JFMSE, 36(1), pp. 11~20, 2024.
수산해양교육연구, 제36권 제1호, 통권127호, 2024.
www.ksfme.or.kr
https://doi.org/10.13000/JFMSE.2024.2.36.1.11

Relationships Between Single Nucleotide Polymorphism and Body Weight of Rainbow Trout (*Oncorhynchus mykiss*) IGF-1 Gene

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무지개 송어 (*Oncorhynchus mykiss*) IGF-1 유전자의 단일염기다형성과 체중 증가 간의 상관관계

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Abstract

Traditional rainbow trout breeding technology, such as SNPs (single nucleotide polymorphisms), assists rainbow trout fish strains. For the study, 28 male fish, 237 female fish and a total of 265 rainbow trout were used for genetic sample collection. We found five single nucleotide polymorphisms and two single nucleotide polymorphisms in the promoter. The two are located in the 3' untranslated region (UTR) at the terminal position. In this study, we found a total of 10 genotypes. Because there are few male samples, these genotypes also occur in female fish and co-exist. By analyzing the correlation between body weight and GBs(Genotype Blocks), effective GBs were selected. And the selection of fast-growing new species, shortening the breeding cycle, achieves the greatest economic benefits and realises the important economic properties of the rainbow trout. The excellent breeding system of the rainbow trout provides a reference for molecular genetics. It also provides a guarantee and basis for future human protection of species diversity, food and nutrition security, and the selection of high-quality species for breeding.

Key words : Rinbow trout , Body weight , Principal component analysis , Insulin-like growth factors -1

Ⅰ. Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a species of Salmon belonging to the family Salmonidae and the genus Pacific Salmon. Insulin-like growth factor 1 (IGF-1) is a member of the insulin family and is an important gene involved in animal growth.

This study uses the current SNP technology of molecular genetics(Yáñez et al. 2020; A. Elkatatny 2016; Hu et al. 2020; Zeng et al. 2021), uses PCR experiments to interpret the gene sequence(Lin et al. 2014) and uses collective comparison of the overall big data to find the

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This work was supported by a Research Grant of Pukyong National University (2022)

single nucleotide polymorphism in the IGF-1 growth factor gene (A. Elkatatny 2016; Sharif et al. 2015), Through this process, the IGF-1 growth factor gene and its relationship with fish growth and weight were discovered in rainbow trout (Ashton, Ritchie, and Wellenreuther 2017). to provide a scientific basis for the domestication of wild animals and plants by humans and to make outstanding contributions to human development in the future.

Ⅱ. Materials and Methods

1. Sample collection and extraction of gDNA

A total of 265 fin samples were taken from the Jang Hang fish farm in Seocheon, South Korea. The rainbow trout samples used in this experiment were diploid and tetraploid, and the triploid sample was produced by hybridization. The fishery has 12 cylindrical breeding tanks with a diameter of 6m and a depth of 1.5m. The fry hatch from the same batch of fertilised eggs and are raised for five months. About 5,000 - 6,000 fish are put into each tank, and a total of 5,000 - 6,000 finished products are raised. 60,000 fish were randomly selected for this research experiment. A total of 265 blood samples were collected from rainbow trout in the study, including 28 males and 237 females. We use the DNeasy blood and Tissue Kit (QIAGEN) to extract genomic DNA from the collected blood samples. When a blood sample is taken, we use it for anticoagulation and we add heparin.

2. PCR amplification and DNA sequencing analysis

In this research activity, specific primers were designed for the PCR of the IGF-1 gene promoter, 5'UTR, and 3'UTR regions (<Table 1>). We tracked the sequence according to the NCBI database and designed rainbow trout-specific primers (IGF-1, GenBank accession number NC_048579). DNA amplification is carried out with the help of polymerase chain reaction (PCR). The composition of the PCR master mix: 0.25ul Ex-Taq DNA polymerase, 5ul buffer 10x, 4ul dNTPs, 2ul forward primer, 2ul reverse primer, 0.5ul genomic DNA, 36.25ul distilled water, and annealing temperature for each pair of primers (<Table 1>). The thermal cycle program was set to 94°C for 5 minutes (pre-denaturation), 94°C for 30 seconds for denaturation, annealing temperature (<Table 1>) for 30 seconds, and 72°C extension time depending on the length of the PCR product (<Table 1>), 72°C for 7 minutes. Use FavorPrep (gel/PCR purification mini) kit to electrophoresis and gel-purify the PCR products, and then submit them to Macrogen.

3. Discovery of SNP and their genotyping

To detect SNPs in this study, the BioEdit (7.0.9.0) alignment tool was used to align the sequences, and ABI sequencing technology was used to achieve SNP identification. If we consider SNP, its allele frequency must be 1% or more in the population (Vigal et al., 2008). In this work, 1% represents two rainbow trout. In the case of parental genotype assignment called genotype blocks (Vignal et al., 2008), clusters of SNP regions are divided into individual blocks. With the help of the genotypic block (GB) structure hypothesis, we discovered a hypothetical SNP marker block related to the weight and point of the IGF-1 gene. Estimates of allele and genotype frequencies, including genotype block inference and their frequencies, have been performed in SNP discovery.

<Table 1> Primer designs for PCR in IGF-1 genes devise

Design primers for IGF-1				
Region	Primers	Primer Length	Annealing Temperature	Annealing Time
Promoter and 5'UTR	Forward primer 5'-TTACGCACGAGCTTCGTGGAC-3' / Reverse primer 5'-AATGCCACTGGAAGAAATGACCGC-3'	499bp	65°C	30sec
A:3'UTR	Forward primer 5'-CTGTTCAGCTATGCTAATGCCGCT-3' / Reverse primer 5'-CAAAGGTTTCTCAAAAGTGCCTGC-3'	854bp	64°C	52sec
B:3'UTR	Forward primer 5'-GATCAGCTGTTCAGCTATGCTAATG-3' / Reverse primer 5'-AAAGGTTTCTCAAAAGTGCCTGC-3'	859bp	61°C	52sec

Ⅲ. Experimental results

This study contributes to the exploration of efficient techniques for the discovery of SNPs in the IGF-1 gene, which regulates the properties of muscle growth. In the IGF-1 gene, we found a total of 2 SNP change sites, the first change position is 22113bp (GG/GA/AA), and the second change position is 22292bp (TT/CT/CC)([Fig. 1]). In particular, we have also calculated the fraction of SNPs in the male and female samples, which gives a better indication of the distribution of SNPs and provides a basis for the study(<Table 2>, <Table 3>). SNPs were screened for both homozygosity and heterozygosity. The average value of polymorphism is higher in the exon region than in the intron and promoter regions. We calculated the total average values to be 1.5 and 0.88 for 28 males and 237 females, respectively. The average score was -4.1080 for males and -8.418 for females.

2. Experimental data analysis

Based on the above analysis of the experimental data, the results were combined, samples with the same duplicated genotype were combined, and duplicated similar genes were removed. A total of 10 fish samples with different genotypes are available for reference. The proportion of each SNP in male and female fish was also counted to obtain the results(<Table 4>). Among them, GBF1 to GBF7 is the genotype blocks shared by male and female samples, GBF8 to GBF13 are the unique genotypes of the female samples, and GBF2 genotype is the whiteboard genotype. A total of 62 fish were caught, representing 23.40% of the total sample.

The genotypes of some of the male samples were taken and the mean and percentage of the total number of male samples were calculated based on the weight scores. Figureshows the corresponding mean body weight data for each of the seven genotypes of male fish, with GBF-1 having the heaviest mean body weight of 1.66 kg. The lightest average weight is 0.96kg for GBF-4 and GBF-7(<Table 5>). We were able to select an

excellent genotype: GBF1. Among them, GBF1 are particularly prominent and can locate special observation objects([Fig. 2]).

The genotypes of some of the female samples were taken and averaged by weight, which accounted for half of the total number of female samples. Figureshows the corresponding RMS data for each of the 10 genotype blocks of female fish, with the heaviest RMS being GBF-7 at 1.27 kg. The lightest one is GBF-2 with an average weight of 0.84kg(<Table 6>). The excellent genetic samples we were able to screen were: GBF7, GBF8, GBF10. Among them, GBF8 has outstanding phenotypes and can be used as specific observation objects([Fig. 3]).

In summary, we observed that the weight of the male rainbow trout was relatively larger than that of the female rainbow trout during the same period, being 58.67% heavier than the average female rainbow trout. The common genotypes are GBF1 and GBF2, which are excellent genotypes shared by both males GBF1 and females GBF8 is an outstanding observation genotype, suitable for special observation and cultivation(<Table 2>). Allele ratio of each SNP in rainbow trout males.

This plot shows the relationship between IGF-1 gene and weight difference, divided by sex. This plot shows the statistics for the male sample. The X-axis represents the genotype-categorized male sample and the Y-axis the weights. In this plot, the dashed line represents the overall mean value for the male sample. Green is the coloring of high-quality samples, since each sample exceeds the mean. Red is the coloring of samples with poor quality. Each sample is below the mean value. Black and white are samples close to the mean. mediocre.

<Table 2> Allele ratio of each SNPs in Rainbow trout male

Gene Name	Gene Location	Gene Region	SNP Location	Base Type	Percentage
Insulin-like growth factors −1 (IGF-1)	Male	3UTR	PS1	G	91.98%
				A	8.02%
			PS2	T	55.27%
				C	44.43%

<Table 3> Allele ratio of each SNPs in Rainbow trout female

Gene Name	Gene Location	Gene Region	SNP Location	Base Type	Percentage
IGF-1	Female	3UTR	PS1	G	91.33%
				A	8.33%
			PS2	T	59.52%
				C	40.48%

<Table 4> Genotypic block as a whole distinguishes genotype divisions according to SNP type

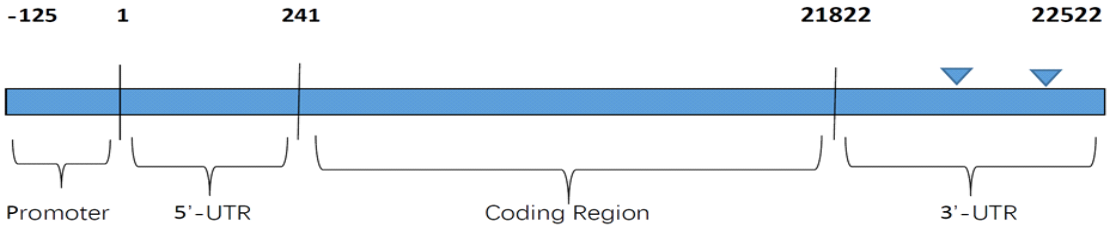
Rainbow Trout	22113bp (GGG)	22292bp (TTT)	Total Quantity	Male	Female
GBF-1	-/-/-	C/C/T	83	35.71% 10	30.8% 73
GBF-2	-/-/-	-/-/-	72	35.71% 10	26.16% 62
GBF-3	-/-/-	C/C/C	46	7.14% 2	18.57% 44
GBF-4	-/-/-	C/T/T	29	7.14% 2	11.39% 27
GBF-5	G/A/A	C/C/T	6	7.14% 2	1.68% 4
GBF-6	G/A/A	-/-/-	16	3.57% 1	6.33% 15
GBF-7	G/G/A	C/C/T	2	3.57% 1	0.42% 1
GBF-8	G/G/A	-/-/-	7	0	2.95% 7
GBF-9	A/A/A	-/-/-	2	0	0.84% 2
GBF-10	G/A/A	C/T/T	2	0	0.84% 2

<Table 5> Genotype segment weight and average weight, score status, and average score segment of male Rainbow trout

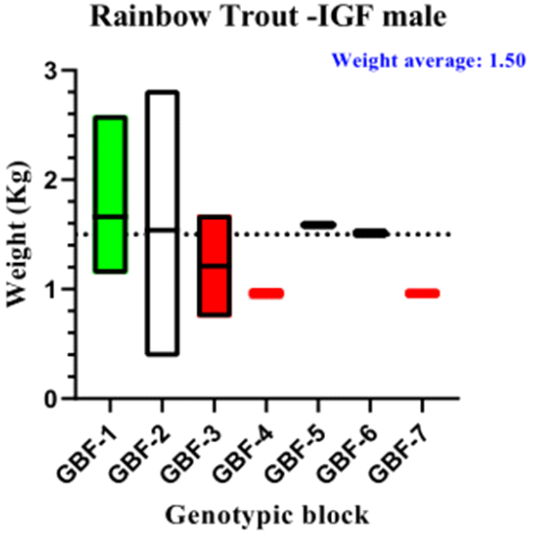
M	Number	Total Weight	Weight Average(kg)
GBF-1	10	16.62	1.66
GBF-2	10	15.39	1.54
GBF-3	2	2.42	1.21
GBF-4	2	1.92	0.96
GBF-5	2	3.17	1.59
GBF-6	1	1.51	1.51
GBF-7	1	0.96	0.96

<Table 6> Genotype segment weight and average weight, score status, and average score segment of female Rainbow trout

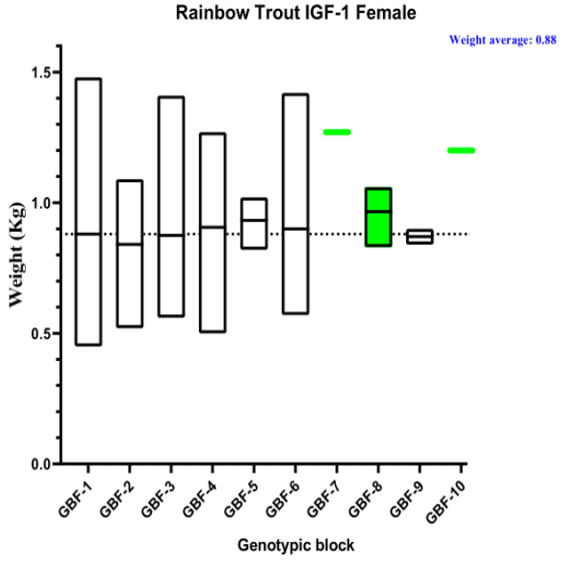
F	Number	Total Weight	Weight Average(kg)
GBF-1	73	64.24	0.88
GBF-2	62	52.13	0.84
GBF-3	44	38.5	0.88
GBF-4	27	24.46	0.91
GBF-5	4	3.73	0.93
GBF-6	15	13.5	0.9
GBF-7	1	1.27	1.27
GBF-8	7	6.76	0.97
GBF-9	2	1.74	0.87
GBF-10	2	1.4	1.20



[Fig. 1] SNPs and their location in the promoter, 5'-UTR and 3'-UTR of IGF-1.
In this diagram we can find the approximate position and position relation of the two SNPs. (3'-UTR) White arrows indicate the location of each SNP and its position on the whole gene. The two blue inverted triangles in Figure indicate the two SNPs found at positions 22113bp and 2292bp.



[Fig. 2] Weight difference according to Genotypic block of IGF-1 male.

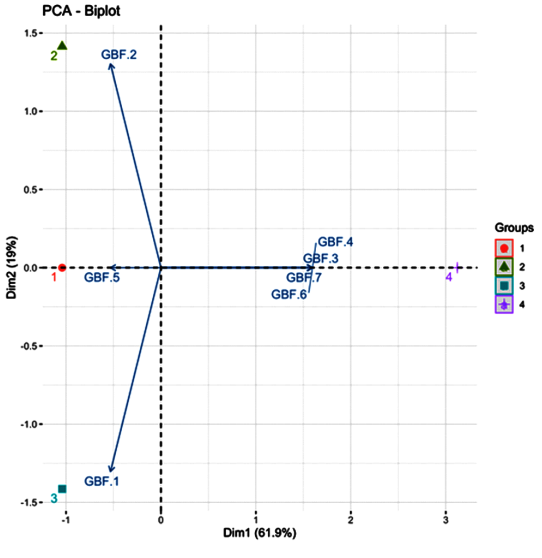


[Fig. 3] Weight difference according to Genotypic block of IGF-1 female.

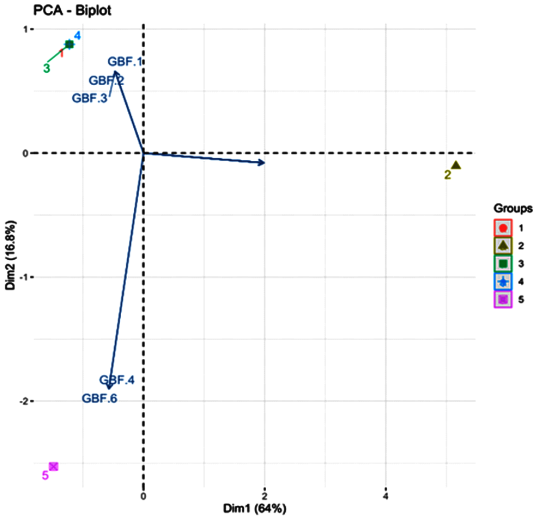
This plot shows the relationship between IGF-1 gene and weight difference, divided by sex. This figure shows the statistics of the female sample. The X-axis represents the sample of females classified by genotype and the Y-axis weights. In this chart, the dotted line represents the overall average of female samples, green is to highlight high-quality samples, because each sample exceeds the average of females, red is for inferior samples, each sample is lower than the average of females, and black and white are close to the average, the sample is mediocre.

This is a statistical method. A set of possibly correlated variables is transformed by an orthogonal transformation into a set of linearly uncorrelated variables, and the set of transformed variables is called the principal component. In practical subjects, many variables related to this are often proposed in order to comprehensively analyze the problem, as each variable reflects some information about the

subject to varying degrees. When using statistical analysis methods to study multiple topics, too many variables can increase the complexity of the topics. One would naturally hope that fewer variables would yield more information. In many cases, there is some correlation between the variables. When there is some correlation between two variables, it can be interpreted that the two variables reflect some degree of overlap in the subject's information. Principal component analysis removes all redundant variables from the original proposal and constructs as many new variables as possible such that these new variables are pairwise uncorrelated. These new variables reflect the original information in the subject's information as much as possible. Try to recombine the original variables into a new set of uncorrelated synthetic variables. At the same time, depending on the actual needs, fewer synthetic variables can be extracted from them to reflect as much information as possible about the original variables. This statistical method is known as



[Fig. 4] Principal component analysis of IGF-1 gene distribution in male Rainbow trout.



[Fig. 5] Principal component analysis of IGF-1 gene distribution in female Rainbow trout.

1. Measure and record the bodyweight of Rainbow trout to extract sample DNA to further determine genotype and classification.
2. Single nucleotide polymorphism is the measurement point of the genotypic variables of each sample in this study.
3. In Figure 4, the Y-axis represents dimension 1 (Dim1), and the calculated percentage is 19%, while the X-axis represents the calculated percentage of dimension Dim2 is 61.9%. In Figure 5, the Y-axis represents dimension 1 (Dim1), and the calculated percentage is 16.8%, while the X-axis represents the dimension Dim2's calculated percentage is 64%. The closer the Y-axis and X-axis percentages are, the more sufficient sample collection is and the more universal it is.

principal component analysis or PCA. PCA is also used in mathematics to reduce dimensionality. It was created by taking a blood DNA sample from the tail of a rainbow trout and calculating its weight as a function of mean value([Fig. 4], [Fig. 5]).

Ⅳ. Discussion

In the 21st century, human aquaculture research and development of rainbow trout (*Oncorhynchus mykiss*) has become more and more important in aquaculture worldwide. Other applications of the QTL techniques developed by bioengineering techniques in aquatic research have also been extensive and far-reaching. We used healthy and excellent individual samples of rainbow trout to explore the relationship between SNPs in the IGF-1 gene and fish growth(Garikipati, Gahr, and Rodgers 2006). In the contemporary research and application of IGF-1 (Fuentes et al. 2013), research on other human domesticated animals also helped our experiments (Li et al. 2017). We find a total of 2SNPs. Among them, it is common for males to outnumber females. In terms of growth differences (Gabillard et al. 2013), the average growth rate varies with gender(Østbye et al. 2001). We classify individual fish by sex and weight. Due to the increasing demand for rainbow trout muscle growth gene research, food source optimization, and aquaculture industry, many countries have focused on rainbow trout breeding, enriching human food nutrition sources, high-quality bio-commodity breeding, and quality improvement projects for other species superior (Carlton et al. 2006; Xu et al. 2003). The study of genetic markers provides key information, which is helpful to select excellent genotype individuals and conduct experiments and explorations in special excellent breeding and species improvement (Vignal et al. 2002). This article shows the link between growth-promoting factor (IGF-1) aggregation and its weight and average point(Aljuboory 2018). Judging from the data collected, the transformation and importance of genetic research explained in this study, also plays an important regulatory role in the research of other fish growth genes(Mohammed, Raji, and Igwebuike 2020; Tsai et al. 2014; feng, Yu, and Tong 2014). Rainbow trout has a more prominent external performance as a marker-assisted sample selection point of view.

V. Conclusion

According to the data content studied in this article, a scientific conclusion can be drawn that growth factor (IGF-1) plays a vital regulatory role in the growth and development of rainbow trout. Based on the 2 controllable SNPs found in the IGF-1 gene at the change point, we can choose to use the good genotype species. With specially tailored breeding, we can easily obtain the improvements and superior offspring that humans need as high-quality biological products. And an excellent source of protein nutrients for humans. In a suitable growth environment, the body weight of a sample monomer of a male fish at the same growth stage should be much greater than that of a sample monomer of a female fish. Single nucleotide polymorphism screening is an efficient and fast experimental method for species improvement. We can use it, and it will be extended. It also lays the theoretical data foundation for the future development of human bioengineering technologies and also explores future research and development directions.

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• Received : 23 November, 2023
• Revised : 08 January, 2024
• Accepted : 01 February, 2024