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Polymorphism analysis of the intron one of insulin-like growth factor 2 receptor gene (*IGF2R*) in FFRC strain common carp (*Cyprinus carpio* L.) and its relationship with growth performance

Z.J. Dong[1,2,3], S.Y. Su[2], W.B. Zhu[2], C.F. Zhang[2], M. Ding[3], W.X. Chen[3], X.H. Yuan[2,3] and Z. Xie[1]

[1]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China
[2]Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, China
[3]Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, China

Corresponding author: Z.J. Dong
E-mail: dongzj@ffrc.cn

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ABSTRACT. The insulin-like growth factor 2 receptor gene (*IGF2R*) encodes a transmembrane protein receptor and acts to sequester and degrade excess circulating insulin-like growth factor 2, which is critical for normal mammalian growth and development. Thus, *IGF2R* may serve as a candidate gene underlying growth trait in the common carp. In this study, we isolated the intron one of common carp *IGF2R* and detected the diversity in 3 continuous generations of FFRC strain common carp. A total of 8 loci were detected within this region, which were named in accordance with their location (i.e., Loc84, Loc106, Loc119,

Loc130, Loc145, Loc163, Loc167, and Loc265). Loc106, Loc119, and Loc145 were moderately polymorphic; while Loc84, Loc130, Loc163, Loc167, and Loc265 exhibited slight level of polymorphism. However, significant differences between polymorphism information content values were not observed among the different generations. For Loc145, all generations deviated from Hardy-Weinberg equilibrium. The total number of significant linkage disequilibria for all generations equaled 40. Among them, 4 pairs were detected in each population, while 8 pairs were found in the 2nd and 3rd generations. For Loc130, the G/T genotype exhibited higher body weight when compared to that of the G/G genotype. The frequency of the homozygous G/G genotype reached 87.96%; thus, we can improve FFRC strain common carp growth performance by increasing the percentage of the G/T genotype within a breeding population. Therefore, the G/T genotype could be used as a molecular marker for superior growth traits.

Key words: FFRC strain common carp; *IGF2R*; Intron one; Single nucleotide polymorphisms

INTRODUCTION

The common carp is a major species in aquaculture production, especially in China, where production reached 2539 thousand tons (i.e., ranked No. 4 with regard to the production of freshwater species) per year according to data from the Food and Agriculture Organization (FAO, 2011). At the same year, a new species [i.e., Freshwater Fisheries Research Center (FFRC) strain common carp] was certified by the National Certification Committee for Aquatic Varieties in China. The species has higher growth performance and an improved body shape. Currently, the FFRC strain common carp occupies a pivotal position in China because it is rapidly disseminated to most of the provinces and brings a significant economic profit (Dong, 2011). However, maintaining its status in the market and further genetic improvements have become primary foci for further research on this strain. Of the many breeding methods available, marker assisted selection is popular for increasing the accuracy of selection programs and rate of genetic gain, for which many loci that affect performance traits are regarded as important tools in breeding programs. Thus, selecting suitable candidate genes and identifying performance-correlated loci have stimulated the use of best linear unbiased prediction (BLUP) as the main selective breeding technology for the FFRC strain common carp.

The insulin sample growth factor (IGF) system is a category of small molecular single-chain polypeptides, which include insulin-like growth factor 1 (IGF1), insulin-like growth factor 2 (IGF2), insulin-like growth factor binding protein (IGFBP), insulin-like growth factor 1 receptor (*IGF1R*), and insulin-like growth factor 2 receptor (*IGF2R*). IGFs had been reported to play important regulatory roles in fetal growth and development of numerous vertebrate species. Invasive placentation and gestational fetal growth are not required for imprinted genes - mannose 6-phosphate/IGF2R (M6P/IGF2R) - to evolve (Killian et al., 2000). Considering the transition of the M6P/IGF2R-mediated protein and transmembrane signal transduction, M6P/IGF2R plays an important role in lysosome transportation

and the clearing or activation of growth factors, including the regulation of IGF2 (Kiess et al., 1993). Gu et al. (2009) found that back muscle content changes depending on the developmental stages of *IGF2R* mRNA. Braunschweig (2012) investigated 2 synonymous single nucleotide polymorphisms (SNPs) (c.5403G>C and c.5421G>T) in *IGF2R* exon 37 (which is related to biallelic *IGF2R* expression) where a new cluster of imprinted genes was identified, including *IGF2R*, *Slc22a2*, and *Slc22a3*; the repression of these genes via bidirectional action of the Region2-imprint control element is independent of the transcript overlap via Air RNA (Zwart et al., 2001).

It has been reported that the *IGF2R* structure in fish is similar to that in mammals (Méndez et al., 2001). *IGF2R* in mammals has been widely studied; research in fish, however, is still in the early stages of development. Herein, we report a novel SNP in intron one of FFRC strain common carp *IGF2R*. Moreover, we analyzed the association of polymorphisms in *IGF2R* with growth traits, which will potentially contribute to improvements in fish genetics and breeding.

MATERIAL AND METHODS

Animals stocks, mating design, and rearing of fry

Three successive generations of FFRC strain common carps were obtained from the FFRC Yixing farm in Wuxi, China. The mating scheme for each generation was determined according to the BLUP selective breeding approach. For each stock, females were stimulated for spawning with hormones and fertilized with the milt from each male brooder. The fertilized eggs were transferred to separate hatching hapas. After 1 week, fries from each group were stocked in separate nursery hapas. Reproduction and fry rearing prior to tagging were conducted in fine-mesh hapas inside concrete tanks. Each tank was installed with 2 rows of hapas, leaving a 40-cm passage along the middle of the tank for inspection and feeding. The size of the hapas was as follows: 120 (length) x 80 (width) x 100 (depth) cm, and each hapa was used for a single genetic combination. The tanks were supplied with filtered well water and equipped with air stones for continuous aeration. Throughout the experiment, dissolved oxygen, pH, and temperature ranged from 3.8 to 8.6 mg/L, 7.2 to 8.5, and 18° to 20°C, respectively; the fish were fed to satiation. After ~3 months of *ad libitum* feeding, 50 fish were randomly sampled from each family and tagged individually with passive integrated transponder tags implanted into the abdominal cavity. The tagged fish were reared in a single pond after temporary culture for 3 days. Fish were fed a commercial feed with 30% protein under the conditions described above. For each genetic combination, spawning time and body weight (tagging weight) were recorded at the time of tagging; at harvest, the body weight (harvesting weight) of each individual was also recorded to analyze growth gain, which corresponded to the period between tagging and harvesting. Thus, growth information for the whole population of FFRC strain successive generations was recorded.

A total of 239 individuals belonging to 3 generations were obtained randomly from the FFRC base population as follows: 1st generation (N1, N = 54), 2nd generation (N2, N = 54), 3rd generation (N3, N = 131). The fish were anesthetized and immediately dissected for collection of the caudal fins, which were fixed in 95% alcohol. This research was conducted in accordance with the Code of Ethics of the World Medical Association.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Caudal fin were grinded using mortar and pestle under -20 frozen in ice-cold pack. Then, genomic DNA was isolated from common carp caudal fin by using the Universal Genomic DNA Extraction Kit Version 3.0 (TaKaRa, Japan) according to manufacturer instructions. The concentration and quality of the DNA were verified by spectrophotometry and electrophoresis on 1.0% agarose gel.

According to the genomic sequence of zebrafish *IGF2R* (NM_001039627), PCR primers were designed to amplify 500-bp PCR products for common carp *IGF2R* intron one (Table 1). The 50-μL volume contained 50 ng genomic DNA, 6 μL 10X PCR buffer, 3 μL MgC_{l2}, 3 μL dNTPs, 2 μL IGF2R-F upstream primer, 2 μL IGF2R-F downstream primer, 0.5 μL Taq DNA polymerase, and filled with deionized water to the final volume (50 μL). The cycling protocol was 2 min at 95°C; 35 cycles of 94°C for 30 s, 55° to 65°C for 30 s, and 72°C for 30 s; an extension at 72°C for 10 min; and then stored at 4°C. The size of the amplification products were confirmed by 1.5% agarose gel electrophoresis stained with ethidium bromide. The potential SNP sites were judged by the occurrences of overlapping peaks in the color maps and verified by amplifying and sequencing DNA from individuals within a corresponding pool.

Table 1. Primer sequences used in analyses of the intron one of the *IGF2R* gene.

Primer name	Primer sequence (5'-3')
IGF2R-F	ATGACAGCCCATGGTACAAGGATCTGT
IGF2R-R2	ACCGCGCTTTCTTTTCCACATTCTGT

Statistical analysis

SNPs were detected by Chromas 2.22 and polymorphism information contents (PICs) were calculated according to Nei's methods. The formula was as follows:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2 \qquad \text{(Equation 1)}$$

The least square mean estimates with standard errors for genotypes and growth traits were used. p_i and p_j are the frequencies of the i^{th} and j^{th} alleles, ''n'' is the number of alleles. Statistical analysis was performed on records of growth traits in samples (N = 239). Pedigrees of core animals in the breeding population were traced back 3 generations. The PopGene 1.32 software and statistical analysis SAS were used to analyze the genetic parameters, the observed number of alleles, effective number of alleles, and Shannon's information index:

$$I = -\frac{\sum_{i=1}^{n} \sum_{j=1}^{n} p_{ij} \ln p_{ij}}{n} \qquad \text{(Equation 2)}$$

where n is the SNP maker points, p_{ij} is the frequency of the i^{th} and j^{th} alleles. Cluster analysis based on non-weighted average of UPGMA (un-weighted pair-group method using arithme-

tic average) methods was used to built the Dendrogram by the PHYLIP NEIGHBOR Version 3.5 software (Felsenstein, 1989). The association between each SNP and performance was quantified using general linear models in SAS 8.0, with sex included as a factor:

$$Y_{ij} = \mu + x_I + x_j + e_{ij} \quad \text{(Equation 3)}$$

where Y_{ij} indicates the i^{th} genotype and the j^{th} sex individual, μ is used instead of the mean value, x_i is used instead of the i^{th} genotype, x_j represents the j^{th} sex (j = male/female), and e_{ij} is the residual error.

RESULTS

Isolating the *IGF2R* FFRC strain common carp intron one and detecting the SNPs

In this study, intron one of common carp *IGF2R* with 500 bp was isolated by PCR amplification (Figure 1), and 108 samples from the FFRC strain common carp *IGF2R* were successfully sequenced. All of the sequences above were aligned using DNAMAN V6; the results showed that they had 77.17% similarity by multiple alignment. A total of 8 loci were detected within this region and named according to their location (i.e., Loc84, Loc106, Loc119, Loc130, Loc145, Loc163, Loc167, and Loc265). Only 3 SNPs had a homozygous genotype (Table 2); for example, Loc130 had the G/G and G/T genotypes, and the frequency of the homozygous G/G genotype was as much as 87.96% (Table 2). According to the PIC of each locus (Anderson et al., 1993; Botstein et al., 1980), Loc106, Loc119, and Loc145 were moderately polymorphic, whereas Loc84, Loc130, Loc163, Loc167, and Loc265 exhibited slight level of polymorphism (Table 3). However, no significant differences between PIC values were observed among the different generations.

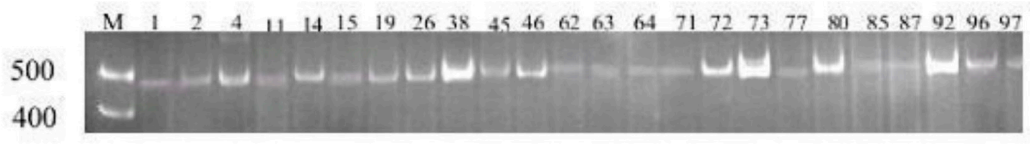

Figure 1. PCR results from the primers of IGF2R-F and IGF2R-R2. *Lane M* = DL 500-bp DNA marker; *lanes 1-97* = 24 samples of the FFRC strain common carp.

Genetic polymorphism analysis of intron one of the *IGF2R* in FFRC strain common carp

According to the assessment method of polymorphisms, the number of alleles observed, effective number of alleles, and Shannon diversity index (Nei, 1987) of the FFRC strain common carp were estimated (Table 4). For each population, the Shannon diversity index analysis revealed an abundance of genetic diversity, except for Loc84 in the 1st generation, and Loc167 and Loc265 in the 2nd generation.

Table 2. Results of single nucleotide polymorphism (SNP) detection for *IGF2R*.

SNP	Ratio of different genotypes			Ratio of different alleles	
	Genotype	N	Ratio (%)	Allele	Ratio (%)
Loc84	A/A	3	2.78	A	8.80
	G/G	92	85.19	G	91.20
	A/G	13	12.04		
Loc106	A/A	4	3.70	A	18.06
	T/T	73	67.59	T	81.94
	A/T	31	28.70		
Loc119	A/A	13	12.04	A	27.31
	G/G	62	57.41	G	72.69
	A/G	33	30.56		
Loc130	G/G	82	75.93	G	87.96
	G/T	26	24.07	T	12.04
Loc145	A/A	58	53.70	A	63.89
	T/T	28	25.93	T	36.11
	A/T	22	20.37		
Loc163	G/G	89	82.41	G	90.28
	T/T	2	1.85	T	9.72
	G/T	17	15.74		
Loc167	T/T	89	82.41	A	8.80
	A/T	19	17.59	T	91.20
Loc265	G/G	89	82.41	G	91.20
	G/T	19	17.59	T	8.80

Table 3. Polymorphism information content of *IGF2R* across generations.

	Multi-population	1st	2nd	3rd
Loc84	0.15	0.13	0.15	0.35
Loc106	0.25	0.33	0.38	0.42
Loc119	0.32	0.47	0.52	0.48
Loc130	0.19	0.35	0.28	0.19
Loc145	0.35	0.54	0.57	0.48
Loc163	0.16	0.30	0.25	0.19
Loc167	0.15	0.28	0.08	0.27
Loc265	0.15	0.31	0.15	0.22
Average	0.22	0.34	0.30	0.33

Table 4. Summary of genetic variation statistics for all loci in all populations.

Generation	SNP	N	N_A	N_E	I
1st	Loc84	80	2	1.0778	0.1599
	Loc106	80	2	1.3423	0.4227
	Loc119	80	2	1.6318	0.5757
	Loc130	80	2	1.5031	0.5173
	Loc145	80	2	1.8089	0.6394
	Loc163	80	2	1.3740	0.4438
	Loc167	80	2	1.2800	0.3768
	Loc265	80	2	1.3423	0.4227
2nd	Loc84	56	2	1.1529	0.2573
	Loc106	56	2	1.3694	0.4409
	Loc119	56	2	1.7326	0.6139
	Loc130	56	2	1.1529	0.2573
	Loc145	56	2	1.9975	0.6925
	Loc163	56	2	1.1128	0.2089
	Loc167	56	2	1.0364	0.0896
	Loc265	56	2	1.0364	0.0896
3rd	Loc84	80	2	1.3423	0.4227
	Loc106	80	2	1.5355	0.5332
	Loc119	80	2	1.6318	0.5757
	Loc130	80	2	1.1327	0.2338
	Loc145	80	2	1.6632	0.5882
	Loc163	80	2	1.1327	0.2338
	Loc167	80	2	1.2195	0.3251
	Loc265	80	2	1.1611	0.2664

N_A = observed number of alleles; N_E = effective number of alleles; I = Shannon's Information index.

The observed heterozygosity (H_O), expected heterozygosity (H_E), Nei's expected heterozygosis, and average heterozygosis are shown in Table 5. In the 1st generation, the H_O for Loc106, Loc119, Loc130, and Loc265 ranged from 0.3000 to 0.4250; the H_O for Loc145, Loc163, and Loc167 ranged from 0.2250 to 0.2500. H_E, and Nei's expected heterozygosis for Loc106, Loc119, Loc130, Loc145, Loc163, and Loc265 ranged from 0.2582 to 0.4528 and 0.2550 to 0.4472, respectively. In the 2nd generation, only the H_O for Loc119 reached 0.3, whereas H_E, and Nei's expected heterozygosis for Loc119 and Loc145 also had values >0.3. In the 3rd generation, only Loc106 had a higher value for H_O, which was >0.3. Similar to the 2nd generation, H_E, and Nei's expected heterozygosis for Loc119 and Loc145 were also >0.3.

Table 5. Summary of heterozygosity statistics for all loci in all 3 populations.

Generation	SNP	N	H_O	H_E	Nei	H_A
1st	Loc84	80	0.0750	0.0731	0.0722	0.1533
	Loc106	80	0.3000	0.2582	0.2550	0.2912
	Loc119	80	0.3250	0.3921	0.3872	0.3991
	Loc130	80	0.4250	0.3389	0.3347	0.1948
	Loc145	80	0.2250	0.4528	0.4472	0.4484
	Loc163	80	0.2250	0.2756	0.2722	0.1636
	Loc167	80	0.2500	0.2215	0.2188	0.1446
	Loc265	80	0.3000	0.2582	0.2550	0.1429
2nd	Loc84	56	0.0714	0.1351	0.1327	0.1533
	Loc106	56	0.1786	0.2747	0.2698	0.2912
	Loc119	56	0.3929	0.4305	0.4228	0.3991
	Loc130	56	0.1429	0.1351	0.1327	0.1948
	Loc145	56	0.1786	0.5084	0.4994	0.4484
	Loc163	56	0.1071	0.1032	0.1014	0.1636
	Loc167	56	0.0357	0.0357	0.0351	0.1446
	Loc265	56	0.0357	0.0357	0.0351	0.1429
3rd	Loc84	80	0.2000	0.2582	0.2550	0.1533
	Loc106	80	0.3500	0.3532	0.3487	0.2912
	Loc119	80	0.2250	0.3921	0.3872	0.3991
	Loc130	80	0.1250	0.1187	0.1172	0.1948
	Loc145	80	0.2000	0.4038	0.3987	0.4484
	Loc163	80	0.1250	0.1187	0.1172	0.1636
	Loc167	80	0.2000	0.1823	0.1800	0.1446
	Loc265	80	0.1500	0.1405	0.1387	0.1429

Nei = Nei's expected heterozygosis; H_A = average expected heterozygosis; H_O = observed heterozygosity; H_E = expected heterozygosity.

Deviation from Hardy-Weinberg equilibrium (HWE) was tested for all generation-locus combinations. The results of the chi-square test indicate that the common carp *IGF2R* gene frequencies of Loc84 in the 2nd generation and Loc119 in the 3rd generation were not in accordance with HWE (Table 6). For Loc145, all generations deviated from HWE ($P < 0.05$). The total number of significant linkage disequilibria for all generations was equal to 40. Among them, 4 pairs were detected in each population, while 8 pairs were found in both 2nd and 3rd generations (Table 7).

Genetic similarity and genetic distance

Based on Nei's genetic similarity coefficient and genetic distance (Nei, 1978) (Table 8), the 1st and 3rd generations exhibited minimum genetic distances and the highest similarities, followed by the 2nd and 3rd generations. In the constructed cluster tree, the 1st and 3rd generations were initially clustered together (Figure 2).

Table 6. Summary of Hardy-Weinberg equilibrium (HWE) for all loci in all 3 populations.

	χ^2 (HWE)			
	Multi-population	1st	2nd	3rd
Loc84	7.28*	0.04	8.15*	2.20
Loc106	0.13	1.13	3.78	<0.01
Loc119	6.00*	1.22	0.23	7.56*
Loc130	1.94	2.72	0.12	0.14
Loc145	34.31*	10.45*	12.22*	10.59*
Loc163	1.30	1.45	0.06	0.14
Loc167	0.95	0.73	<0.01	0.43
Loc265	0.85	1.13	<0.01	0.22

*P < 0.05 and the SNP locus in this population deviated from HWE.

Table 7. Linkage disequilibrium analysis for all loci in all 3 populations.

Different generations					
1st		2nd		3rd	
Loc106_A	Loc130_G	**Loc84_A**	**Loc106_A**	**Loc84_A**	**Loc106_A**
Loc106_A	Loc130_T	**Loc84_A**	**Loc106_T**	**Loc84_A**	**Loc106_T**
Loc106_T	Loc130_G	**Loc84_G**	**Loc106_A**	**Loc84_G**	**Loc106_A**
Loc106_T	Loc130_T	**Loc84_G**	**Loc106_T**	**Loc84_G**	**Loc106_T**
Loc106_A	Loc163_G	**Loc130_G**	**Loc163_G**	Loc119_A	Loc145_A
Loc106_A	Loc163_T	**Loc130_G**	**Loc163_T**	Loc119_A	Loc145_T
Loc106_T	Loc163_G	**Loc130_T**	**Loc163_G**	Loc119_G	Loc145_A
Loc106_T	Loc163_T	**Loc130_T**	**Loc163_T**	Loc119_G	Loc145_T
Loc130_G	**Loc163_G**	**Loc167_A**	**Loc265_G**	**Loc130_G**	**Loc163_G**
Loc130_G	**Loc163_T**	**Loc167_A**	**Loc265_T**	**Loc130_G**	**Loc163_T**
Loc130_T	**Loc163_G**	**Loc167_T**	**Loc265_G**	**Loc130_T**	**Loc163_G**
Loc130_T	**Loc163_T**	**Loc167_T**	**Loc265_T**	**Loc130_T**	**Loc163_T**
				Loc167_A	**Loc265_G**
				Loc167_A	**Loc265_T**
				Loc167_T	**Loc265_G**
				Loc167_T	**Loc265_T**

The letters in bold type indicate that corresponding linkage disequilibrium pairs can be found at least twice in the 3 generations.

Table 8. Nei's original measures of genetic identity and genetic distance.

Generation	1st	2nd	3rd
1st	****	0.9877	0.9922
2nd	0.0123	****	0.9894
3rd	0.0078	0.0107	****

Nei's genetic identity above the diagonal; Nei's genetic distances below the diagonal.

FFRC strain common carp *IGF2R* intron one SNP correlation with body weight

For the original parents of the FFRC strain common carp, sex has been shown to significantly affect body weight (Su et al., 2012); thus, we conducted a similar analysis for FFRC strain common carp (Figure 3A). Similar to prior research, females had higher body weights when compared to the those of the males. When the Loc130 genotype was considered to influence the effect of sex on body weight, the difference in body weight between females and males increased (Figure 3B). In the same model, individuals with the G/T genotype for

Loc130 exhibited higher body weights when compared to those with the G/G genotype (Figure 3C). No significant differences were observed between these 2 genotypes in each generation (Table 9).

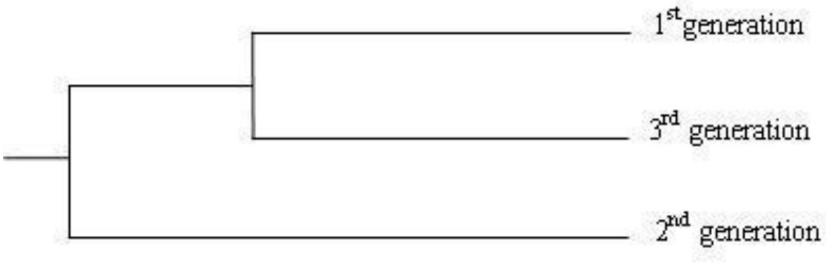

Figure 2. Dendrogram based on Nei's genetic distance.

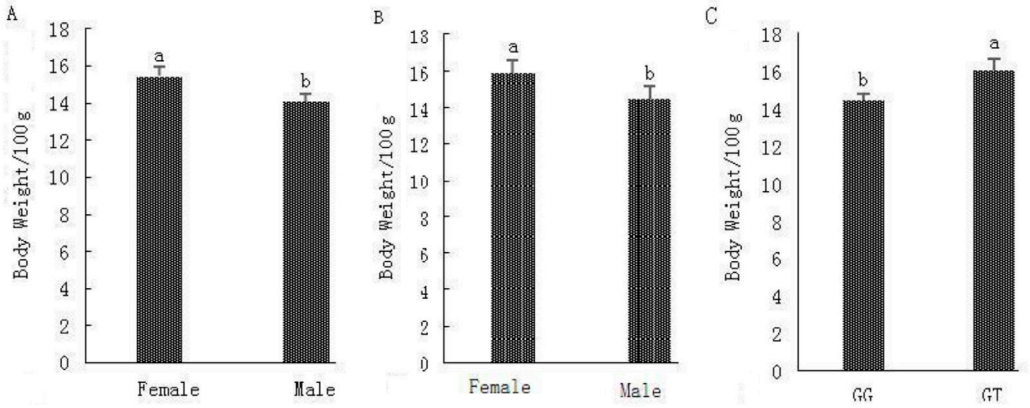

Figure 3. Significance test of sex effects on body weight and significance test of Loc130 effects on body weight.

Table 9. Significance test of sex effects on body weight and significance test of Loc130 effects on body weight distribution of Loc130 in all 3 generations.

Generation	G/G		G/T	
	N	Average body weight	N	Average body weight
1st	23	1726.41 ± 47.59	17	1794.34 ± 64.77
2nd	24	1399.86 ± 64.77	4	1304.48 ± 111.95
3rd	35	1223.95 ± 44.21	5	1231.03 ± 112.92

Only significant associations ($P < 0.05$) between the 4 assayed SNPs and the performance traits investigated were observed; non-significant associations among the different genotypes were not identified. The same genotypes for all loci in the 1st generation were associated with heavier body weights when compared to those of the 2nd and 3rd generations. In the same generation, different genotypes for the same locus did not exhibit significant differences.

DISCUSSION

FFRC strain common carp *IGF2R* intron one polymorphisms

In the present study, 3 loci of *IGF2R* intron one in the FFRC strain common carp population deviated from HWE ($P < 0.05$), which could be explained by intensive selection via long-term commercial breeding programs. Furthermore, only one of the 3 loci deviating from HWE was identified in the 1st generation, while 2 loci were identified in the 2nd and 3rd generations. In other words, the results may be related to further genetic breeding of FFRC strain common carp. Our finding was similar to that reported by other study indicating deviations in genotype frequencies from HWE that would be expected for loci with an impact on the traits under selection in selected populations (Goliásová and Wolf, 2004).

Of the 8 loci (Loc84, Loc106, Loc119, Loc130, Loc145, Loc163, Loc167, and Loc265), Loc106, Loc119, and Loc145 were intermediate polymorphism in the FFRC strain common carp population according to the classification conducted via PIC ($PIC < 0.25$, slight level of polymorphism; $0.25 < PIC < 0.5$, moderate level of polymorphism; and $PIC > 0.5$, high polymorphism). These loci indicate that the intermediate genetic diversity and genetic variation within the carp *IGF2R* intron one were higher than those of other loci. However, each generation yielded similar PICs for these loci, which may be caused by strictly controlling for the inbreed coefficients obtained during the selection of FFRC strain common carp. In combination with the HWE data revealed in this paper, the locus with higher polymorphism may allow for easier detection of deviations in genotype frequencies under intensive selection.

A dendrogram based on Nei's genetic distance showed that the 1st and 3rd generations formed a polyphyletic group, which then clustered with the 2nd generation. This report illustrates allele separation and a homozygous allele that occurred in the 2nd and 3rd generations, respectively.

FFRC strain common carp *IGF2R* intron one polymorphisms and effect of sex on body weight

Berkowicz et al. (2011) quantified genotype-phenotype associations between 3 intronic bovine *IGF2R* SNPs and body size traits in Irish Holstein-Friesian sires. Kaku et al. (2007) also observed a significant difference in birth weight among the 3 neonatal c.901C>G genotypes of the *IGF2R* gene (i.e., CC, CG, and GG). However, it is difficult to use fish as a reference, especially in carp for which the genotype-phenotype associations of *IGF2R* have been rarely reported. In this study, screening the region of FFRC strain common carp *IGF2R* intron one, 8 SNP polymorphisms were identified; one SNP located at Loc130 was detected, whereas the G/T genotype of Loc130 exhibited higher body weight when compared to that of the G/G genotype. The frequency of the homozygous G/G genotype was as high as 87.96%; thus, we can improve FFRC strain common carp growth performance by increasing the percentage of the G/T genotype within a breeding population. Therefore, the results suggest that the G/T genotypes could be used as a molecular marker for superior growth traits. However, further analysis should be performed in order to validate the observed associations and the physiological significance of variation in the intron one of *IGF2R*.

Loc130 analyzed in this study was intronic, and, therefore, it is unlikely that it directly modulates *IGF2R* expression levels or alters the efficiencies of IGF2/IGF2R interactions, thus

leading to heritable differences in carp growth. However, mutations (intron 3 g.3072G>A) occurring in maternally imprinted porcine IGF2 are believed to prevent the binding of an IGF2 repressor protein, resulting in increased IGF2 expression that, in turn, stimulates increased muscle growth (Van Laere et al., 2003; Goodall and Schmutz, 2007; Stinckens et al., 2007, 2010). It is interesting that Braunschweig (2012) found ESTs that mapped to *IGF2R* intron one in sense orientation, which likely represents internally primed transcript artifacts. Therefore, this SNP may be related to the spliced *IGF2R* variant in common carp. Moreover, the heterozygous status of both SNPs, c.5403G>C and c.5421G>T in pigs, is presented, which demonstrates biallelic *IGF2R* expression. Thus, it is possible that the status of *IGF2R* SNPs in the current study may affect *IGF2R* expression levels by preventing or enhancing the binding of repressor/activator molecules to *IGF2R* DNA sequences.

The original parents of the FFRC strain common carp, the Huanghe and Jian carp, exhibited significant differences in body weight between the males and females (Su et al., 2013). Similarly, FFRC strain females also exhibited higher body weights. When the effect of Loc130 was considered in the general linear model, the body weight gain between females and males increased in comparison to other loci. In addition, the estimated male and female body weights were also higher. This illustrated that *IGF2R* imprinting may be an effective way to explain the differences in body weight among the different sexes, which was supported by maternally expressed QTL that can affect muscle depth. Boysen et al. (2010) found a QTL that showed a clear parent-of-origin-dependent effect on the form of maternal polar overdominance. In this article, no significant differences were observed between the 2 genotypes among the 3 generations. However, the same genotype had significant effects on body weight among the different generations, whereas the G/T genotype exhibited improved performance when compared to that of the G/G genotype. This may be caused by the lower number of individuals with the G/T genotype that were detected.

Consequently, *IGF2R* may serve as a candidate gene for growth-related traits in common carp populations. These findings, together with the documented biological roles of the *IGF2R* gene in animal growth and development, suggest that *IGF2R* genes represent important reservoirs of molecular markers for future genetic improvements in livestock populations.

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