QTL for Short-Duration Vigorous Swimming Movements in Common Carp (Cyprinus carpio L.) Based on LDH Activity

Lashari Punhal,1,2 Muhammad Younis Laghari,1,2 Naeem Tariq Narejo,2 Xiaofeng Zhang,1 Peng Xu,1 Xiaowen Sun1* and Yan Zhang1*

1Centre for Applied Aquatic Genomics, Chinese Academy of Fishery Sciences, Beijing, China
2Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro, Pakistan

Abstract.- Lactate dehydrogenase (LDH) enzyme activities in muscle was significantly related with burst swimming speed and endurance swimming capacity in a variety of fish. Quantitative trait loci (QTLs) associated with LDH were analyzed based on F1 family crossed by Hebao and Heilongjiang carp. A total of seven QTLs (qLDH1, qLDH6, qLDH7, qLDH21 qLDH22, qLDH45a and qLDH45b) were detected on six linkage groups (LG1, LG6, LG7, LG21, LG22, LG45), and the explained variances of LDH activity by these QTLs was more than 36%. Confidence intervals ranged from 2.0 to 10 cM in the present study. To increase the intensity of QTL mapping, four new microsatellite markers (CAFS02457, CAFS02458, CAFS02460, and CAFS02461) were developed from common carp genomic fragments. Intensity of candidate QTL intervals was increased (by > 5 cM) in four of the seven QTLs. The p-value of qLDH7 was 0.0007 analyzed by an ANOVA and explained 42.0% of the variance suggesting this QTL maybe a major QTL.

Key words: LDH activity, QTL, vigorous swimming.

INTRODUCTION

Swimming ability is considered as a main character, determining survival in many species of fish. Thus, it is suggested that swimming capability is a major trait affecting fitness (Reidy et al., 2000; Plaut, 2001). Generally, oxygen uptake occurs at the critical swimming speed. It is also relatively close measure of maximum aerobic capacity of the fish and gives a good estimate for swimming ability. In general, it includes aerobic and anaerobic swimming (Hammer, 1995; Tudorache and Viaenen, 2008). Physiology, predation pressure, and natural variation influence the stress response of prey species. The level of response also may be related to aspects of individual antipredator performance, such as escape speed, defense, or locomotor performance (Guderley, 2004; Kaufman and Gunn, 2006; Monclus et al., 2009; Selch and Chipp, 2007; Slos and Stoks, 2008; Sullivan and Somero, 1983). In fish, myotomes contain two basic types of muscle fiber, red and white muscle (George, 1962; Ogata, 1958; Ogata and Mori, 1964). White muscle is mainly involved in short-duration vigorous swimming movement and red muscle is for slowly swimming movement.

Muscle glycolytic lactate dehydrogenase (LDH) enzyme activities have been shown to be correlated with burst swimming speed and endurance swimming capacity in a variety of fish (Garenc and Couture, 1999; Martinez and Guderley, 2003). LDH, an anaerobic cytosolic enzyme that is involved in glycolysis, provides a continuous supply of NAD+ oxidized during anaerobic glycolysis, thus maintaining redox balance. LDH activity is significantly and consistently correlated with the overall morphology of fish (Anders, 2005). The lactate generated in white muscle during bursts of high-speed swimming is generally believed to be transported via the circulatory system from muscle to tissues such as the heart, gill, and liver, where aerobic metabolism dominates (Bilinski, 1974; Driedzic and Hochachka, 1978). Activity of LDH reflects the increased glycolytic capacity of the white myotomal muscle of large animals, which allows fish to produce more power per gram muscle during sprint swimming (Childress and Somero, 1990). Activities of metabolic enzymes have been studied using qualitative trait loci (QTL) analysis in various plants and animals. For example, ATPase
enzyme activities have been examined in the pig Sus scrofa using this technique (Klaus et al., 2006). Post-feeding related enzyme activities, such as those of a-hydroxybutyrate dehydrogenase isocitrate, citrate synthase, LDH, aspartate amino transferase, glutamine synthetase, and Na+/K+ ATPase, have been studied in the dogfish shark (Squalus acanthias) using QTL analysis (Patrick and Walsh, 2006). QTL analysis also has been used to investigate primary carbohydrate metabolism enzymes, including hexose phosphate pool, fructose-6-phosphate, α-D-glucose-6-phosphate (G6P), and α-D-glucose-1-phosphate, in the mouse-ear cress Arabidopsis thaliana (Joost et al., 2008; Saeed-ul-Hassan et al., 2012).

Common carp is widely distributed in China and is cultured in many strains including tolerance to cold temperature and low oxygen level, activity of LDH, various body shape and color, different kind of meat etc (Sun and Liang, 2004; Mao et al., 2009; Liu et al., 2009; Li et al., 2009; Zhang et al., 2011, 2013; Zheng et al., 2011; Jin et al., 2012; Wang et al., 2012; Laghari et al., 2013a,b,c). The Hebao carp (Cyprinus carpio var. wayuanensis) is a major strain cultured in China, and it is well known for its high growth rate, tolerance to low oxygen levels, and red color (Sun et al., 1994; Sun and Zhang, 1994; Zhang and Sun, 1994). Heilongjiang carp (Cyprinus carpio haematopterus) is slow growing, with steel-gray color and spindle-shaped body, distributes in Heilongjiang River of China (Hu et al., 2010). Moreover, a genetic linkage map of the common carp (Cyprinus carpio L.) was constructed using 307 markers covering 51 linkage groups with a marker interval of 11 cM in the consensus map (Zhang et al., 2013). This linkage map provides the necessary tool for Quantitative trait loci (QTL) analysis of LDH enzyme activity. In the present study, we used the genetic linkage map to identify QTLs affecting LDH activity within the F1 family. This study will contribute to the development of marker-assisted selection in common carp. There are no previous studies that investigated the QTL analysis associated with LDH activity in fish. However, further research is required to elucidate the functional basis of the genes related to LDH activity based on QTL information.

**MATERIALS AND METHODS**

F1 family of common carp (female Hebao carp X male Heilongjiang carp) was produced at the Aquaculture Experimental Station, Harbin, China. A total of 92 F1 individuals were transferred in to 120 L rectangular tanks with water circulation system and all conditions in the tanks were maintained constantly, i.e. water temperature was 20°C and fish were fed four times per day at 8:00 a.m., 12:00 p.m., 4:00 p.m. and 8:00 p.m. at satiation. We discouraged fish from resting to motivate them for short duration swimming constantly.

White muscle tissue (10.0 g) from 92 F1 individuals were homogenized for 10 s in a 1:50 (wt/vol) dilution of LDH extraction buffer containing 50 mM Tris-HCl, 0.3 mM sucrose, 0.1 M KCl, 1 mM EDTA, 5 mM MgCl₂, and 0.5 mM phenylmethanesulfonyl fluoride at pH 7.4 with a polytron homogenizer. Homogenates were then centrifuged at 4°C for 5 min at 600 RPM. The resulting supernatant produced an enzyme sample that was decanted and assayed for LDH activity. The LDH activity was measured spectrophotometrically using a Monotest LDH kit (Jiancheng Bioengineering Institute, Nanjing, China). The reaction mixture was composed of 50 mM phosphate buffer (pH 7.5), 0.6 mM sodium pyruvate, and 0.18 mM NADH. After pre-incubation at 30 °C for 10 min, the enzyme reagent was added to the reaction mixture and the reaction was carried out at 30°C. The average decrease in absorbance of NADH at 365 nm was measured. The extinction coefficient of 9,118 was used to convert the absorbance change to the molar concentration of the product formed, and the values obtained were converted to those at 25°C using a conversion factor of 0.75. One unit of enzyme activity was defined as 1 mmol of NADH reduced per minute at 25°C. Each sample was analyzed three times. Fin clips of the parents and 92 progeny were collected for DNA isolation. DNA was isolated by the standard phenol–chloroform protocol method (Sambrook and Russell, 2001). The quality of DNA was checked on 1% agarose gel, and the quantity measured using Nanodrop 2000 (Thermo Fisher, USA).

A linkage map of common carp, constructed by Zheng et al. (2011), was used for QTL analysis.
Generally, map is consisted of 307 markers including 109 Simple Sequence Repeats (SSRs), 31 Expressed Sequence Tag-SSR (EST-SSR), and 167 Single Nucleotide Polymorphism (SNP) markers derived from ESTs, which were mapped into 51 linkage groups (Zhang et al., 2013). The linkage map was constructed using JoinMap 4.0 (Stam, 1993) with default significance levels ranging from 4.0 to 10.0 LOD. A threshold of 4.0 was set to detect linkages that may have resulted from allele-coding errors. QTL analysis was carried out using the MapQTL 4.0 program (Van Ooijen et al., 2002). Multiple interval mapping was employed to detect any significant associations between LDH activity and marker loci in the data sets. The LOD score significance thresholds were calculated by permutation (n=10,000) tests in MapQTL 4.0.

A one-way ANOVA was carried out to characterize the QTL allele substitution effects, to evaluate the differences among the genotypes of markers that were nearest to each QTL. The genotypes of the marker locus lying closest to the peak in each of the QTL-containing genomic regions were defined as m1f1, m1f2, m2f1, and m2f2, where m1, m2 and f1, f2 denote the genotypes of the father and mother, respectively. Since abundant genomic resources had been developed in the past years in our research center, including BAC library, BAC end sequences, transcriptome data and draft genome of common carp (Xu et al., 2011, 2012; Ji et al., 2012). These resources provide more chance to develop new microsatellite markers in the region where QTL was located. To fulfill the purpose, we compared the sequences of QTLs markers into common carp genome draft, and then find microsatellite sequence by software Msatfinder V 2.0.9 (Thurston, 2010). Thus, new SSR markers were developed and genotyped in the mapping family to further reduce the distance of the QTL interval. PCR reactions of SSR genotyping were conducted with the following thermo-profile: an initial step at 94°C for 2 min, followed by 30 cycles of 94°C for 30 sec, annealing at Tm temperature for 30 s, and extension at 72°C for 30 s, with a final step of 72°C for 10 min. Each PCR reaction consisted of 1x PCR buffer (Takara, Dalian) with 1.5 mm MgCl2, 200 nm of each PCR primer, 50 mm of each dNTP, 10 ng genomic DNA and 1 unit of Taq DNA polymerase (Takara, Dalian). PCR products were analyzed using 6% PAGE gels on a Genetic Analyzer (ABI 377, Applied Biosystems, Foster City, CA). Genotypes were determined by using a molecular size standard GSROX-500 (Applied Biosystems) with the software GENESCAN 2.1 (Applied Biosystems) (Shen et al., 2005).

RESULTS AND DISCUSSION

A total of four new microsatellite markers were developed to increase marker intensity of the QTL intervals. These new markers, with GenBank accession numbers CAFS02457 (X523660), CAFS02458 (JX523661), CAFS02460 (JX523663), and CAFS0261 (JX523664), were grouped in LG6, LG7, LG22, LG45 (Fig. 1). Unfortunately we could not found available microsatellite markers in LG1 and LG21 respectively. These QTLs intervals were significantly reduced after adding new markers, that qLDH6 interval was reduced from 13.0 into 7.0, qLDH7 interval was reduced from 17.0 into 10.0, qLDH22 interval was reduced from 15.0 into 10.0 and qLDH45b interval was reduced from 6.8 into 2.0 (Fig. 2). Hence, increased markers enhanced the QTL strength and provided the opportunity for gene prediction based on QTL information.

The average value of LDH activity of 92 offspring of the F1 individuals was calculated 2740.54±1037.27 mmol. A total of seven QTLs (qLDH1, qLDH6, qLDH7, qLDH21, qLDH22, qLDH45a and qLDH45b), were found localized on LG1, LG6, LG7, LG21, LG22 and LG45 (Table I). All LGs were found with individual QTL except LG45 that consist of multi QTLs. LOD threshold, by permutation test, was calculated as 4.3, 3.8, 5.5, 4.2, 4.9, 6.0 and 6.0 for each QTL separately. The LOD score was detected 4.6, 4.2, 5.8, 4.5, 5.5, 6.1 and 6.3 corresponding to each QTL. These QTLs covered less than 10 cM with confidential interval ranged from 2.0 to 10 cM. Explained variances of LDH activity by these QTLs were more than 36%, i.e. 49.4, 44.30, 42.0, 40.40, 41.60, 38.2 and 36.1% separately.

In addition to investigate the association between the LDH trait and genotype, ANOVA was performed from the nearest marker of each QTL.
Fig. 1. Six linkage groups (LG1, LG2, LG3, LG4, LG5 and LG6) consisted on QTLs associated with LDH. Each linkage group is highlighted with the QTL name. Graphs located on left side of each linkage group, shows LOD value of QTL. Each graph, shows LOD value of QTL.
Fig. 2. Mapping of QTL for LDH trait detected with genome wide threshold in *Cyprinus carpio*. Red line indicates original QTL region; Blue line shows increased QTL intensity after adding new microsatellite markers: QTL region on each chromosome are related with LDH trait. (LG1, LG6, LG7, LG21, LG22 and LG45).
P-value of $q_{LDH7}$ was 0.0007 and explained variance trait was 42.0%, and it suggested this QTL maybe major QTL (Table I). Female alleles from Hebao carp were associated with LDH activity. Among these QTLs, the neighboring markers (SNP1105, SNP1282, HLJE334 and SNP1454) of four QTLs, was only contained two alleles. This homozygosis of markers as parent of hebao carp, showed high phenotypic value (3212.48±318.6, 3438±430.5, 3277.11±465.5 and 3332.84±148.4). Moreover, nearest markers (HLJ483, HLJE338 and HLJ563) of the other three QTLs was with four alleles, which advantage alleles was from f1 or f2.

In summary, we identified seven QTLs in common carp linkage map, which explained variances of LDH activity. Novel SSR markers were developed and mapped into the QTL intervals, increasing the intensity of candidate QTL intervals. This identified QTL would provide useful initiation on discovering genes that associate with vigorous swimming ability related traits in *C. carpio*.

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