tcatgagttgtgattaagtgactgactgaaattgagaaagtgatggggaagaacaagaaacgagaaat
caaggaaaaaaaatacagattcagttttagatcttgtctagacagcctgcatgtga ataacttgtggt
gcacagtcatttatcctctgctgagcctggacattgtgttatgagtaggctgccaacgcaaatatgaa
actaccacctattgcacccctcgccaagagcatctatgactcgctgaaagagtggggactctcttatt
ggcaagctcgagtataaaaattgtgaatgtttgacacagccagcttcatgtgcctttaaggagaaatg
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aactatcataggcagcagcagcagcagcagcagtgtgtacgtgcttcagtacctccgtgaacacgcac
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ttttgtttttgcacggaatgaatgccgttttaaaaacaaatatattatacatataaaatgtatgatat
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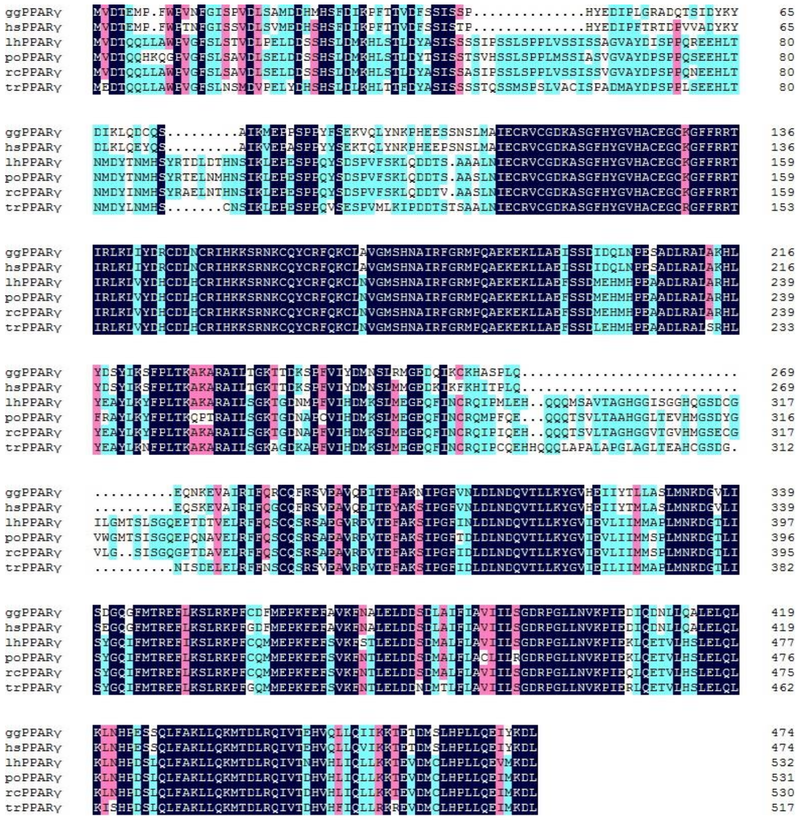
tttcaaattctgtaacacctgttgagttataatttttactatgaagttattttgcatctatggaaact
gtcttctctgtctgatccatcagatgttgttactgtgggtgggtggggctgcgcatacaaagactgga
ttgatagaaatttgttgta***aataaa***atattttttaactttttacttcaaaaaaaaaaaaaaaaaaaaa
aaaaaaa

Figure 1. Nucleotide and deduced amino acid sequences of *lhPPARγ* (GenBank accession number: KJ848473.1). 5'UTR and 3'UTR were showed in low case letters. Start and stop codons are indicated by bold letters. The potential polyadenylation signal (AATAAA) were showed in bold and italic letters. The N-terminal region was underlined, the DBD was boxed, while the LBD was indicated by arrow.



```
ggPPARγ   MVDTEMP.FWPVNFGISPVDLSAMDDHMHSFDIKPFTTVDFSSISSP..............HYEDIPLGRADQTSIDYKY    65
hsPPARγ   MVDTEMP.FWPTNFGISSVDLSVMEDHSHSFDIKPFTTVDFSSISTP..............HYEDIPFTRTDPVVADYKY    65
lhPPARγ   MVDTQQLLAWPVGFSLSTVDLPELDDSSHSLDMKHLSTLDYASISSSSIPSSLSPPLVSSISSAGVAYDISPPQREEHLT    80
poPPARγ   MVDTQQHKQGPVGFSLSAVDLSELDDSSHSLDMKHLSTLDYTSISSTSVHSSLSPPLMSSIASVGVAYDPSPPQSEEHLT    80
rcPPARγ   MVDTQQLLAWPVGFSLSAVDLSELDDSSHSLDMKHLSTLDYASISSASIPSSLSPPLVSSISSVGVAYDPSPPQNEEHLT    80
trPPARγ   MEDTQQLLAWPVGFSLNSMDVPELYDHSHSLDLKHLTTFDYASISSSSTQSSMSPSLVACISPADMAYDPSPPLSEEHLT    80

ggPPARγ   DIKLQDCQS.........AIKMEPPSPPYFSEKVQLYNKPHEESSNSLMAIECRVCGDKASGFHYGVHACEGCKGFFRRT   136
hsPPARγ   DLKLQEYQS.........AIKVEPASPPYYSEKTQLYNKPHEEPSNSLMAIECRVCGDKASGFHYGVHACEGCKGFFRRT   136
lhPPARγ   NMDYTNMHSYRTDLDTHNSIKLEPESPPQYSDSPVFSKLQDDTS.AAALNIECRVCGDKASGFHYGVHACEGCKGFFRRT   159
poPPARγ   NMDYTNMHSYRTELNMHNSIKLEPESPPQYSDSPVFSKLQDDTS.AASLNIECRVCGDKASGFHYGVHACEGCKGFFRRT   159
rcPPARγ   NMDYINMHSYRAELNTHNSIKLEPESPPQYSDSPVFSKLQDDTV.AASLNIECRVCGDKASGFHYGVHACEGCKGFFRRT   159
trPPARγ   NMDYLNMHS.......CNSIKLEPESPPQVSESPVMLKIPDDTSTSAALNIECRVCGDKASGFHYGVHACEGCRGFFRRT   153

ggPPARγ   IRLKLIYDRCDLNCRIHKKSRNKCQYCRFQKCLAVGMSHNAIRFGRMPQAEKEKLLAEISSDIDQLNPESADLRALAKHL   216
hsPPARγ   IRLKLIYDRCDLNCRIHKKSRNKCQYCRFQKCLAVGMSHNAIRFGRMPQAEKEKLLAEISSDIDQLNPESADLRALAKHL   216
lhPPARγ   IRLKLVYDHCDLHCRIHKKSRNKCQYCRFQKCLNVGMSHNAIRFGRMPQAEKEKLLAEFSSDMEHMHPEAADLRALARHL   239
poPPARγ   IRLKLVYDHCDLHCRIHKKSRNKCQYCRFQKCLNVGMSHNAIRFGRMPQAEKEKLLAEFSSDMEHMHPEAADLRALARHL   239
rcPPARγ   IRLKLVYDHCDLHCRIHKKSRNKCQYCRFQKCLNVGMSHNAIRFGRMPQAEKEKLLAEFSSDMEHMHPEAADLRALARHL   239
trPPARγ   IRLKLVYDHCDLHCRIHKKSRNKCQYCRFQKCLNVGMSHNAIRFGRMPQAEKEKLLAEFSSDLEHMHPEAADLRALSRHL   233

ggPPARγ   YDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLRMGEDQIKCKHASPLQ...........................   269
hsPPARγ   YDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKIKFKHITPLQ...........................   269
lhPPARγ   YEAYLKYFPLTKAKARAILSGKTGDNMPFVIHDMKSLMEGEQFINCRQIPMLEH..QQQMSAVTAGHGGISGGHQGSDCG   317
poPPARγ   FRAYLKYFPLTKQPTRAILSGKTGDNAPCVIHDMKSLMEGEQFINCRQMPFQE...QQQTSVLTAAHGGLTEVHMGSDYG   316
rcPPARγ   YEAYLKYFPLTKAKARAILSGKTGDNAPFVIHDMKSLMEGEQFINCRQIPIQEH..QQQTSVLTAGHGGVTGVHMGSECG   317
trPPARγ   YEAYLKNFPLTKAKARAILSGKAGDKAPFVIHDMKSLMEGEQFINCRQIPCQEHHQQQLAPALAPGLAGLTEAHCGSDG.   312

ggPPARγ   ..........EQNKEVAIRIFQRCQFRSVEAVQEITEFAKNIPGFVNLDLNDQVTLLKYGVHEIIYTLLASLMNKDGVLI   339
hsPPARγ   ..........EQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVHEIIYTMLASLMNKDGVLI   339
lhPPARγ   ILGMTSLSGQEPTDTVELRFFQSCQSRSAEGVREVTEFAKSIPGFINLDLNDQVTLLKYGVIEVLIIMMAPLMNKDGTLI   397
poPPARγ   VWGMTSISGQEPQNAVELRFFQSCQSRSAEAVREVTEFAKSIPGFTDLDLNDQVTLLKYGVIEVLIIMMSPLMNKDGTLI   396
rcPPARγ   VLG..SISGQGPTDAVELRFFQSCQSRSAEAVREVTEFAKSIPGFIDLDLNDQVTLLKYGVIEVLIIMMSPLMNKDGTLI   395
trPPARγ   ..........NISDELELRFFNSCQSRSVEAVREVTEFAKSIPGFIDLDLNDQVTLLKYGVIEILIIMMAPLMNKDGTLI   382

ggPPARγ   SDGQGFMTREFLKSLRKPFCDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQL   419
hsPPARγ   SEGQGFMTREFLKSLRKPFGDFMEPKFEFAVKFNALELDDSDLAIFIAVIILSGDRPGLLNVKPIEDIQDNLLQALELQL   419
lhPPARγ   SYGQIFMTREFIKSLRKPFCQMMEPKFEFSVKFSTLELDDSDMALFLAVIILSGDRPGLLNVKPIEKLQETVLHSLELQL   477
poPPARγ   SYGQIFMTREFLKSLRKPFCQMMEPKFEFSVKFNTLELDDSDMALFLACLILRGDRPGLLNVKPIEKLQETVLHSLELQL   476
rcPPARγ   SYGQIFMTREFLKSLRKPFCQMMEPKFEFSVKFNTLELDDSDMALFLAVIILSGDRPGLLNVKPIEQLQETVLHSLELQL   475
trPPARγ   SYGQIFMTREFLKSLRKPFGQMMEPKFEFSVKFNTLELDDNDMTLFLAVIILSGDRPGLLNVKPIERLQETVLHSLELQL   462

ggPPARγ   KLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQIIKKTETDMSLHPLLQEIYKDL                           474
hsPPARγ   KLNHPESSQLFAKLLQKMTDLRQIVTEHVQLLQVIKKTETDMSLHPLLQEIYKDL                           474
lhPPARγ   KLNHPDSLQLFAKLLQKMTDLRQIVTNHVHLIQLLKKTEVDMCLHPLLQEVMKDL                           532
poPPARγ   KLNHPDSLQLFAKLLQKMTDLRQIVTDHVHLIQLLKKTEVDMCLHPLLQEIMKDL                           531
rcPPARγ   KLNHPDSLQLFAKLLQKMTDLRQIVTDHVHLIQLLKKTEVDMCLHPLLQEIMKDL                           530
trPPARγ   KISHPDSLQLFAKLLQKMTDLRQIVTDHVHFIQLLRKREVDMCLHPLLQEIMKDL                           517
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Figure 2. Alignment of PPARγ deduced amino acid sequences between *Liza Haematocheila* (lhPPARγ: KJ848473.1) with *Gallus gallus* (ggPPARγ: NP_001001460.1), *Homo sapiens* (hsPPARγ: CAA62153.1), *Paralichthys olivaceus* (poPPARγ: ACO55651.1), *Rachycentron canadum* (rcPPARγ: ABC50163.1), *Takifugu rubripes* (trPPARγ: NP_001091096.1). The shared residues represented the similar regions between the different species.

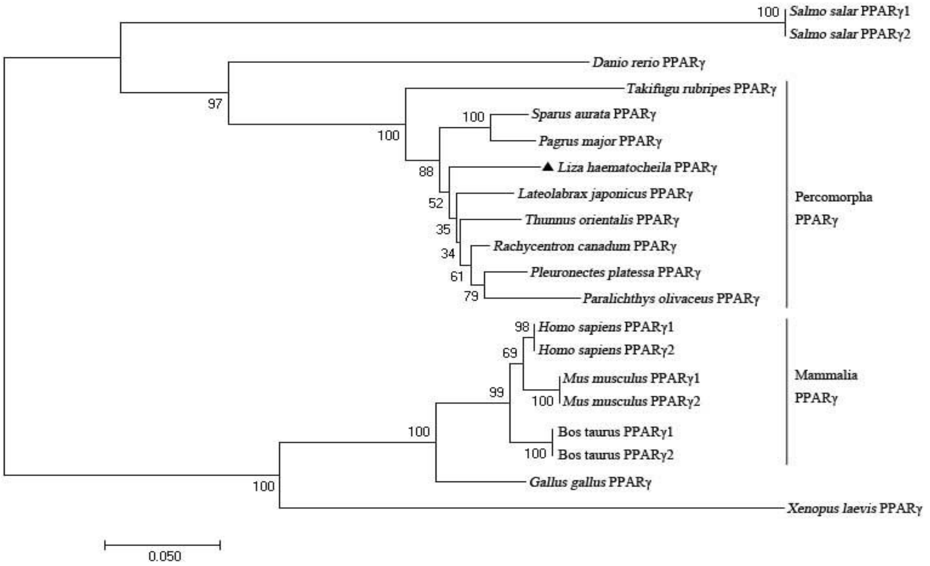

Figure 3. Phylogenetic analysis of PPARγ sequences in vertebrates. An unrooted phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replications. The numbers shown at branches indicated the bootstrap values (%). The sequences were extracted from GenBank: *Salmo salar* PPARγ1 (CAC95230.1), PPARγ2 (ACZ62641.1), *Danio rerio* PPARγ (NP_571542.1), *Takifugu rubripes* PPARγ (NP_001091096.1), *Sparus aurata* PPARγ (AAT85618.1), *Pagrus major* PPARγ (BAF80459.1), *Lateolabrax japonicus* PPARγ (ABC70398.1), *Thunnus orientalis* PPARγ (BAK20459.1), *Rachycentron canadum* PPARγ (ABC50163.1), *Pleuronectes platessa* PPARγ (CAD62449.1), *Paralichthys olivaceus* PPARγ (ACO55651.1), *Homo sapiens* PPARγ1 (CAA62153.1); PPARγ2 (AAC51248.1), *Mus musculus* PPARγ1 (NP_001120802.1); PPARγ2 (NP_035276.2), *Gallus gallus* PPARγ (NP_001001460.1), *Xenopus laevis* PPARγ (NP_001081312.1), and *Liza Haematocheila* PPARγ (KJ848473.1) which was marked with triangle.

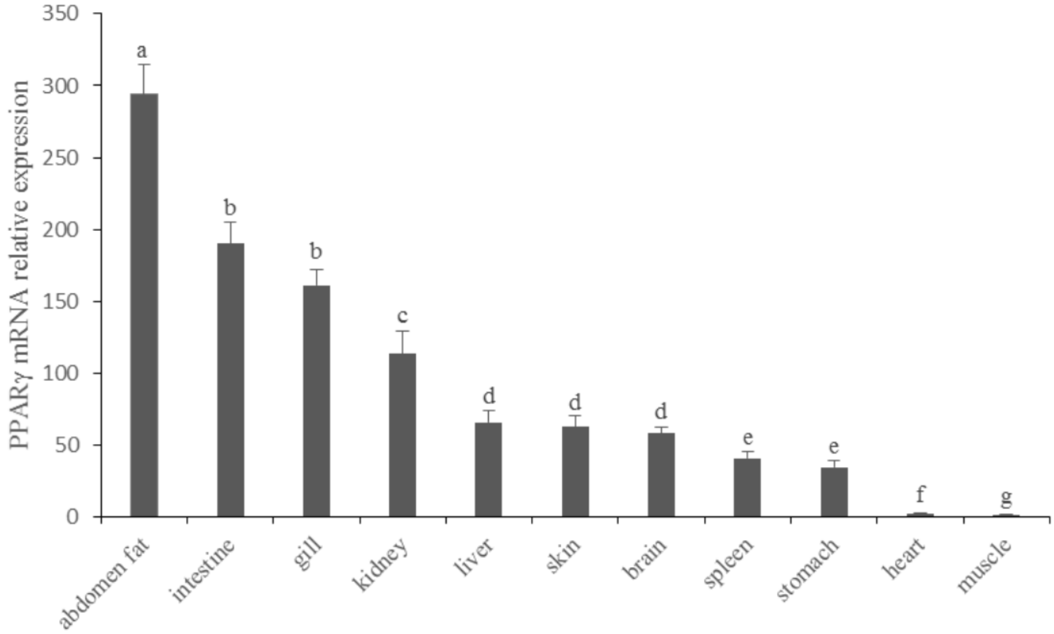

Figure 4. Tissue-specific expression of the *lhPPARγ* mRNA determined by real time quantitative RT-PCR. The relative *lhPPARγ* mRNA expression of each tissue was calculated by the $2^{-\Delta\Delta Ct}$ methods. *β-actin* was selected as a reference gene and expression level of *lhPPARγ* mRNA in muscle was arbitrarily defined as 1. The values represented the means ± SD (n = 9). Significant difference was showed in different low case letters at P< 0.05.

Table 3. lh*PPARγ* mRNA expression response to dietary lipid levels

Tissues	Dietary lipid levels (%)					
	2.0	4.8	7.5	9.8	12.0	14.6
Liver	1.01±0.08	1.04±0.15	1.25±0.19	1.24±0.14	1.27±0.21	1.37±0.20
Abdomen fat	1.03±0.13	1.01±0.09	1.03±0.06	1.37±0.16	1.32±0.12	1.39±0.14
Muscle	1.09±0.11	1.22±0.13	1.17±0.07	1.23±0.12	1.43±0.11	1.48±0.16

Note: The relative *lhPPARγ* mRNA expression at different lipid levels was calculated by the $2^{-\Delta\Delta Ct}$ methods. At the lipid content of 2.0%, expression level of *lhPPARγ* mRNA in liver, abdomen fat and muscle was arbitrarily defined as 1. The values represented the means ± SD (n = 9).

including the N-terminal region, DBD, flexible domain and CBD. Although the overall primary sequence of lhPPARγ shows 64.8% identity with human PPARγ, the DBD and LBD show 95.2% and 72.2% identities, respectively. Similar results were also found in many other studies (Boukouvala *et al*., 2004, Cho *et al*., 2009; Luo *et al*., 2015; Zheng *et al*., 2015) indicating that the DBD and LBD of these receptors were conserved in the lower vertebrates. Multiple sequence alignment demonstrated that the fish PPARγ protein is approximately 30 amino acid residues longer than its mammalian counterpart mainly inserting in N-terminal and LBD. lhPPARγ was more closely related to the Percomorpha PPARγ than to other species, which was also demonstrated by the phylogenetic analysis.

lhPPARγ mRNA was expressed in abdomen fat with the highest level, followed by intestine, gill, kidney, liver, skin, brain, spleen, stomach, liver, heart, while the lowest expression of *lhPPARγ* mRNA was observed in muscle. The widely spatial distribution indicated that most tissues were potential targets of *lhPPARγ* due to its role in many metabolic pathways. The highest mRNA level of *lhPPARγ* observed in abdomen fat was in agreement with its physiological role in fat accumulation and adipocyte differentiation (Rosen & Spiegelman, 2006). Similar findings were also observed in other fish such as yellow catfish (Zheng *et al*., 2015), orange-spotted grouper (Luo *et al*., 2015) and olive flounder (Cho *et al*., 2009). In contrast, the highest *PPARγ* mRNA expression level was observed in liver and intestine in adult and immature *Megalobrama amblycephala*, respectively (Li, Gui, Wang, Qian, & Zhao, 2013). In humans, *PPARγ* is highly expressed in adipocytes and weakly expressed in the bone marrow, spleen, testis, brain, skeletal muscle, and liver (Elbrecht *et al*., 1996). These discrepancies may be due to differences in organ constitution and functions, and different rates of dietary lipid intake and metabolism among species. Interestingly, high mRNA expression was also found in gill which implied *lhPPARγ* might be involved in osmoregulation process.

The expression of fish *PPARγ* is known to be regulated by nutritional status and several hormones such as insulin and growth hormone in a tissue-specific manner. In mammals, when energy intake is greater than energy expenditure, adipose tissue swells through increasing the numbers and/or enlarging the size of adipocytes (Rosen & Spiegelman 2006). More studies have indicated that *PPARγ* mRNA expression was increased when fed with high lipid diet and a number of PPARγ-targeted genes was also involved in lipogenesis regulation. Lian, Luo, Sui, Li, & Hua. (2015) found that the expression of PPARγ mRNA was significantly up-regulated in n-3 PUFA-enriched diet-fed mice. Jones *et al*. (2005) found that *PPARγ* knockout mice displayed diminished weight gain despite when fed a high lipid diet. Yuan *et al*. (2016) found that the highest expression of hepatic *PPARγ* mRNA existed in grass carp fed with high lipid diet. In this study, we found redlip mullet fed with a high lipid diet showed higher *PPARγ* mRNA expression in the liver, intestine and muscle. However no significant difference was observed, which implied lipogenesis may not activated by the current levels of dietary lipid and redlip mullet may adapt to the current lipid level.

Conclusion

In summary, we cloned the *lhPPARγ* gene, analyzed phylogenetic tree, and characterized tissue distribution and response to dietary lipid levels of the gene. The widespread distribution of *lhPPARγ* (high constitutive expression in different organs) suggests its important biological function. Although insignificant, slightly increased expression of *PPARγ* in response to dietary lipids suggests that *PPARγ* may be possibly involved in lipid metabolism in redlip mullet, but this has to be confirmed in further studies.

Acknowledgments

This research was supported by the Fund Research Project of ‘‘333 Talent Project’’ of Jiangsu Province (No: BRA2015386), the Joint Forward-looking Research Project of Jiangsu Province and Fisheries Tree New Project of Jiangsu Province (No: D2016-18), Open Fund Project of Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization (No: KF201503), Student Innovation Training Program of Yancheng Institute of Technology (2016).

References

Abbott, B.D. (2009). Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. *Reprod Toxicol,* 27 (3-4), 246-257. doi: 10.1016/j.reprotox. 2008.10.001

Adeogun, A.O., Ibor, O.R., Regoli, F., & Arukwe, A. (2016). Peroxisome proliferator-activated receptors and biotransformation responses in relation to condition factor and contaminant burden in tilapia species from Ogun River, Nigeria. *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology*, 183-184, 7-19. doi: 10.1016/j.cbpc.2015.12.006.

Bogacka, I., Bogacki, M., & Wasielak, M. (2013). The effect of embryo presence on the expression of peroxisome proliferator activated receptor (PPAR) genes in the porcine reproductive system during periimplantation. *Acta Vet Hung*, 61(3), 405-415. doi: 10.1556/AVet.2013.013.

Boukouvala, E., Antonopoulou, E., Favre-Krey, L., Diez, A., Bautista, J.M., Leaver, M.J., …Krey, G. (2004). Molecular characterization of three peroxisome proliferator-activated receptors from the sea bass (*Dicentrarchus labrax*). *Lipids*, 39(11),1085-1092.doi:10.1007/s11745-004-1334-z.

Cho, H.K., Kong, H.J., Nam, B.H., Kim, W.J., Noh, J.K, Lee, J.H., … Cheong, J. (2009). Molecular cloning and characterization of olive flounder (*Paralichthys olivaceus*) peroxisome proliferator-activated receptor gamma. *General and Comparative Endocrinology*, 163(3),251-258. doi:10.1016/j.ygcen.2009.04.018.

Cruz-Garcia, L., Sánchez-Gurmaches, J., Monroy, M., Gutiérrez, J., & Navarro, I. (2015). Regulation of lipid metabolism and peroxisome proliferator-activated receptors in rainbow trout adipose tissue by lipolytic and antilipolytic endocrine factors. *Domestic Animal Endocrinology*, 51, 86-95. doi:10.1016/j.domaniend. 2014.11.002.

Desvergne, B., & Wahli, W. (1999). Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews*, 20(5), 649-688. doi: 10.1210/er.20.5.649.

Elbrecht, A., Chen, Y., Cullinan, C.A., Hayes, N., Leibowitz, M.D., Moller, D.E., & Berger, J. (1996). Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma-1 and gamma-2. *Biochemical and Biophysical Research Communications*, 224(2), 431-437. doi:10.1006/bbrc.1996.1044

Fang, C., Wu, X., Huang, Q., Liao, Y., Liu, L., Qiu, L., … Dong, S. (2012). PFOS elicits transcriptional responses of the ER, AHR and PPAR pathways in *Oryzias melastigma* in a stage-specific manner. *Aquatic Toxicology*, 106-107(1), 9-19. doi:10.1016/j.aquatox.2011.10.009.

He, S., Liang, X.F., Qu, C.M., Huang, W., Shen, D., Zhang, W.B., & Mai, K.S. (2012). Identification, organ expression and ligand-dependent expression levels of peroxisome proliferator activated receptors in grass carp (*Ctenopharyngodon idella*). *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology*, 155 (2), 381-388. doi:10.1016/j.cbpc.2011.10.008.

Ibabe, A., Grabenbauer, M., Baumgart, E., Fahimi, H.D., & Cajaraville, M.P. (2002). Expression of peroxisome proliferator-activated receptors in zebrafish (*Danio rerio*). *Histochemistry and Cell Biology*, 118(3), 231-239. doi:10.1007/s00418-002-0434-y.

Jones, J.R., Barrick, C., Kim, K.A, Lindner, J., Blondeau, B., Fujimoto, Y., … Magnuson, M.A. (2005). Deletion of *PPARγ* in adipose tissues of mice protects against high fat diet- induced obesity and insulin resistance. *Proc Natl Acad Sci U S A*, 102(17), 6207-6212. doi: 10.1073/pnas.0306743102.

Livak, K.J., & Schmittgen, T.D. (2001). Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25(4), 402-408. doi: 10.1006/meth.2001.1262.

Kidani, Y., & Bensinger, S.J. (2012). Liver X receptor and peroxisome proliferator-activated receptor as integrators of lipid homeostasis and immunity. *Immunological Reviews*, 249(1), 72-83. doi:10.1111/j.1600-065X.2012.01153.x.

Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., ... Evans, R.M. (1994). Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci U S A*, 91(15), 7355-7359. doi:10.1073/pnas.91.15.7355.

Kondo, H., Misaki, R., Gelman, L., & Watabe, S. (2007). Ligand-dependent transcriptional activities of four torafugu pufferfish *Takifugu rubripes* peroxisome proliferator-activated receptors. *General and Comparative Endocrinology*, 154(1-3), 120-127. doi:10.1016/j.ygcen.2007.05.034.

Krey, G., Keller, H., Mahfoudi, A., Medin, J., Ozato, K., Dreyer, C., & Wahli, W. (1994). Xenopus peroxisome proliferator activated receptors: genomic organization, response element recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *Journal of Steroid Biochemistry and Molecular Biology*, 47(1-6), 65-73. doi:10.1016/0960-0760(93)90058-5.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., … Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23(21), 2947-2948. doi: 10. 1093/bioinformatics/btm404.

Leaver, M.J., Ezaz, M.T., Fontagne, S., Tocher, D.R., Boukouvala, E., & Krey, G. (2007). Multiple peroxisome proliferator-activated receptor beta subtypes from Atlantic salmon (*Salmo salar*). *Journal of Molecular Endocrinology*, 38(3), 391-400. doi:10.1677/JME-06-0043.

Li, S., Gui, Y., Wang, W., Qian, X., & Zhao, Y. (2012). PPARγ, an important gene related to lipid metabolism and immunity in *Megalobrama amblycephala*: cloning, characterization and transcription analysis by GeNorm. *Gene*, 512(2), 321-30. doi:10.1016/j.gene. 2012.10.003.

Lian, M., Luo, W., Sui, Y., Li, Z., & Hua, J. (2015). Dietary n-3 PUFA protects mice from Con A induced liver injury by modulating regulatory T cells and PPAR-γ expression. *PLos One*, 10(7), e0132741. doi:10.1371/journal.pone.0132741.

Liang, X., Zhao, Y., Li, Y., & Gao, J. (2015). Identification and structural characterization of two peroxisome proliferator activated receptors and their transcriptional changes at different developmental stages and after feeding with different fatty acids. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 193, 9-16. doi:10.1016 /j.cbpb.2015.12.002.

Luo, S., Huang, Y., Xie, F., Huang, X., Liu, Y., Wang, W., & Qin, Q. (2015). Molecular cloning, characterization and expression analysis of complement component C8 beta in the orange-spotted grouper (*Epinephelus coioides*) after the Vibrio alginolyticus challenge. *Gene*, 43(7), 310-324. doi:10.1016/j.gene.2015.01. 018.

Montagner, A., Wahli, W., & Tan, N.S. (2015). Nuclear receptor peroxisome proliferator activated receptor (PPAR) β/δ in skin wound healing and cancer. *European Journal of Dermatology*, 25(1), 4-11. doi:10.1684/ejd.2014.2505.

Pavlikova, N., Kortner, T.M., & Arukwe, A. (2010). Peroxisome proliferator-activated receptors, estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and second messenger activator. *Aquatic Toxicology*, 99(2), 176-185. doi: 10. 1016/j.aquatox.2010.04.014.

Poulsen, L., Siersbaek, M., & Mandrup, S. (2012). PPARs: fatty acid sensors controlling metabolism. *Seminars in Cell & Developmental Biology*, 23(6), 631-639. doi:10.1016/j.semcdb.2012.01.003.

Rosen, E.D., & Spiegelman, B.M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature*, 444(7121), 847-853. doi:10.1038/nature 05483.

Shao, X., Wang, M., Wei, X., Deng, S., Fu, N., Peng, Q., … Lin, Y. (2016). Peroxisome proliferator-activated receptor-γ: master regulator of adipogenesis and obesity. *Current Stem Cell Research & Therapy*, 11(3), 282-289. doi: 10.2174/1574888X 106661505 28144905.

Shen, A., Jiang, K., Wang, J., & Shen, X. (2015). Effects of salinity on growth, feeding and the mRNA expression of Na+/K+-ATPase and HSP 90 in *Liza haematocheila*. *Environment and Ecology Research*, 3(3), 51-59.doi: 10.13189/eer.2015.030301.

Sher, T., Yi, H.F., McBride, O.W., & Gonzalez, F.J. (1993). cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry*, 32(21):5598-5604. doi:10.1021/bi00072a01.

Yuan, X., Liang, X.F., Liu, L., Fang, J., Li, J., Li, A., … Wang, Q. (2016). Fat deposition pattern and mechanism in response to dietary lipid levels in grass carp, *Ctenopharyngodon idellus*. *Fish Physiology & Biochemistry*, 1-13. doi: 10. 1007/s10695-016-0240-4.

Zheng, J.L., Zhuo, M.Q., Luo, Z., Pan, Y.X., Song, Y.F., Huang, C., … Chen, Q.L. (2015). Peroxisome proliferator-activated receptor gamma (PPARγ) in yellow catfish *Pelteobagrus fulvidraco*: molecular characterization, mRNA expression and transcriptional regulation by insulin in vivo and in vitro. *General and Comparative Endocrinology*, 212:51-62. doi:10.1016/j.ygcen.2014.12.020.