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Genetic Analysis Of Insulin Growth Factor-1 And Growth Hormonegenes Of Labeo Rohita From Ponds Of Gambat, Sindh, Pakistan

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ABSTRACT

In the anterior pituitary gland, growth hormone (GH) is an ancestral hormone released periodically by somatotroph cells, under the control of the growth hormone-releasing hormone receptor (GHRHR). IGF-1 causes both hypertrophy (increase in cell size) and hyperplasia (increase in the number of cells) in most tissues, including bone, due to growth hormone stimulation. Growing, reproducing, and lactating features in vertebrates and invertebrates are known to be modulated by genes governing growth hormone (GH), the growth hormone-releasing hormone receptor (GHRHR), and the insulin-like growth factor 1 gene (IGF1). Labeo rohita (Fish) from ponds in Gambat Sindh, Pakistan, studied genetic diversity in the GH-1 regulatory gene and IGF1 genes. The Gh-1 and IGF1 genes of Labeo rohita were assessed for genetic polymorphism using blood samples, PCR, and a Nanodrop spectrophotometer. The PCR product was genotyped using Gel electrophoresis and DNA sequencing (Fish). This study found a 01-nucleotide mutation in the IGF-1 gene of Labeo rohita fish. The Gh1 gene in Labeo rohita fish was found to be unaffected by mutations. These changes are classified as a missense point mutation because of how they occurred: a truncated TTA codon was changed to an inert TTC codon, which codes for the essential amino acid leucine, while TTC codon codes for phenylalanine, a vital amino acid that is nonpolar and neutral owing to the inert and hydrophobic nature of the benzyl side chain. It is envisaged that our findings will serve as a guide and reference for selecting high-quality Labeo rohita for industrial usage and genetic development activities in fish based on our findings in this study.

Keywords: Growth Hormone, Insulin Growth Factor-1, Labeo rohita, SNPs

Introduction

Improvements in fish growth are critical for the country's economy, as is keeping tabs on the fish's genetic variability, according to (Lupchinskijr. et al., 2011). It was predicted that studies conducted at

the DNA level to find out such DNA distinctions linked to production-related phenotype to use them as a tool early on in helping the descendants select and employ their entire performance in aquaculture would be successful (De-Santis & Jerry, 2007). Finding SNPs and productive traits for many fish species has been accomplished through the somatotropic axis genes (De-Santis and Jerry 2007).

Labeo rohita is a popular table fish in Southeast Asian countries. Particularly prevalent throughout Asia, particularly in Pakistan, Bangladesh, India, Nepal, and Myanmar (Talwar and Jhingran., 1991). It can grow up to 200 centimeters in length. Males spawn in rivers when floodwater extends into additional or fewer limpid shallows enclosing the productive flats, high above tidal reach. L.Rohita conducted many genetic experiments, including DNA fingerprinting using sex-specific satellite DNA and mini-satellite DNA studies. The fact that it provides vast amounts of protein for human consumption and employment and business prospects for a vast number of individuals who are either directly or indirectly involved in this line of work or tourism makes it an important activity.

Although many genes are involved in controlling growth in animals, the IGF-1 and GH genes are critical for all species' overall health and development. IGF-1-stimulated GH action on muscles and bone growth (Sellier 2000). There are seventy amino acids in IGF-1, which is a 7.5-kDa polypeptide (Daughadayand Rotwein1989). Alternate splicing of the coding region 1&2 results in an IGF-1 gene length of 90 kb (Steenbergh et al., 1991). The first exon has 1155 nucleotides, while the second exon has 750. Growth hormone 1, also called hypophysis development hormone or simply growth hormone, is a protein encoded by the GH gene.

The encoded factor, a hormone from the luteotropin family, keeps the growth going. Chromosome seventeen contains the factor and four distinct genes. Barash et al., (2017). GH is an anabolic hormone synthesized and released by the adenohypophysis' lactotroph cells in a time-unit and pulsatile pattern. It is essential for postnatal growth and advancement, lactation, reproduction, and tissue growth, just as it is for fat, carbohydrate, and protein metabolism. Ayuk et al., (2006). While growth hormone generation in some tissues is still experimental, the GH factor has been widely employed in livestock as a marker in many stock species, including goats, sheep, and cows, because of its sensible and point potential. (Thomas., 2007, Zhou et al., 2005)

According to the research, GH-Restriction TaqI's Fragments Length Polymorphism was shown to be associated with body mass in Belgian White Blue bulls seven and thirteen months old (Sneyers et al., 1994). Extensive possessions initiated bovine growth hormone genotype on annual body weight, and the valine/leucine genotype delivers fruitful benefits on Canadian cattle (Pereira et al., 2005). As a potential candidate gene for growth Boer goat buck characteristics, this factor's single nucleotide

polymorphism was assessed (Naylor et al., 2009). Indian sheep breeds have not had any research done on this particular gene marker. The current study attempted to detect GH gene variation in indigenous sheep breeds because Indian native sheep are so important in mutton production.

Despite extensive research into the genotypic effects of IFG-1 and the Gh factor of Labeo rohita and all over the world, there is very little known about this gene in Pakistani society. Genetic mutations in the IFG-1 and Gh genes are linked to Labeo growth (Labeo rohita), and this study aims to find out more about them in the Khairpur district.

Methodology

Sampling

In order to collect blood samples of around 2-3 ml with a syringe from Rohu fish weighing 1 to 3 kilograms, tubes containing an anticoagulant called EDTA will be used in the ponds of Gambat city. A permanent catheter will be injected into the snout and blood arteries of the fish. The collected blood samples were kept at a temperature of -4°C in a refrigerator at the Shah Abdul Latif University, Khairpur Molecular Genetics Laboratory of the Zoology Department.

DNA Extraction

A novel polymorphism in the IGF-1 gene and the GH gene of Labeo rohita was identified using the whole genome DNA Extraction Kit, and the mutation types were classified based on the Following the standard protocol set forth by the National Institutes of Health, the extracted DNA was verified using a 1.5 percent Agarose gel and then heated to 20°C. (Sambrook et. et al., 2002).

Primer Designing and PCR Amplification

The primers were synthesized at CEMB Lahore using Primer Premier 3 software, which was used to create them. The PCR amplification described by employed a 20-L reaction mixture containing 50 mg template DNA, 10 pM of each primer, 0.20 mM of dNTP, 2.5 mM of $MgCl_2$, and 0.5 U of Taq DNA polymerase (Zhang et al. 2007). A 5-minute denaturation at 95°C will be followed by 32 cycles of 30-second denaturation at 94°C, followed by 30-second annealing temperature at 58°C, followed by a final 10-minute extension at 72°C for 10-seconds, and a final 10-minute extension will take place at 72°C for 10-seconds.

Gel –Electrophoresis

The PCR result was examined in the electrophoresis chamber using Yang et al. (2009) protocol's 1.5 percent agarose gel and 200mg/ml (Ethidium bromide) dye.

DNA Sequencing and Data Analysis

Centre of Excellence in Molecular Biology, Lahore sequenced the PCR result. Polymerase chain reaction. A genetic code-based computer algorithm assessed the mutation proportion of each goat breed.

RESULTS AND DISCUSSION

The study of genetic variation caused by genes with significant effects has contributed significantly to our understanding of animal metabolic regulation mechanisms. It is possible to study the metabolic control of growth using the high growth (hg) gene in mice since it considerably boosts post-weaning growth rate and mature body size (Bradford & Famula, 1984). Dwarfism is a typical phenotype in numerous well-used mouse models of endocrine growth regulation. The high-growth gene appears to have a broad impact on all aspects of growth chemistry without significantly impacting body composition as a whole (Calvert et al. 1985). The gene increases the efficiency of energy metabolism. Without the growth hormone/insulin-like factor 1 (IGF-1) growth axis, there is no growth or metabolism. This process includes several steps, including binding of human growth hormone (GH) to its receptor, activation of the transcription factor Stat5b, nuclear translocation, and interaction with DNA binding sites in chromatin.

Researchers have discovered new information about a particular fish breed, the Labeo rohita from District Khairpur, thanks to the findings of this study, which provide extensive genomic data and explain the comparison of mutations and percentages in two genes. We found one mutation in the Labeo rohita breed's two genes using DNA sequencing and gel electrophoresis. This was made possible using a thermal cycler, which rapidly cools and heats a tube.

PCR was used to amplify 210bp segments of each fish breed gene, then sequenced to reveal genetic alterations. Genetic testing on the IGF1 and GH genes has shown one mutation in total, thanks to DNA sequencing, electrophoresis, and polymerase chain reaction (PCR). One deletion mutation was identified, as was a second missense one as well. Additionally, the Labeo rohita igf1 gene showed a variance of 3.33%, whereas the GH gene showed a variance of 0.47%, as seen in Tables 1 and 2. Figures 1 and 2 illustrate the quantification of the IGF1 and Gh genes using PCR-amplified DNA, respectively. According to the most recent research, the Labeo rohita fish breed is ideal for industrial and genetic modification reasons

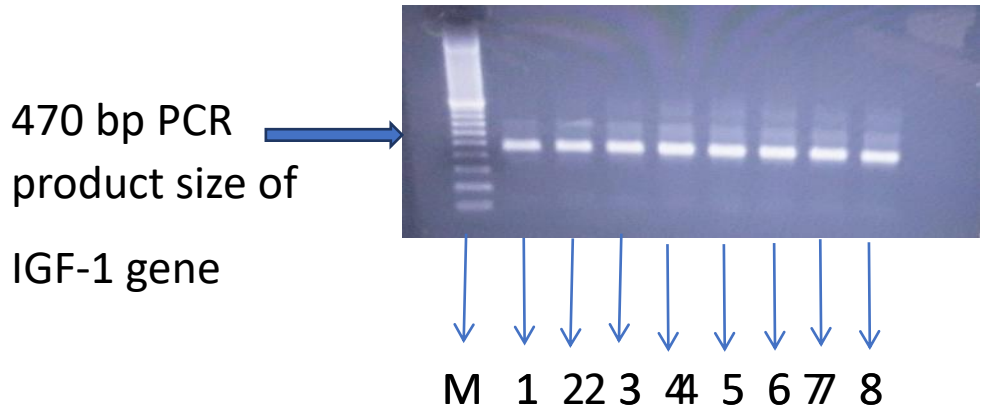

Figure 1 PCR product size of DNA of IGF-1 Gene on Gel (EZ Gel-documentation)

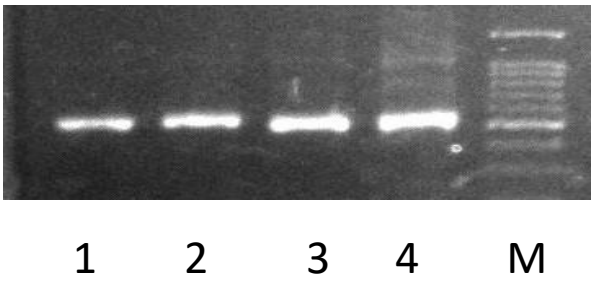

Figure 2 **PCR product size of DNA of Gh1 Gene on Gel EZ Gel-documentation**

Table 01 showing the detailed position and types of SNPs in Labeo Rohita Fish

Name of Genes in Labeo rohita	Position of change bases	Original codon	Original amino acid	Changed codon	Changed amino acid	Type of Point Mutation
IGF1 gene	341	TTA	Leucine (Essential	TTC	Phenyl alanine	Missense point

			amino acid)		(Essential Amino acid)	mutation
	No mutation was identified in other samples					
Gh1 gene	No mutation was identified					

Table 02 Showing the total percentage of mutation in IGF1 gene based on Genetic code

Name of Gene	Name of Fish	No. of SNP's Identified	Percentage Formula	Percentage
IGF1	Labeo rohita	01	01/470*100	0.212

Polymorphism occurs when the mutation rate exceeds one in a population. However, if the population's rate of change is smaller than 1, the mutation is referred to as a single point. In the IGF1 gene of Labeo rohita, using the population genetics criterion outlined above, a Point mutation can be seen to exist.

Conclusion

It was found that when blood samples were collected, PCR amplified and then quantified with a Nanodrop spectrophotometer, along with the PCR product itself, genetic polymorphism in the IGF-1 gene and the Gh1 gene could be estimated in Labeo rohita fish. The IGF-1 gene in Labeo rohita fish had a 01 nucleotide change as a result of this investigation. The Gh1 gene in Labeo rohita fish was found to be unaffected by mutations. These changes are classified as a missense point mutation because of how they occurred: a truncated TTA codon was changed to an inert TTC codon, which codes for the essential amino acid leucine, while TTC codon codes for phenylalanine, an important amino acid that is nonpolar and neutral due to the inert and hydrophobic nature of the benzyl side chain. It is envisaged that our findings will serve as a guide and reference for selecting high-quality Labeo rohita for industrial usage and for genetic development activities in fish based on our findings in this study.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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