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Genetic diversity and association analysis of two duplicated ODC genes polymorphisms with weight gain in *Cyprinus carpio* L.

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ABSTRACT

Ornithine decarboxylase (ODC) is a rate-limiting enzyme in polyamine synthesis, and its activity can be used as an indicator of protein synthesis. In this study, we cloned two ODC1 genes from *Cyprinus carpio* var. Jian, namely *CcODC1a* and *CcODC1b*, identified five and three single nucleotide polymorphism (SNP) loci in the *CcODC1a* and *CcODC1b* sequences, respectively. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to discriminate the genotypes of these SNP loci in a Jian carp breeding base population. Except for *a-E6-A212G* and *b-I3-A126G*, all other SNP loci strayed from Hardy–Weinberg equilibrium. The polymorphism information content ranged from 0.28 to 0.38. The expected heterozygosity and observed heterozygosity were 0.34–0.50 and 0.347–0.626, respectively. So the population had medium heterozygosity and abundant variation, suggesting that sufficient breeding potential existed in the population. Stepwise regression method results indicated the additive effects of three SNPs (*a-E6-A98C*, *a-E9-A19G* and *b-I3-A126G*) on body weight gain were extremely significant at 0.01 level, and the dominant effects of *b-I3-A126G* was also significant at 0.05 level. All the three additive effects SNPs had body weight gain advantageous genotype AA. The individuals inherited advantageous genotype AA of *b-I3-A126G* with the highest body weight gain, showed the greatest potential of being important candidate molecular marker, which could be used in fast growing common carp breeding in the future.

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1. Introduction

Ornithinede carboxylase (ODC) is a rate-limiting enzyme in polyamine synthesis, which converts ornithine into putrescine (Bruno et al., 2014; Hyang et al., 2014). ODC plays important roles in diverse biological processes, including cell growth, differentiation, transformation and apoptosis (Pendeville et al., 2001; Lange et al., 2014). ODC levels were found to be altered in response to many growth factors, oncogenes, tumor promoters and changing in polyamine levels (Thomas and Thomas, 2001; Pegg, 2006). It has been reported that the activity of ODC in the vigorous growth tissues was significantly higher than its activity in slow growing tissues, and could therefore be used as an index of cell proliferation (Tsuji et al., 1998, Favre et al., 2001; Qingsong et al., 2012; Bruno et al., 2013).

The ODC gene is a candidate gene for growth and carcass traits. In chickens, *ODC* transcription levels were associated with the rate of body weight gain (Johnson et al., 1995). Parsanejad et al. (2004) reported that *ODC* polymorphisms were associated with growth traits in White Leghorn chickens. Uemoto et al. (2011) showed that *ODC1* was located in a quantitative trait loci (QTL) region of growth in chicken, and its promoter polymorphisms were significantly correlated with growth and characteristic of body frame. A study on *Salmo salar* confirmed that the muscular ODC activity could be used as an indicator of protein synthesis and specific growth rate (Benfey et al., 1994). Arndt et al. (1994) proved that both the RNA concentration and ODC activity were highly significantly correlated to specific growth rate in Atlantic salmon (*S. salar*), while the RNA concentration was slightly higher than the ODC activity. It had also been suggested that the RNA concentration and enzyme activity of ODC1 can be used as an indicator of protein synthesis. Therefore, it is feasible to screen weight gain-related molecular markers on *ODC1*.

Molecular marker-assisted selection (MAS) can increase selection precision and reduce the number of generations required to achieve a desired improvement (Dekkers, 2004, Collard and Mackill, 2008). It is essential to identify the molecular markers linked to economic traits

Abbreviation: *CcODC1a*, ornithine decarboxylase 1a of *Cyprinus carpio*; *CcODC1b*, ornithine decarboxylase 1b of *Cyprinus carpio*; Jian carp, *Cyprinus carpio* var. Jian; MAF, minimum allele frequency; MAS, molecular marker-assisted selection; ODC, ornithine decarboxylase; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; SNPs, single nucleotide polymorphisms; H-W, Hardy–Weinberg equilibrium; He, expected heterozygosity; Ho, observed heterozygosity; PIC, polymorphism information content.

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during MAS. The screening of traits associated with markers on candidate genes can narrow down the scope, making the process more efficient. Many valuable markers have been identified using this approach. For example, Szydlowski et al. (2012) found a SNP locus in the 5′ flanking region of the pig fat mass and obesity-associated (*FTO*) gene that was associated with fatness in Polish Landrace pigs. Wang et al. (2013) identified SNP loci in F-box only protein 32 (*FBXO32*) that were associated with growth traits in Nan Yang cattle. El-Magd et al. (2014) screened a non-synonymous mutation in the insulin-like growth factor 2 receptor gene that was significantly related to the daily weight gain of Egyptian buffalo. Cong et al. (2014) confirmed that two SNPs in the insulin receptor-related receptor gene were significantly related to growth of the Pacific oyster *Crassostrea gigas*. Klinbunga et al. (2015) found SNPs on the vitellogenin receptor gene, which were significantly associated with gonad index and ovary weight in the giant tiger shrimp *Penaeus monodon*.

In view of the relationship between *ODC1* and growth, we first cloned two common carp *ODC1* genes (*CcODC1*s). Eight SNP loci were identified by comparing the *CcODC1* sequences from six Jian carp (*Cyprinus carpio* var. Jian). Then the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to identify individual genotypes. The Stepwise Regression Method was applied to evaluate the genetic effects of eight loci on the body weight gain in a Jian carp breeding base population that was composed of 12 families.

2. Materials and methods

2.1. Animals

The Jian carp used in this study were from the Jian carp breeding base population, which consists of 12 families and cultured in the Yixing Breeding Base of the Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences (Wuxi, China). Fishes were propagated simultaneously in April 2013 and cultured in different cages for the first month. About 100 individuals per family were randomly selected, PIT tagged and mixed in the same pond when the juvenile Jian carp was about 50 g in weight. Phenotype including body weight and length were collected at the same time. And these data were collected again in November 2014. Accounting for accidents like marker loss and fish death during the culture process, the total experimental fish were 933 individuals. The numbers of individual fish in the different families ranged from 41 to 92. Tail venous blood was collected by disposable syringes from each individual for genomic DNA extraction.

2.2. DNA extraction

Genomic DNA was extracted using an EZNA™ SE Blood DNA Kit (Omega, Norcross, USA) according to manufacturer's protocol. DNA was examined using 1% agarose gel electrophoresis, and the concentration was determined by photometry. The DNA working concentration was 50 ng/μL.

2.3. Cloning and genotyping SNP loci of CcODC1 DNA sequences

Three primer pairs E1F-E6R (located at exons 1 and 6 respectively), E6F-E9R (located at exons 6 and 9 respectively), and E8F-E11R (located at exons 8 and 11 respectively) were designed to clone the two *CcODC1* DNA sequences based on the *CcODC1* cDNA sequence (GenBank accession no. JQ342671) (Table 1). Amplifications were carried out in 25 μL volume with 100 ng template DNA using LA Taq polymerase (Takara, Dalian, China). The polymerase chain reaction (PCR) thermal cycles were as follows: pre-denaturation at 94 °C for 2 min, 94 °C for 30 s, 55–58 °C for 30 s, and 72 °C for 1–2 min for 30 cycles, and a final extension at 72 °C for 10 min. The PCR products were analyzed using 1% agarose gel electrophoresis. The target DNA was recovered from the gel using a gel extraction kit (Takara), then cloned in a pMD18-T vector and introduced into *Escherichia coli* DH5a competent cells. Positive clones were identified by *Eco*RI and *Hin*dIII (Takara) double digestion. At least three clones per plate were sequenced by Shanghai Biosune Biotechnology Co. Ltd. (Shanghai, China). A locus was considered to be a positive SNP locus if it had an alternative base in two or more individuals out of the six samples.

Table 1
Primers used for sequencing the *CcODC1* gene fragments.

Primer	Sequence	Tm (°C)	PCR fragment
E1F	CTCTGGTCTAATGGGAACACTCTTTG	55	exon 1 to 6
E6R	GTACGTCTCAGGATCAGTGCAGC		
E6F	GTTCTCCGCATCGCCACAGA	56	exon 6 to 9
E9R	CGCCGTCATTCACGTAGTACATCAG		
E8F	GATAAGTATTTCCCTGTTGACTGCGG	58	exon 8–11
E11R	CACGAATCTGCTGCATGCACTG		

PCR-RFLP was performed to discriminate different alleles in the SNP loci. SNPs were named as gene name; the number of the exon or intron; followed by the two different nucleotides and their location in the exon or intron of the gene. For example, *a-E6-A98C* indicated that there were A and C alleles at the ninety-eighth base in the sixth exon of *CcODC1a*. The primers and restriction enzymes used in the PCR-RFLP, and the endonuclease fragments of the different alleles were listed in Table 2. The enzyme digestion products were analyzed in 2.0–3.5% agarose gels.

2.4. Sequence analysis and data processing

The sequences were assembled using DNA Star (Lasergene, San Francisco, USA), and aligned using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/). For the polymorphism analysis, the genotypes were determined based on the results of electrophoresis using GenAlEx6.2 (www.anu.edu.au/BoZo/). The Populations software package was used to calculate allele frequency, the Hardy–Weinberg (H–W) equilibrium, heterozygosity (He) and polymorphism information content (PIC). The calculation methods for the different parameter were referred to Nei and Kumar (2000) and Avise (2000). The mixed linear model was used to simultaneously analyze genetic association between multiple markers and growth traits, which was denoted as

$$y_{ij} = \mu + sex + fam + b_o \cdot W_0 + \sum_{i=1}^{8} (a_i \cdot z_i + d_i \cdot w_i) + e$$

Where y_{ij} is the observation for the ith individual in the jth family; μ is population mean; *sex* and *fam* are sex effect and family effect respectively; W_0 is the initial body weight; a_i and d_i are additive and dominant genetic effects, respectively; z_i and d_i are the indicator variables corresponding to the kth marker genotype. w_i is 0 for homozygote and +1 for heterozygote; e is the random residual effect. Generally, z_i is defined as 0 for heterozygote, +1 for the homozygote of lower MAF value and −1 for the other homozygote. Genetic effects for markers were estimated by Stepwise Regression Method in the R project for statistical computing (Shacham and Brauner, 2014). Genetic contribution ratio for the marker was calculated by the formula:

$$\hat{h}_i^2 = \frac{\mathrm{var}(a_i * z_i)}{\sum (a_i * z_i + d_i * w_i) + \mathrm{var}(fam) + \mathrm{var}(e)}$$

Where *fam* and *e* are family variance and residual error variance respectively.

The GLM model of SPSS v17.0 software (SPSS Inc., USA) was used to analyze the association between different genotypes and diplotype of SNPs with body weight gain of Jian carp, $P < 0.05$ was considered statistically significant.

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