General and Comparative Endocrinology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Short Communication

Characterization and sexual dimorphic expression of Cytochrome P450 genes in the hypothalamic–pituitary–gonad axis of yellow catfish

Shuting Xiong [a], Jing Jing [a], Junjie Wu [a], Wenge Ma [a], Farman Ullah Dawar [a], Jie Mei [a,*], Jian-Fang Gui [a,b,*]

[a] College of Fisheries, Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Huazhong Agricultural University, Wuhan 430070, China
[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, University of the Chinese Academy of Sciences, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 8 December 2014
Revised 18 April 2015
Accepted 21 April 2015
Available online xxxx

Keywords:
Cytochrome P450 (*CYP*)
Pelteobagrus fulvidraco
Phylogenetic analysis
Sexual dimorphism
Hormone treatment

ABSTRACT

Yellow catfish (*Pelteobagrus fulvidraco*) is an important freshwater fish species in China. In particular, an all-male population has been commercially produced for the males grow faster than females. However, the molecular mechanisms underlying sexual dimorphism of body size and sex differentiation are still unclear in yellow catfish. This study attempts to characterize and analyze the expression of Cytochrome P450 (*CYP*) family members that have been shown to play an important role in sex differentiation and metabolism in teleosts. A total of 25 *CYP* genes were identified from our transcriptomes by 454 pyrosequencing and Solexa sequencing, including 17 genes with complete open reading frame (ORF). Phylogenetic analyses were conducted to compare these genes with their counterparts from other teleosts. In the tissues of hypothalamic–pituitary–gonad (HPG) axis, most of the genes were expressed at uniform level in both sexes. However, multiple *CYP* genes displayed sexual dimorphic expression, such as *cyp2AD*, *cyp4b*, *cyp8a*, *cyp11b2*, *cyp17a* and *cyp27a* expressed at higher level in testis than in ovary, whereas *cyp2g*, *cyp7a*, *cyp8b*, *cyp19a1a* and *cyp26a* expressed at higher level in ovary than in testis. The expression response of six *CYP* genes in ovary was also assessed after 17α-methyltestosterone (MT) treatment. Testis-biased expressed *cyp11b2* and *cyp17a* were significantly up-regulated, while *cyp11a* and *cyp19a1a* were reduced in ovary after MT treatment. Our work is helpful for understanding molecular evolution of *CYP* genes in vertebrates and the mechanism of sexual dimorphism in teleosts.

1. Introduction

The Cytochrome P450 (*CYP*) super-family genes encode multiple enzymes with diverse functions in vertebrates. They usually synthesize or metabolize endogenous molecules and interact with exogenous chemicals from the diets or environments (Baldwin et al., 2009). Recently, *CYP* family members are also reported as essential factors for sex differentiation. *Cyp19a1a*, a gene that is highly expressed in ovaries and aromatizes androgens into estrogens, plays a conserved role for ovary development in teleosts (Heule et al., 2014; Huang et al., 2009). The sexual dimorphic expression of *Cyp19a1a*, *Cyp1a1*, *Cyp2e1*, and *Cyp7b1* has been partially explained by different patterns of epigenetic modifications (Penaloza et al., 2014; Zhang et al., 2013), although some microRNAs are potentially correlated with the female-biased expression of *Cyp2b9* via post-transcriptional regulation (Xie et al., 2013). Transcriptional factors *Sf1* and *Sox9* are co-expressed with *Cyp26b1* in Sertoli cells and up-regulate its expression to ensure the male fate of germ cells, whereas *Cyp26b1* and *Sf1* are co-expressed in Leydig cells. The FOXL2 antagonizes *Cyp26b1* expression in ovaries (Kashimada et al., 2011). Male-biased expression of *CYP2C11* in adult rats is irreversibly imprinted shortly after birth that was likely resulted by growth hormone other than the usual testosterone (Das et al., 2014). Cyp17, an enzyme for androgens and estrogens synthesis in vertebrates (Attard et al., 2008), plays a key role for ovarian development in *Cynoglossus semilaevis* (Chen et al., 2010). Sex-specific expression of *cyp19a1a* in XX gonad at 5 dah and *cyp11b2* in XY gonad from 35 dah was observed during early gonad differentiation in Nile tilapia (Ijiri et al., 2008). However, the regulation and function of CYPs in sexual dimorphism are still unclear.

Sexual dimorphism is widely defined as the morphological, physiological and behavioral differences between individuals of

* Corresponding authors at: College of Fisheries, Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Huazhong Agricultural University, Wuhan 430070, China. Fax: +86 27 68780707 (J.-F. Gui).
E-mail addresses: jmei@mail.hzau.edu.cn (J. Mei), jfgui@ihb.ac.cn (J.-F. Gui).

http://dx.doi.org/10.1016/j.ygcen.2015.04.015
0016-6480/

different sexes in the same species (Desjardins and Fernald, 2009; Leinonen et al., 2011). Sex dimorphic growth pattern and male priority phenomenon have been observed in yellow catfish, and an all-male population has been produced in this fish by crossing YY super-male and XX female (Dan et al., 2013; Gui and Zhu, 2012; Wang et al., 2009). However, the exact molecular mechanism for sexual dimorphism remains unclear. Yellow catfish will provide a promising research model to study candidate genes and possible pathways responsible for sexual dimorphism and sex differentiation (Chen et al., 2014; Jing et al., 2014; Mei and Gui, 2015). Here, we reported the identification and characterization of yellow catfish *CYP* genes and investigated their phylogenetic relationships to other vertebrates. We also examined the sexual dimorphic expression pattern of *CYP*s in hypothalamus-pituitary-gonad axis of yellow catfish. Our study will provide a clue to reveal molecular mechanism of sexual dimorphism and sex differentiation in teleosts.

2. Materials and methods

2.1. Experimental animals and hormone treatment

One-year-old sexually mature yellow catfish individuals with similar size were collected from our breeding center at Jingzhou, Hubei province, China. All fish were acclimatized in the laboratory facility for one week, the water was aerated and temperature was maintained at 26 °C. The genetic sex of experimental fish was determined as described previously (Dan et al., 2013). Three kinds of tissues including pituitary, hypothalamus and gonads of each sex were collected from 3 individuals. The experimental operations were conducted as the requirement of the institution animal care and use committee of Huazhong Agricultural University.

For the hormone treatment experiment, healthy female individuals were injected with 17α-methyltestosterone (MT, sigma–Aldrich) and male with 17α-ethinylestradiol (EE2, sigma–Aldrich) behind the pectoral fin. The MT and EE2 were dissolved in 5% ethanol/95% saline prior to injection. Control groups were injected only with 5% ethanol/95% saline solution. In previous studies, injection of 1–100 μg MT per gram body weight into fish body has been shown to affect gonadal development and induce female-to-male sex inversion (Kelly et al., 1996; Sarter et al., 2006; Tan-Fermin et al., 1994). When different doses of MT (0, 1, 10 and 100 μg/g body weight) were injected into female yellow catfish, the levels of 17β-estradiol (E2) and 11-ketotestosterone (11-KT) in the blood were measured using the fish E2 ELISA Kit and 11-KT ELISA Kit (Enzyme-linked Biotechnology Company, Shanghai) as described previously (Parikh et al., 2006). We found that 10 μg MT/g body weight is an optimal dose that gives a relative more stable effect on endogenous sex hormones, as indicated by a durative reduction of endogenous E2 and increase of endogenous 11-KT. Moreover, 10 μg EE2/g body weight is a usual concentration for fish injection (Carrera et al., 2007; Jing et al., 2014), that had significant estrogenic effect on male darkbarbel catfish (*Pelteobagrus vachelli*), a close relative species of *Pelteobagrus fulvidraco* (Li et al., 2009). For the following experiment, both MT and EE2 were injected at a dose of 10 μg per gram body weight. Gonadal tissues were sampled at 0, 12, 24, 36, 48, 72, 96 and 120 h post treatment (4–5 fish individuals per group). The collected tissues were immediately stored in liquid nitrogen container for RNA extraction.

2.2. Sequence analysis

Our 454 pyrosequencing library was constructed from multiple tissues of yellow catfish (NCBI accession number: SRP032172), while Solexa sequencing data (unpublished data) was generated from libraries constructed from gonads. The assembled sequences were performed local BLAST searches against the NCBI non-redundant (nr), STRING and GENE databases (cutoff value $E \leqslant 1e{-}5$). Gene names were assigned to each sequence based on the highest alignment score among BLAST matches. A set of *CYP* family genes was retrieved and translated using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The predicted ORFs were then verified by BLASTP against nr protein sequence database. Finally, the output protein sequences were put into The Simple Modular Architecture Research Tool website (SMART) (http://smart.embl-heidelberg.de/) to predict the conserved domains based on sequence homology.

2.3. Phylogenetic analysis

Seventeen *Cyp* genes with full length were identified in *P. fulvidraco*, and ninety-five other *Cyp* genes were obtained from GenBank including *cyp1a1*, *cyp1b1*, *cyp4b*, *cyp4v*, *cyp7a*, *cyp7b*, *cyp8a*, *cyp8b*, *cyp11a*, *cyp11b*, *cyp17a*, *cyp19a1a*, *cyp19b1b*, *cyp20a*, *cyp26a*, *cyp27* and *cyp51*. The coding sequences of total 112 *CYP* genes were initially aligned in MEGA6 by MUSCLE method with default parameters (Tamura et al., 2013). Poorly aligned positions were removed by Gblocks (Castresana, 2000). After Gblocks treatment, the best-fit nucleotide substitution model among 88 models was chosen in jModelTest (Darriba et al., 2012). As a result, the parameters (i.e. partiton, -lnL, proportion of invariable sites and Gamma distribution shape) were optimized using with the Akaike Information Criterion (AIC) (Posada and Buckley, 2004), and the model GTR + I + G was determined as the most likely model of nucleotide substitution. Phylogenetic relationships were reconstructed in Maximum Likelihood (ML) and Bayesian inference (BI). In ML pipeline, PhyML 3.0 (Guindon et al., 2010) were performed with parameters obtained from AIC (The proportion of invariable was fixed to 0.007 and the shape of the gamma distribution parameter was fixed to 1.173). The number of distribution categories was set to 4 and the median approximation was performed for each rate class. The random BioNJ tree and the SPR moves were regarded as starting tree and topological searching operation. Each internal branch of the phylogeny was estimated using non-parametric bootstrap with 1000 replicates. The Bayesian analysis was performed in Mrbayes with following settings by AIC: lset nst = 6 rates = gamma, prset statefreqpr = fixed (0.2275, 0.2747, 0.2840, 0.2139), revmatpr = fixed (rAC = 1.9346, rAG = 3.4645, rAT = 1.6537, rCG = 1.1964, rCT = 4.0525, rGT = 1.0000), Shapepr = fixed (1.1730), pinvarpr = fixed (0.0070). The Markov chains Monte Carlo search was performed with 4 heated chains for 50,000,000 generations, with Samplefreq = 1000. The initial 1250 trees were burn-in and calculated posterior probabilities by the rest of stable trees.

2.4. RNA extraction, reverse transcription and quantitative RT-PCR (qRT-PCR)

The total RNA was extracted using miRNeasy Mini Kit (QIAGEN, USA) and treated with RNase-free DNase (Qiagen) according to the manufacturer's instruction. 1 μg total RNA was reverse transcribed using GoldScript cDNA Synthesis Kit (Invitrogen, USA). Primers were designed using Primer 5.0 and all were listed in Table 1. The primers were confirmed without primer dimers in qPCR test and the sequences were also confirmed by sequencing. qRT-PCRs were performed on a Bio-Rad PCR system by CFX96 Optics Module (Bio-Rad, Singapore) with SYBR Green I Dye as described previously (Wang et al., 2013; Zhong et al., 2014). All qPCR reactions were performed in 20 μL reactions, containing 10 μL 2 × SYBR green master mix (Bio-rad, USA), 0.5 μL (10 μM) of each primers, 1 μL the RT synthetic