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Research Article

Single Nucleotide Polymorphisms in Skeletal Muscles of Myogenin and Myostatin Genes of the Nile Tilapia (*Oreochromis niloticus*)

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The present study was conducted on the Nile tilapia (*Oreochromis niloticus*) and screened for single nucleotide polymorphisms (SNPs) in the muscle hormone genes myogenin (MyoG) and myostatin (MSTN). Samples were collected from the JangHang fish farm and categorized by body weight and sex. The average total weight was 1.02 kg for the 82 males and 0.63 kg for the 70 females. A body weight in the range of the average weight ±10% was considered as normal weight. Target regions of each gene were categorized, such as the promoter region, 5′-untranslated region (5′UTR) and 3′UTR. Genomic deoxyribonucleic acid (gDNA) was extracted, amplified, sequenced, aligned, and screened for the presence of nucleotide variations. Three SNPs, 1,875T>A, 1,892C>T, and 1,946A>G, and one insertion (TGACATATTTCCCTCACA) at 2,138–2,145 were detected in the 3′UTR of the MyoG gene. However, within the MSTN gene, one insertion at 450 (T) and two SNPs at 536 (G>A and A>A) were found in the 5′UTR. In addition, four insertions at 1,278–1,279 (GT, T, TGT, and G) and one short tandem repeat (STR) at 1,129 (TG) in Exon 1, two SNPs at 4,438 (3′UTR; A>C and C>C), and two SNPs at 4,514 (3′UTR; G>A and A>A) were identified. Principal component analysis (PCA) and statistical analyses were performed to evaluate the relationships between the SNPs and body weight, forming 6 and 41 genotype blocks in the MyoG and MSTN genes, respectively. We found significant correlations between the SNPs and body weight traits for the effective genotype blocks GBG-1, GBG-3, and GBG-4 of MyoG and GBN-1, GBN-2, GBN-3, GBN-4, GBN-13, GBN-22, and GBN-24 of MSTN. These outcomes would be significant for fish breeding and may help obtain data for use in genetic marker kits. In some instances, genetic marker kits have a distinct advantage for improvement in genetics, parentage control, identification of species, and enhancing breeding stock and its applications in aquaculture.

1. Introduction

Globally, Nile tilapia (*Oreochromis niloticus*) is one of the most extensively cultured fish. It is farmed in freshwater [1] and represents 8.4% of the total finfish aquaculture production, which amounts to 3.7 million tons harvested in 2014 (Food and Agriculture Organization 2016) [2]. Tilapia farming, which is considered acceptable for market farming, involves many environmental factors and can be adapted to different settings [3]. Production costs depend on improvements to the farming system. The genetic breeding program aims to produce animals that will grow quickly. With the help of this program, different Nile tilapia strains have been developed [4–6]. In addition, skeletal muscle tissues are an essential part of fish that are mostly consumed by humans. The determination of genetic changes is a necessary step in implementing genetic improvement programs aimed at selecting faster-growing fish with lower feed conversion rates and higher disease resistance [7].

Skeletal muscle growth and development are regulated by two genes, namely, myostatin (MSTN) and myogenin (MyoG). In rainbow trout, the diameter distributions of

skeletal muscle fibres and adipocytes have been investigated [8]. MSTN is a member of the transforming growth factor-β (TGF-β) protein superfamily [9] and negatively regulates the growth of skeletal muscles. However, in fish, its function may not be restricted to muscle growth regulation [10–14]. The gene structure of MSTN in teleost fish, such as Nile tilapia, is highly conserved and consists of three exons (300–400 nucleotides) and two introns [15]. MSTN-1 and MSTN-2 transcripts have been reported in barramundi, zebrafish, and gilthead seabream [16–19]. MyoG expression has been reported in the Wuchang bream (*Megalobrama amblycephala*), with white muscle having the highest level of expression compared to the other types of muscle [20].

Significant associations have been observed between g.2770C>A polymorphisms and three growth traits: total length, body length, and body weight. In addition, two SNPs were discovered in intron 2 and the 3′UTR within the MSTN gene in bighead carp (*Aristichthys nobilis*) [21]. In *Ancherythroculter nigrocauda* MSTN, five SNPs are known, two of which exhibit a positive effect on body weight [22]. In the MyoG gene, a nucleotide variation (T>C) is found. These findings indicate that MyoG polymorphisms are linked to various growth traits. Accordingly, their potential use as assisted selection markers can be evaluated in relation to tilapia growth traits [1].

In the current study, a panel of polymorphic SNPs was screened for the purpose of identification and characterization. The screened SNPs within the MyoG and MSTN genes in tilapia were utilized to investigate the association of polymorphisms in the muscle hormone genes with body weight. SNP markers give a more accurate picture of how genetically similar breeding candidates are, making it easier to select the best candidates and serving as a valuable tool for developing a genetic marker kit. Selective breeding is becoming more important in aquaculture, helping to increase its sustainability and advantages for an abundant supply of healthy seafood.

2. Materials and Methods

2.1. Ethical Statement. The care and use of experimental animals complied with the Institutional Animal Care and Use Committee of Pukyong National University. Animal welfare laws, guidelines, and policies are approved by the "Chairman of the Institutional Animal Care and Use Committee, Pukyong National University and permit reference number: PKNUIACUC (20180352)." The fish were collected as part of faunal surveys; experimental conditions caused severe distress or lasting harm to sentient fishes (e.g., predation studies, toxicity testing, and disease trials); surgical procedures were used; sentient unanaesthetised animals were subjected to chemical agents that induce neuromuscular blockade, such as muscle relaxants. The fish were not killed for fish fin sampling at the end of the experiment.

2.2. Tilapia Farming for SNP Assessment. The JangHang fish farm comprises an air-recirculating aquaculture system (AIRRAS). The major factor limiting fish growth and well-being is the oxygen concentration. Fish need oxygen to breathe; dissolved oxygen (DO) concentration should be 2–10 ppm (parts per million), and tilapia ponds should be managed to keep the ammonia concentrations at 1–10 mg/L. When DO declines below this level for an extended period, metabolism, growth, and disease resistance are compromised. Tilapias reproduce best at temperatures above 27°C and do not reproduce in water at temperatures below 20°C. The optimal water temperature for the growth of tilapias was determined to be between 16°C and 40°C. The fish were fed on a commercial diet (Raguife Diet-Top Fish 32% crude protein, Sào Paulo, Brazil) twice a day in the winter season and 4–6 times a day in the summer season.

2.3. Experimental Design and Sample Collection. In this study, 24-month-old Nile tilapias (*Oreochromis niloticus*; length, 30–40 cm) were used to explore SNPs within the MyoG and MSTN genes. Stocking and harvesting of the fish were performed in July 2017 and July 2019, respectively. A total of 152 fish fin samples were collected from JangHang Fish farm, Seocheon, South Korea. Fish were grouped by body weight (heavy vs. light) and sex (male vs. female). Body weight was measured using a precision balance with a sensitivity of 0.01 g. The sex was determined by manually examining the genital papilla. Fish fin samples were collected in sterile Eppendorf tubes containing 99.9% ethanol and stored at −20°C until DNA extraction. During sample collection, microchips (1.4 × 8.2 mm; KAML8506; CLA International co., Ltd 1203 Rosenville, 1588-3 Seocho-dong, Seocho-gu, Seoul, Republic of Korea) were used to tag each fish subcutaneously under aseptic conditions. The microchip number was tallied using an animal electronic recognizer. A difference of more than 10% from the mean weight was chosen as the criterion to identify SNP and GB effects on weight. SNPs and GBs that influence Nile tilapia weight were identified for both male and female fish.

2.4. DNA Extraction and PCR Amplification. Genomic DNA (gDNA) was extracted from collected fish samples using a DNeasy Blood & Tissue Kit (QIAGEN, Germantown, MD, USA). Quantification of gDNA was performed using a NANODROP LITE Spectrophotometer (Thermo SCIENTIFIC). While utilizing the National Center for Biotechnology Information (NCBI) database, we chose the sequences for the MyoG (GenBank accession no. NC_031970) and MSTN (GenBank accession no. NC_031987). Polymerase chain reaction (PCR) was conducted to amplify a fragment of MyoG [2,241 base pair (bp)], which includes 243 bp of the promoter and the 5′UTR and 713 bp of the 3′UTR, as well as MSTN (4,616 bp), which includes 1,252 bp of the promoter and the 5′UTR and 1,302 bp of the 3′UTR. The primers designed with the help of the Primer-BLAST tool to amplify the MyoG and MSTN genes are shown in Table 1. PCR master mix (50 μL) comprised the following: 0.25 μL Ex Taq DNA polymerase, 5 μL Buffer 10x, 4 μL dNTPs (Deoxynucleotide triphosphates), 2 μL forward primer, 2 μL reverse primer, 0.5 μL of gDNA, and 36.25 μL distilled water (Table 1).

Table 1: Primer sets used for amplification of MyoG and MSTN genes. This table shows the primers used for sequencing of MyoG and MSTN genes. Forward and reverse primers sequences, size of PCR product, annealing temperature and duration, and regions such as promoter, 5′'UTR, and 3′'UTR of MyoG and MSTN genes.

	Primer set	Size of PCR product	Annealing temp/duration	Region
MyoG-1	F: 5′-TTTGCTGCATGAACCCAACACA-3′ R: 5′-ACTGGACAGCGTATTGAAAATGGT-3′	429 bp	62°C/30 s	Promoter and 5′UTR
MyoG-2	F: 5′-AACAATGACTGGGGTAAAATGTCA-3′ R: 5′-CTGAACTGGGCTCGCTTGA-3′	436 bp	60.1°C/30 s	3′UTR
MSTN-1	F: 5′-GCCCGACCTGCCCGATACACTT-3′ R: 5′-GGTAGATGGAGGGAGGACGGGTG-3′	722 bp	70°C/47 s	Promoter and 5′UTR
MSTN-2	F: 5′-GGTCCCTGCTGTACCCCAAC-3′ R: 5′-GGACCTGACAAGACCTAAAGTGG-3′	926 bp	65°C/1 min	Promoter and 5′UTR
MSTN-3	F: 5′-GACAAGCGCCCGGTCCACAATA-3′ R: 5′-AAGTGTATCGGGCAGGTCGG-3′	447 bp	66°C/30 s	3′UTR
MSTN-4	F: 5′-ACAAGCGCCCGGTCCACAATAAC-3′ R: 5′-TGCAGGAAGGTCGGGCTGTTTG-3′	947 bp	66°C/1 min	3′UTR

SimpliAmp™ Thermal Cycler (Applied Biosystems® by Life Technologies™, Marsiling Industrial Estate Road 3 Singapore 739256) and TaKaRa Thermal Cycler Dice Touch (TAKARA BIO INC. Nojihigashi 7-4-38, Kusatsu, Shiga, 525-0058, Japan) were used to amplify gDNA. The thermal cycler program was set as follows: 5 min at 94°C for initial denaturation, 30 s at 94°C for denaturation, 30 s at the annealing temperatures indicated in Table 1, an extension step at 72°C depending on the length of the PCR product, and a final extension step for 7 min at 72°C. The PCR products and a 1000-bp DNA ladder (RAON, 510 Misadaero, Hanam-si, Gyeonggi-do, 1004~1005, Misa IS Biz Tower, Hangang) were loaded onto a 1.5% agarose gel stained with Midori Green Advance DNA Stain (Nippon Genetics Europe GmbH, Mariaweilerstrasse 28–30; 52349 Düren; Germany) and visualized under UV Trans illuminators (DAIHAN Scientific Co., Ltd. 326, Sinpyeongseokhwa-ro, Jijeong-myeon, Wonju-si, Gangwon-do, Korea). Gel purification was performed using FavorPrep (GEL/PCR purification mini kit; Favorgen Biotech Corp. Wembley, WA 6014 Australia), and the samples were submitted to Macrogen for sequencing.

2.5. Sequence Analysis, Discovery of SNP, and Genotyping. BioEdit 7.0.9.0. software was used to identify the SNPs in the Nile tilapia MyoG and MSTN genes. The sequences were aligned using the ClustalW multiple alignment tool to detect nucleotide variations, and the identification was achieved using the ABI sequencing technique. The allelic frequency of an SNP must be 1% or more in a population. In this study, 1% represents two individuals. A cluster of SNP regions in blocks residing in a specific individual in the case of the parent genotyping assignment is called a genotype block (GB). With the help of the GB structure hypothesis, we discovered lumps of SNP putative markers related to body weight. The estimation of the allelic and genotypic frequencies, which included the genotype block inference and its frequency, was also conducted to discover SNPs.

2.6. Analytical Relationship between Genotype Block and Body Weight

2.6.1. Statistical Analysis. One-way ANOVA (analysis of variance) was used to examine the relationships between the genotype blocks of the SNPs and the body weight traits of tilapia using SPSS (Statistical Package for Social Sciences) Statistics software 22.0 based on the general linear model. The data are presented as the mean ± SD. Differences with $P \leq 0.05$ were considered significant. The differences in body weight traits between different genotype blocks were tested by considering the number of SNPs as factors and the body weight traits of tilapia as dependent variables.

2.6.2. Principal Component Analysis. PCA was carried out, and the results were visualized using the R programming language [23]. All calculations were performed using the available built-in packages. The 10 different SNPs of the MyoG and MSTN genes from the $S(k)$ datasets were coded as variables. The $S(k)$ data set was given a k range of 0.30 to 315.00. A particular set of $S(k)$ values was chosen to represent a single data point for each variable in the PCA. The PCA results were additionally visualized using the factoextra packages [23]. The loading plots were produced using the factoextra package, which also displayed a classification of the variables of interest. The quality of representations was determined using the eigenvalues of the quality. Such values are typically represented by a colour gradient and normalized to a range of −1 to 1 using this package.

3. Results

3.1. SNP Discovery. The MyoG and MSTN genes in Nile tilapia were sequenced to identify polymorphic variations and evaluate the relationship between such variations and muscle growth traits. Three SNPs and one insertion were found in the 3′UTR and assigned 3US1 (3′UTR single nucleotide polymorphism 1), 3US2, 3US3, and 3US4 g.1,875T>A, g.1,892C>T, g.1,946A>G, and 2,138–2,145 TGA CATATTTCCCTCACA) based on their positions in the 2,241-bp-long MyoG gene (Figure 1). Other compositions and their percentages are listed in Table 2. The 4,616-bp-long MSTN gene is fragmented into four parts: the sequences of promoter region and 5′UTR into MSTN1 and MSTN2; the 3′UTR into MSTN3 and MSTN4. Six SNPs were detected and named 5US1 (5′UTR single nucleotide polymorphism 1), 5US2, E1-STR (exon 1-short tandem repeat), E1-S4 (exon 1 SNP4), 3US1, and 3US2 according to their positions in the MSTN gene (Figure 2). Screening of the 5′UTR showed one insertion at 450 (T) and one site with heterozygous and homozygous mutations of g.536G>A and g.536A>A, while one short tandem repeats (STR) region (TG) at position 1,129, and four insertions at 1,278–1,279 (GT, T, TGT, and G) were identified in the first exon. Two homozygous and heterozygous variations g.4,438A>C, g.4,438C>C, g.4,514G>A, and g.4,514A>A were in the 3′UTR (Table 3). Three complete nucleotide variations and one insertion in the MyoG gene as well as three SNPs, two insertions, and an STR in the MSTN gene were identified within the discovery population. Compared to the MyoG gene, the MSTN gene exhibited a higher number of genetic variations. Significantly, more polymorphisms were observed in the exon regions compared with the introns and promoter regions in both genes. We calculated the total weight average of 82 male and 70 female fish as 1.02 and 0.63, respectively, and determined the average criterion as having a weight between above and below 10% of the body weight.

3.2. Statistical Analysis. A total of 152 Nile tilapia were genotyped. Genotyping was estimated for the four and six polymorphisms demonstrated within the MyoG and MSTN genes and the body weight of each individual fish. Six and 41 genotype blocks were constructed for MyoG and MSTN, respectively. In successfully genotyped individuals, statistical analysis was conducted to evaluate the relationship between the SNPs and body weight traits. There was a significant

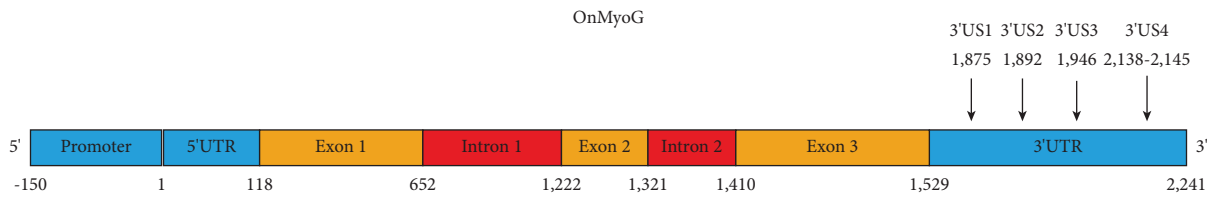

Figure 1: SNPs and their location in the OnMyoG (myogenin gene of *Oreochromis niloticus*). There are four SNPs in the 3′UTR at 1,875, 1,892, +1,946, and 2,138–2,145. Arrows are interpreting the presence of SNPs (3US1, 3US2, 3US3, and 3US4: 3′UTR single nucleotide polymorphism 1, 2, 3, and 4, respectively).

Table 2: SNPs/insertion in MyoG gene. This table shows the SNP name/location, SNPs/insertion, and composition percentage in the MyoG gene of Nile tilapia (*Oreochromis niloticus*).

Region	SNP name/location	SNP/insertion	Percentage (%)
3′UTR	3US1/1,875	T/T	94.7
		T/A	5.26
	3US2/1,892	C/C	98.68
		C/T	1.31
	3US3/1,946	A/A	96.71
		A/G	3.28
	3US4/2,138–2,145	No insertion	86.18
		(TGACATATTTCCCTCACA)	13.81

SNP: single nucleotide polymorphism. MyoG: myogenin.

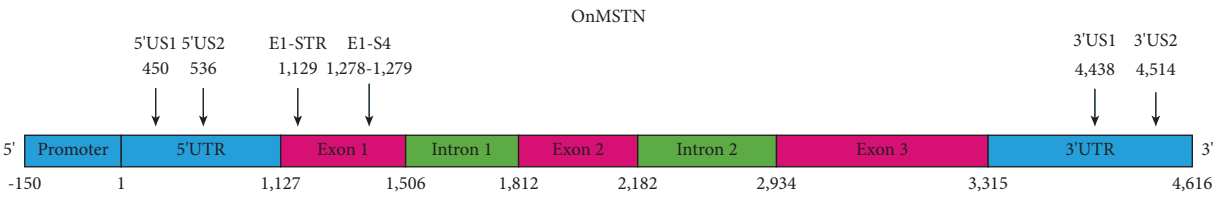

Figure 2: SNPs and their location in the OnMSTN (myostatin gene of *Oreochromis niloticus*). There is one insertion at 450, one SNP at 536, one STR at 1129, one insertion at 1278-1279 in the exon 1 region, and two SNPs in the 3′UTR region at 4,438 and 4,514 nucleotide positions. Arrows are interpreting the presence of SNPs (5US1, 5US2, and 5US3: 5′UTR single nucleotide polymorphism 1, 2, and 3, E1-STR: exon 1-short tandem repeats, 3US1, and 3US2: 3′UTR single nucleotide polymorphism 1 and 2).

Table 3: SNPs/Insertion in MSTN gene. This table shows the SNP name/location, SNPs/insertion/STR, and composition percentage in the MSTN gene of Nile tilapia (*Oreochromis niloticus*).

Region	SNP name/location	SNP/insertion/STR	Percentage (%)
5′UTR	5US1/450	No insertion	94.7
		T	5.26
	5US2/536	G/G	84.21
		G/A	14.47
		A/A	1.31
Exon 1	E1-STR/1,129	No short tandem repeats	66.44
		TG	33.55
		GT	23.03
	E1-S4 1,278-1,279	No insertion	5.26
		T	1.97
		TGT	1.32
		G	0.66
3′UTR	3US1/4,438	A/A	68.42
		A/C	25.65
		C/C	5.92
	3US2/4,514	G/G	53.94
		G/A	39.47
		A/A	6.57

SNP: single nucleotide polymorphism. MSTN: myostatin.

correlation between the body weight and the SNPs of GBG-3 (genotypic block of MyoG) and GBG-4 in males and GBG-1 in females (Figures 3 and 4), while GBG-2, GBG-5, and GBG-6 were not significantly associated with body weight traits of *O. niloticus* (Table 4). The body weights of fish with effective genotype blocks, GBG-3, GBG-4, and GBG-1, were 0.61–1.56 kg, 0.71–1.42 kg, and 1.18 kg, respectively. In the MSTN gene, GBN-22 (genotypic block of MSTN), GBN-1, GBN-2, GBN-3, and GBN-4 in males and GBN-13 and GBN-24 in females were significantly correlated with body weight (Figures 5 and 6), whereas other genotypic blocks were not significantly correlated with body weight traits of Nile tilapia (Table 5). To assess the average weight, an association of the SNPs in MyoG and MSTN genes and the body weight trait values for each genotype block were calculated. Effective genotype blocks GBN-22, GBN-1, GBN-2, GBN-3, GBN-4, GBN-13, and GBN-24 exhibited body weights of 0.78–1.56 kg, 1.41 kg, 1.33 kg, 1.33 kg, 1.31 kg, 0.74–1.42 kg, and 0.82 kg, respectively.

3.3. Principal Component Analysis. To analyse the SNP data, we performed PCA. Genetic variations were calculated using the R programming language. The main axes of variation in this data are represented by the first two principal components (PC): dimension 1 (Dim1) and dimension 2 (Dim2), which explained SNP variations in the MyoG and MSTN genes in males and females, respectively. To visualize these data, we created scatter plots of these components. The calculated nucleotide variations were 51.5% of the first Dim1, distinguished between X3S3.AG (X = SNP, 3S3 = 3rd SNP of 3′UTR, AG = SNP) and X3S1. . ., and X3S1..TGACATATTTCCCTCACA, X3S3.AA with X3S1.TT, X3S2.TC, and 3S1.AT of the MyoG male, while the second PC Dim2 (29.3%) of the MyoG male distinguished between X3S3.AG and X3S1.., and X3S1..TGACATATTTCCCTCACA and X3S3.AA with X3S1.TT, X3S2.TC, and X3S1.AT (Figure 7). However, a difference between the X3S3.AG and X3S1. . .and X3S1. . .and X3S3.AA with X3S1.TT and X3S1.AT of the first Dim1 was calculated with 50% SNP variations while Dim2 (34.4%) differentiated between X3S3.AG and X3S1. . ., and X3S1. . .and X3S3.AA with X3S1.TT and X3S1.AT of the MyoG female (Figure 8). On the other hand, Dim1 plotted against Dim2 was found to be 36.6% and 26% for the MSTN male nucleotide variations distinguished between X3S2.GG., X5S2.GG, 5STR1.8., X5S3.TGT, X5S3.G., X5S3.GT., X3S1.CA., X5S1. . ., and X3S2.AA., X5S1.T., X3S1.AA., X5S3. . ., X5S2.AA., X3S1.CC., X5STR1. . ., X%STR1.10., X5S2.AG., X3S2.GA. Dim2 of MSTN male distinguished between X3S2.AA., X5S1.T., X3S1.AA., X5S3. . ., X5S2.AA., X3S1.CC., X5STR1.8., X5S3.TGT., X5S2.GG., XS2.GG., and X5STR1. . ., X5S3.G., X5STR1.10., X5S3.GT., X3S1.CA., X5S2.AG., X5S1. . ., and X3S2.GA (Figure 9). The Dim1 (39.7%) of MSTN female distinguished between X5STR1. . ., X3S1.CA., X3S1.CA., X3S1.CC., X5S2.AA., X5S2.AG., XS2.GA., and X5STR1.11., and X5S1.T., X3S2.GG., X5S2.GG., X3S2.AA., X5STR1.10., and X3S1.AA while Dim2 (26.9%), X5STR1. . ., X3S1.CA., X3S2.GG., X5S1.T.,

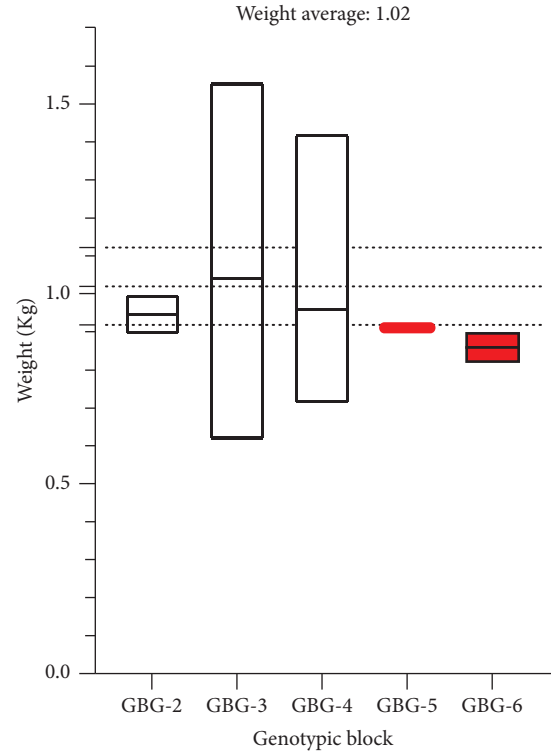

Figure 3: Descriptive statistical analysis of the weight (kg) by a genotype block of OnMyoG male. The pointed line interprets the weight average: 1.02, red bars are interpreting the genotype block whose body weight is below 10%, and GBG: genotype block of MyoG.

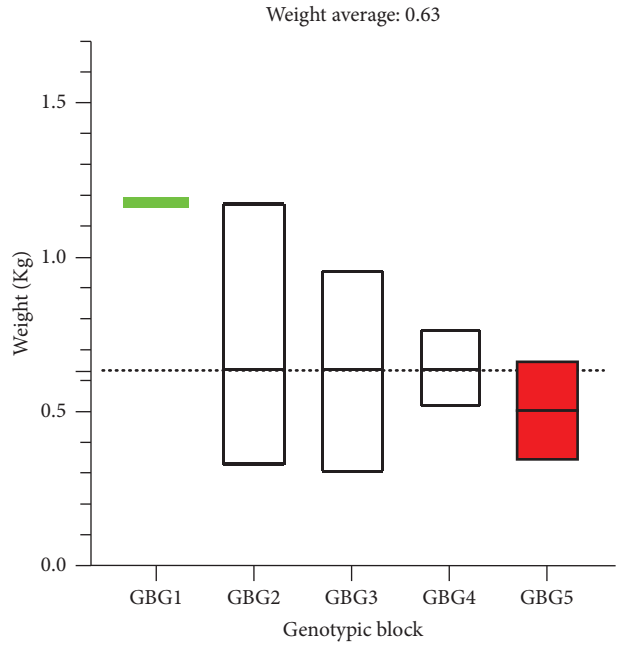

Figure 4: Descriptive statistical analysis of the weight (kg) by a genotypic block of OnMyoG female. The pointed line interprets the weight average: 0.63, the green bar interprets the genotype block whose body weight is above 10%, and the red bar interprets the genotype block whose body weight is below 10%.

Table 4: Genotype block composition of MyoG gene. This table shows the genotype block composition of the Nile tilapia (*Oreochromis niloticus*) MyoG gene. According to screened SNPs and the weight average of each individual, we discovered the genotype blocks.

Genotype blocks	3US1 (T/T)	3US2 (C/C)	3US3 (A/A)	3US4 (—)	Male	Female	Total individual
GBG-1	—	—	A/G	TGACATATTTCCCTCACA	0	1	1
GBG-2	A/T	T/C	—	—	2	0	2
GBG-3	—	—	—	—	70	49	119
GBG-4	—	—	—	TGACATATTTCCCTCACA	7	13	20
GBG-5	A/T	—	—	—	1	5	6
GBG-6	—	—	A/G	—	2	2	4

GBG: genotype block of MyoG.

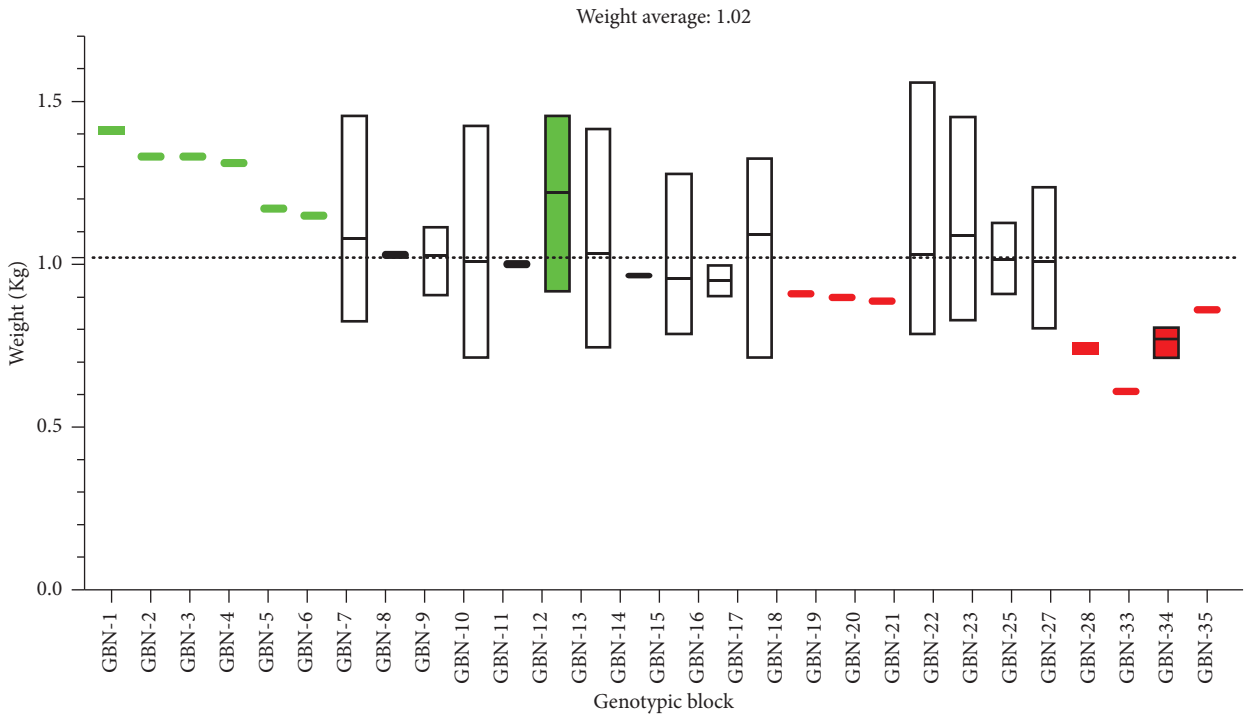

Figure 5: Descriptive statistical analysis of the weight (kg) by a genotypic block of OnMSTN male. The pointed line interprets the weight average: 1.02, green bars are interpreting the genotype block whose body weight is above 10%, red bars are interpreting the genotype block whose body weight is below 10%, and GBN: genotype block of MSTN.

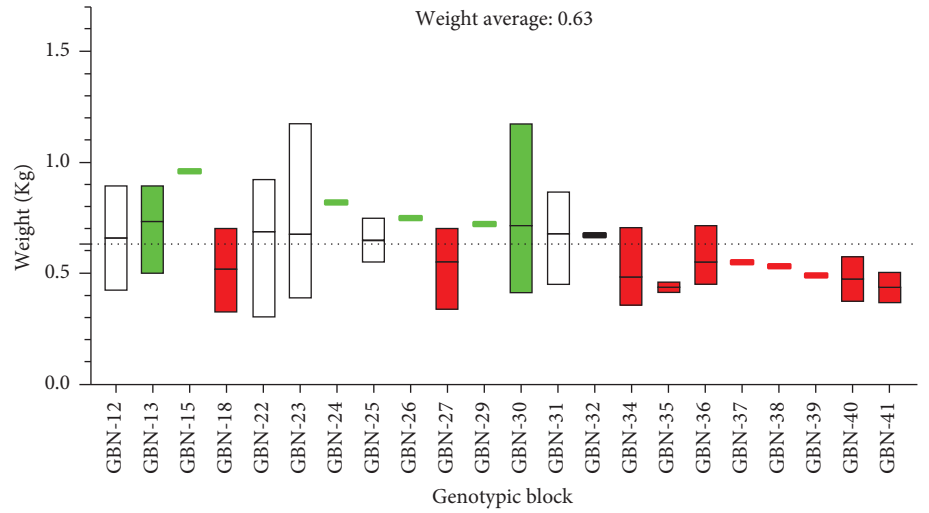

Figure 6: Descriptive statistical analysis of the weight (kg) by a genotypic block of OnMSTN female. The pointed line interprets the weight average: 0.63, green bars are interpreting the genotype block whose body weight is above 10%, red bars are interpreting the genotype block whose body weight is below 10%, and GBN: genotype block of MSTN.

Table 5: Genotype block composition of MSTN gene. This table shows the genotype block composition of the Nile tilapia (*Oreochromis niloticus*) MSTN gene. According to screened SNPs and the weight average of each individual, we discovered a genotype block.

Genotype blocks	5US1 (—)	5US2 (GG)	E1-STR	E1-S4 (—)	3US1 (AA)	3US2 (GG)	Male	Female	Total individual
GBN-1	—	—	—	TGT	CA	—	1	0	1
GBN-2	—	—	—	GT	—	GA	1	0	1
GBN-3	—	—	8	GT	CA	—	1	0	1
GBN-4	—	—	8	GT	—	GA	1	0	1
GBN-5	—	—	—	TGT	—	——	1	0	1
GBN-6	—	—	8	G	—	—	1	0	1
GBN-7	—	—	—	GT	CA	—	5	0	5
GBN-8	—	—	—	—	CC	AA	1	0	1
GBN-9	—	—	10	GT	—	GA	3	0	3
GBN-10	—	—	—	GT	—	—	10	0	10
GBN-11	—	AG	—	GT	CA	GA	1	0	1
GBN-12	T	—	—	—	—	AA	3	2	5
GBN-13	—	—	—	—	CA	GA	8	3	11
GBN-14	—	—	8	GT	—	—	2	0	2
GBN-15	—	—	10	—	—	GA	0	1	1
GBN-16	—	AG	—	GT	—	GA	4	0	4
GBN-17	—	—	10	GT	CA	—	2	0	2
GBN-18	—	—	—	—	CC	—	6	2	8
GBN-19	T	—	8	GT	—	AA	1	0	1
GBN-20	T	—	—	GT	—	AA	1	0	1
GBN-21	—	AA	—	—	—	AA	1	0	1
GBN-22	—	—	—	—	—	—	11	11	22
GBN-23	—	—	—	—	—	GA	5	9	14
GBN-24	—	—	11	—	—	GA	0	1	1
GBN-25	—	AG	—	—	—	GA	2	2	4
GBN-26	—	—	7	—	—	—	0	1	1
GBN-27	—	—	10	—	—	—	3	4	7
GBN-28	—	AG	10	GT	—	GA	2	0	2
GBN-29	—	AG	11	T	—	GA	0	1	1
GBN-30	—	—	11	—	CA	GA	0	6	6
GBN-31	—	—	11	—	—	—	0	7	7
GBN-32	—	AG	—	—	—	—	0	1	1
GBN-33	—	AG	—	—	—	AA	1	0	1
GBN-34	—	—	—	—	CA	—	3	4	7
GBN-35	—	AG	—	—	CA	GA	1	2	3
GBN-36	—	AG	11	—	CA	GA	0	3	3
GBN-37	—	AA	—	—	—	GA	0	1	1
GBN-38	—	—	11	T	—	—	0	1	1
GBN-39	—	—	11	T	—	GA	0	1	1
GBN-40	—	—	11	—	CA	—	0	4	4
GBN-41	—	AG	11	—	—	GA	0	2	2

GBN: genotypic block of MSTN.

X5S2.GG., X3S2.AA., and X3S1.CC. distinguished from X5STR1.10., X5STR1.7., X5S2.AA., X5S3.T., X5S2.AG., X3S2.GA., X3S1.AA., and X5STR1.11 (Figure 10). Therefore, the scatter plot of MyoG in male and female samples showed that GBG-1, GBG-3, and GBG-4 were closely associated with the body weight of tilapia. Similarly, MSTN male and female scatter plot revealed that GBN-1, GBN-2, GBN-3, GBN-4, GBN-13, GBN-22, and GBN-24 were closely correlated with Nile tilapia body weight.

4. Discussion

4.1. Discovery of SNP. Several studies have been performed on the central functions of the MyoG and MSTN genes. Specific traits are investigated through SNP discovery in genes [24–26]. The myogenic regulatory factors (MRFs) have important and varied functions in the myogenic pathway. Since myogenic differentiation antigen (MyoD) and myogenic factor 5 (Myf5) perform similar tasks in the

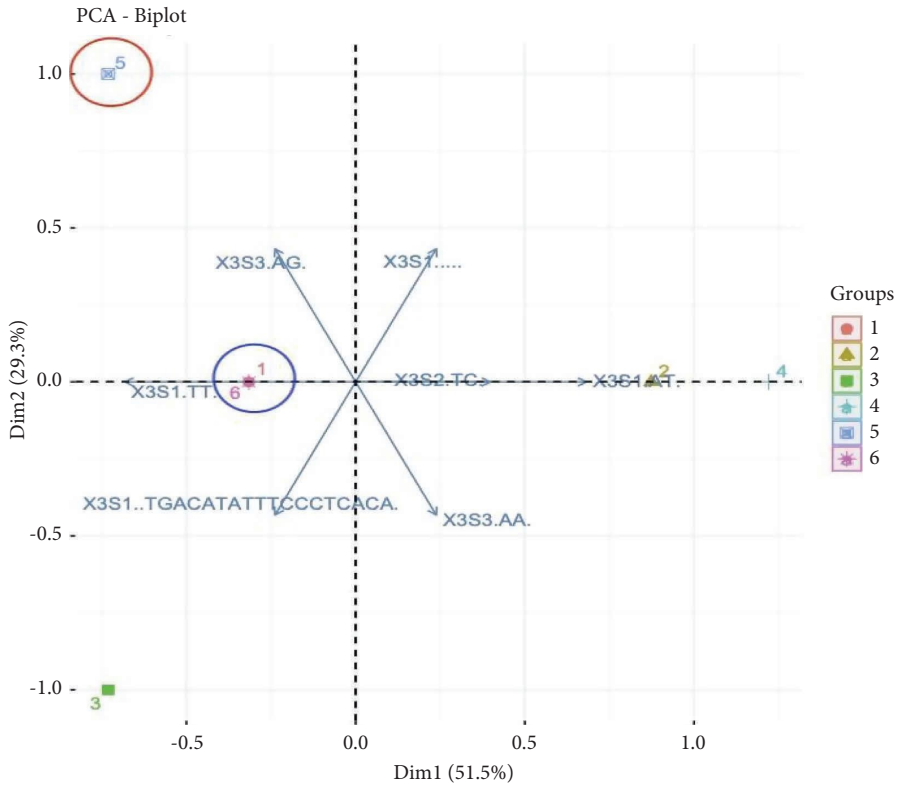

FIGURE 7: Scatter plot of principal component axis one dimension 1 (Dim1) and axis two dimension 2 (Dim2) based on SNP data of OnMyoG male. *Y*-axis indicates Dim1, and the calculated percentage is 51.5% while *X*-axis shows that the Dim2 having a calculated percentage is 29.3%.

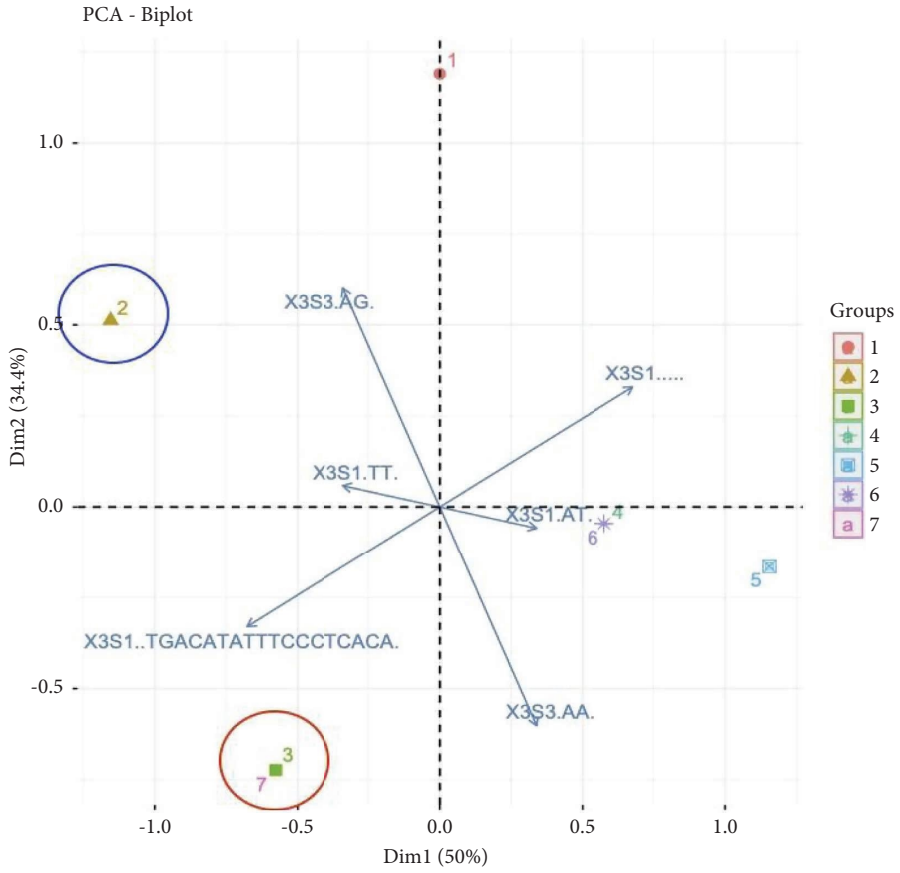

FIGURE 8: Scatter plot of principal component axis one dimension 1 (Dim1) and axis two dimension 2 (Dim2) based on SNP data of OnMyoG female. *Y*-axis indicates Dim1, and the calculated percentage is 50% while *X*-axis shows the Dim2 having a calculated percentage is 34.4%.

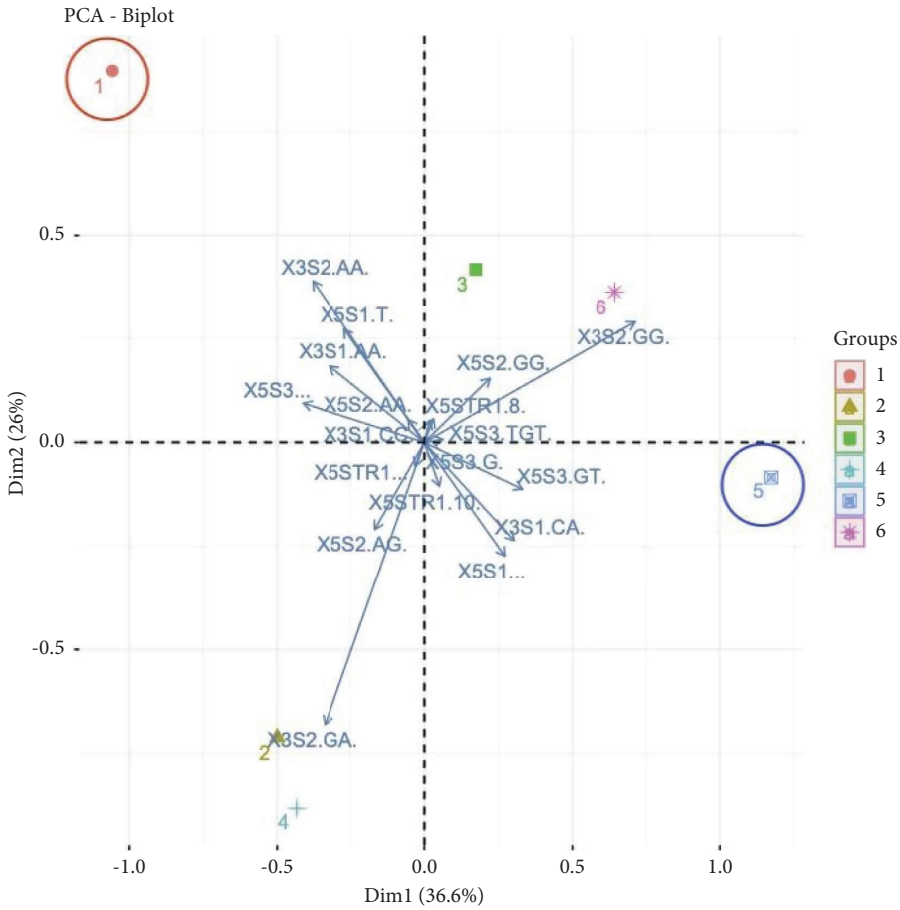

Figure 9: Scatter plot of principal component axis one dimension 1 (Dim1) and axis two dimension 2 (Dim2) based on SNP data of OnMSTN male. *Y*-axis indicates Dim1, and the calculated percentage is 36.6% while *X*-axis shows the Dim2 having a calculated percentage is 26%.

differentiation and proliferation of muscle precursor cells, deleting just one of them has little impact on muscle development [27], while deleting both results in the complete abolition of the skeletal muscle lineage [28]. Myoblasts are correctly positioned and specified in their absence, but there is a severe lack of muscle fibres. Myoblast differentiation into myotubes is regulated by the MyoG factor [29, 30]. Myf6 regulates the formation of myotubes and the continued fusion of muscle fibres, but it is not necessary for muscle growth [31, 32]. The MRFs can also induce skeletal muscle differentiation in nonmuscle cells both *in vivo* [33] and *in vitro* [34]. Mutations influencing myostatin function loss have been discovered in these double-muscled cattle breeds, which is one of the reasons that myostatin is thought to be responsible for double muscling in cattle [35–37]. Similar to that of mammals, the tissue-specific expression pattern of MSTN differs slightly in fish; it is strongly expressed in skeletal muscles and weakly expressed in cardio myocytes, mammary glands [38], and adipose tissue [39] in mammals.

Single nucleotide polymorphisms (SNPs) in the exons of Myf5 and MyoG have been studied in order to identify growth-related molecular markers and provide a theoretical foundation for the breeding and conservation of Bian chickens at the molecular level. Three genotypes are involved in the discovery of a mutation site at exon 1 and exon 3 of the MyoG gene in Jinghai yellow chicken [40]. Six cattle that are native to China were tested for a MyoG gene SNP and its significance, and findings support the hypothesis that in cattle that are native to China, the MyoG gene may affect body measurement traits [41]. Polymorphisms in the MSTN gene have also been investigated in some fish species, and several SNPs and microsatellites are reported in the channel catfish [42]. In domestic animals, MSTN polymorphisms may affect a variety of traits. For example, two SNPs are significantly correlated with muscle depth in commercial Charollais sheep [43]. Two SNPs from almost the entire MSTN gene sequence of *Aristichthys nobilis* are reported in the 3′UTR and intron 2 [21]. There have also been reports of associations between SNPs in the MSTN-2 gene and other genes, including insulin-like growth factor (IGF-1), growth hormone (GH), and prolactin (PRL) and vertebrate growth in pigs [44], gilthead seabream [45], Atlantic salmon [46, 47], and Arctic char [48].

In this study, the MyoG and MSTN genes of Nile tilapia were isolated and sequenced. Four SNPs were found: g.1,875T>A, g.1,892C>T, g.1,946A>G, and an insertion at 2,138–2,145 (TGACATATTTCCCTCACA) in the 3′UTR, with no reported mutations in the promoter and 5′ flanking

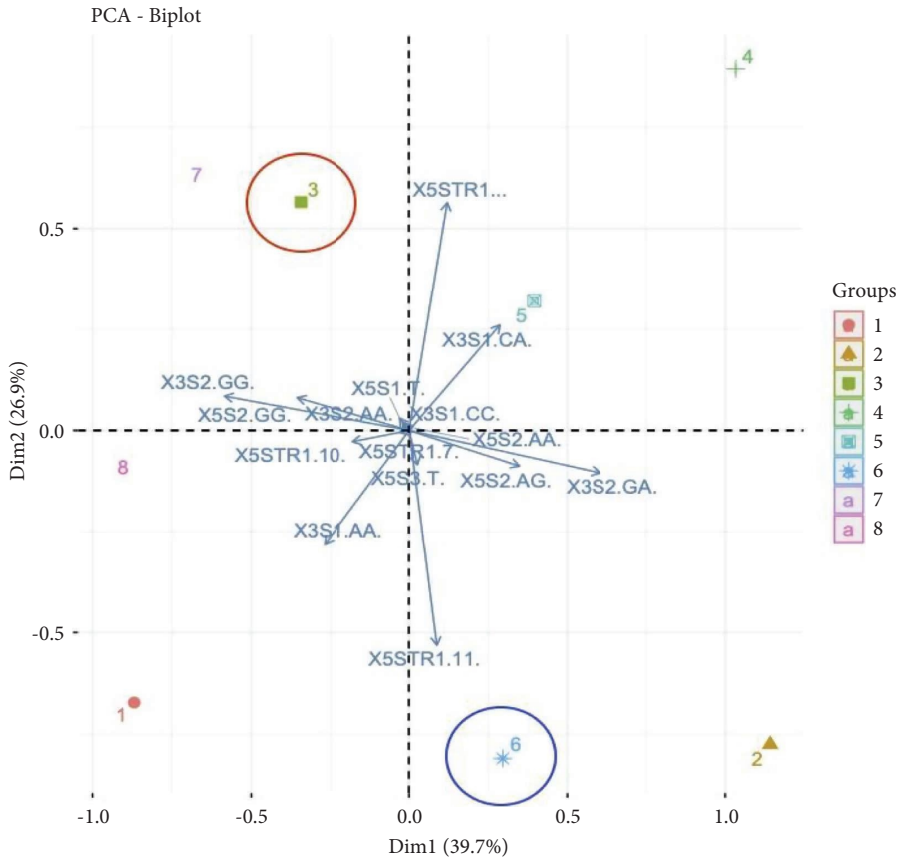

Figure 10: Scatter plot of principal component axis one dimension 1 (Dim1) and axis two dimension 2 (Dim2) based on SNP data of OnMSTN female. *Y*-axis indicates Dim1 and the calculated percentage is 39.7% while *X*-axis shows the Dim2 having a calculated percentage is 26.9%.

region of the MyoG gene. A novel SNP in the first exon of the MyoG gene was also analysed [49]. A few polymorphisms have been observed in the stop codon region of the MyoG gene of European meat pig breeds [50]. The MSTN genes of Nile tilapia and other vertebrate species consist of three exons and two introns and encode a long-chain peptide consisting of 376 amino acids [24, 51–53]. Six polymorphisms were found in the current study: 450 (T) insertion and 536G>A within the 5′UTR; one site of short tandem repeats (TG) at 1,129; 1,278–1,279 (GT, T, TGT, G) insertions in the first exon; and 4,438A>C and 4,514G>A in the 3′UTR, with no variations in the promoter region of the MSTN gene. This pattern reveals six variations, located in exons 2 and 3, associated with the double muscling phenotype. Similarly, a study has reported six known mutations in the MSTN sequence located in exons 2 and 3 that cause double muscling in cattle by down-regulating this gene [37, 54]. Our findings revealed the presence of a higher number of mutations in exons compared with introns and promoter regions in both genes.

4.2. Genotype: Association between SNP and Body Weight. Three SNPs in the MyoG gene are described in Chinese-Tibetan sheep: two in exon regions (C109A and C183T) and one in an intron (A1403C) [55]. The C allele may be linked to an increase in body weight because individuals with genotype CC at the C109A locus have significantly greater body weights compared with individuals with genotype AA in all breeds. In the Jinghai yellow chicken [56], three genotypes (AA, AB, and BB) are found in exon 1 of MyoG. The body weights of chickens with BB genotype at the second, fourth, and sixth weeks were significantly higher than those with AB genotype, and the BB genotype was associated with a higher weight compared with the AA genotype at the eighth week. Two nucleotide variations in exon three of the MSTN gene of common carp are associated with body weight (BW) [57]. An SNP was found to be significantly associated with body weight in Bian chickens [58]. Variations in the MSTN gene associated with growth traits have been demonstrated in other fish species [59]. Several methodologies have been developed, which allow even modest laboratories to utilize

SNPs as a meaningful genetic marker [60]. Alterations in the 5′UTR and their effects on BW and size have also been observed in the MSTN gene in goats [61]. The MSTN-1 gene of rainbow trout has two SNPs in its introns [62]. Spotted halibut's muscle mass and growth performance have been linked to variations in the MSTN gene in many cases (*Verasper variegatus*) [63]. A relationship between specific MSTN-2 promoter SNP alleles and fish size is also reported, indicating that this gene might be a candidate for marker-assisted selection in fish farming [45].

In this study, the average total weight of the 82 male fish was 1.02, and that of the 70 female fish was 0.63. Overall, we discovered six genotype blocks in the MyoG gene and 41 in the MSTN gene. All significant body weight-associated variations in MyoG were in GBG-3 and GBG-4 in males and in GBG-1 in females. Those in the MSTN gene were in GBN-22, GBN-1, GBN-2, GBN-3, and GBN-4 in males and in GBN-13 and GBN-24 in females, revealing the genotype blocks with the highest impact. When we grouped the fish by sex and body weight, we observed differences in growth according to sex. Many countries focus on tilapia-breeding programs because of the increasing demand for genetic research, food sources, and aquaculture industries. Genetic marker studies provide pivotal information that can aid selection programs. Herein, the connection is demonstrated between the polymorphisms in the muscle hormones MyoG and MSTN and the body weight of fish. From the collected data, nucleotide alterations were presented in this work, underlying the importance of using genetic studies of Nile tilapia in marker-assisted selection (MAS). DNA markers that are firmly connected to the quantitative trait loci (QTL) for traits of interest based on QTL mapping or association studies are required for MAS implementation.

Tilapia is a vital food source for people globally. Currently, tilapia farming is practiced on almost all continents, regardless of the climate or other environmental factors. Due to the widespread recognition of this species' significance for industrialized aquaculture as well as food security in developing nations, various genetic improvement programs using conventional selective breeding methods have been established in various countries. The search for DNA markers associated with the traits of economic interest is of the greatest priority to provide information to support selection programs as new molecular approaches to animal breeding become available. Further quantitative investigation of the expression of muscle hormone genes is necessary to confirm the putative associations between the SNPs and body weight reported here. The SNPs described in the present study, and their putative association with tilapia body weight, once confirmed, may prove useful in a marker-assisted selection approach.

5. Conclusion

In conclusion, the SNPs in the MyoG and MSTN genes were examined in 152 Nile tilapia. Six SNPs, three insertions, and one novel STR were analysed in MyoG and MSTN. A higher number of genetic variations were found in the MSTN gene than in the MyoG gene. The tilapia-breeding program should remain a focus due to the massive demand for food sources, aquaculture industrialization, and genetics research. Utilizing all currently available methods for genetic improvement has become necessary because of the improvements of modern aquaculture and aquatic species raised in farms. To produce better products, this initially only involved selection and, in some cases, strain crossing. Scientists started to identify genes linked to traits important to the many aquaculture species with the advent of molecular biology and the development of new genetic markers. There are now more opportunities for aquaculture genetics thanks to sequencing projects, SNP discovery efforts. Novel and screened SNPs described in this research were investigated, and the results may allow the assessment of their associations with Nile tilapia muscle growth using selection marker kits in the future. It is predicted that investment in genetic marker kits will increase along with the value of aquaculture products. Due to the discovery of DNA markers linked to disease, such efforts are likely to result in more efficiently grown products and an increase in disease resistance.

Abbreviations

3US1: 3′UTR single nucleotide polymorphism 1
5US1: 5′UTR single nucleotide polymorphism 1
ANOVA: Analysis of variance
bp: Base pair
Dim1: Dimension 1
Dim2: Dimension 2
dNTPs: Deoxynucleotide triphosphates
E1-S4: Exon 1 SNP4
E1-STR: Exon 1-short tandem repeat
GB: Genotype block
GBG: Genotypic block of MyoG
GBN: Genotypic block of MSTN
gDNA: Genomic deoxyribonucleic acid
MRFs: Myogenic regulatory factors
MSTN: Myostatin
Myf5: Myogenic factor 5
MyoD: Myogenic differentiation antigen
MyoG: Myogenin
NCBI: National center for biotechnology information
PCA: Principal component analysis
PCR: Polymerase chain reaction
SNPs: Single nucleotide polymorphisms
SPSS: Statistical package for social sciences
STR: Short tandem repeat
TGF-β: Transforming growth factor-β
UTR: Untranslated region.

Data Availability

The data supporting the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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