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Polymorphism of the *STAT5A* and *MYF-5* genes in Anatolian water buffalo

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Abstract: The aim of this study was to determine the genetic variation of *MYF-5* and *STAT5A* genes in Anatolian water buffalo which was the only buffalo breed reared in Turkey by using the PCR-RFLP method. In this study, 120 Anatolian water buffalo were examined. After PCR amplification for *MYF-5* gene, 512 bp PCR products were digested with TaqI enzyme. Although no AA genotype was found, the frequency of GG and AG genotypes were 0.77 and 0.33. PCR products of 215 bp for *STAT5A* gene were digested with AvaI enzyme and showed that all of the Anatolian water buffalo examined had monomorphic in terms of CC genotype. Anatolian water buffalo were found in Hardy-Weinberg equilibrium with respect to *MYF-5-TaqI* polymorphism.

Key words: Anatolian water buffalo, polymorphism, *MYF-5*, PCR-RFLP, *STAT5A*

1. Introduction

Water buffalo, which is a semiaquatic farm animal in terms of its feeding and farming area, is a livestock species raised particularly for milk production in Turkey. Anatolian water buffalo, which belongs to a river type, is the only water buffalo breed raised in Turkey. Anatolian water buffalo is known for its ability to adapt to poor environmental conditions and utilize low quality roughage at a significant level. However, it is not a suitable farm animal for intensive breeding because of the low level of lactation milk yield, long gestation period of 320 days, slow growth, and low daily live weight gains of male calves [1]. For this reason, in the breeding of Anatolian water buffalo in terms of yield traits, it is thought that the use of candidate genes in selection studies may be important.

In the last decade, there has been a growing interest in the use of genomic data to assist traditional methods in improving significant yield-related traits in livestock breeding. Anatolian water buffalo is raised for milk and meat production in Turkey. Therefore, it will be a right strategy to give priority to the candidate genes related to these traits in the improvement processes of these traits genetically. Potential candidate genes related to the yield-related traits to be used in the marker-assisted selection processes can be determined with the studies conducted on the candidate genes within a QTL region [2]. In the studies conducted on candidate genes, it is aimed to determine how SNPs affect the phenotype after identifying SNPs found in candidate genes involved in the physiological or endocrinological stages of the process in the emergence of a phenotype [3]. The *myogenic factor 5* (*MYF-5*) gene and the *signal transducer and transcription activator 5A* (*STAT5A*) gene are the candidate genes to be considered first in the improvement of meat and milk yield in farm animals due to their physiological processes [4,5].

Muscle fiber formation during the embryonic development is regulated by the structurally and functionally interconnected *myogenic* (*MyoD*) gene family with 4 members called *myogenic factors 3, 5,* and *6* (*MYF-3, 5,* and *6*) and *myogenin (MyoG*) [4]. *MYF-5* gene controls the growth and differentiation in skeletal muscles [6]. During muscle growth, the *MYF-5* gene is transcribed before the *MyoD* gene family and they are followed by the transcriptions of the *MyoG*, *MYF-6*, and *MyoD* genes [7, 8]. It is reported that the *MYF-5*, which is transcribed first, plays a role in certain factors such as initiating the transcription of the *MyoD* gene family in the process of muscle growth, being an important step for the growth of muscle, determining the type of muscle cell that will occur, and forming the muscle cell differentiation [9,10]. It is stated that the *MYF-5* gene found on chromosome 5 in cattle is related to the 3chromosomal regions associated with carcass and meat quality traits [11]. Therefore, it is argued that the *MYF-5* gene may be a candidate gene for growth and meat quality traits in farm animals due to its role in the growth of muscle cells and the chromosomal regions it is linked to [12,13]. In the studies investigating the relationship between growth, meat yield, and meat

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quality with SNPs found in the *MYF-5* gene in different cattle breeds, it is suggested that the *MYF-5* gene is related to these traits [4, 14–17].

Signal transducer and activator of transcription (STAT) proteins consisting of 8 members mediate the effects of various hormones and cytokines [18]. *STAT5A* protein, which is also known as mammary gland factor, is the main regulator for the effect of growth hormone on target genes [19]. The *STAT* gene family, which is an important member of the cytokine signaling pathway, is also one of the important transcription factor [20,21]. It is specified that *STAT5A*, which was first discovered in the mammary gland, has an effect on the expression of milk protein genes together with prolactin [22].

In cattle, there is a correlation between some polymorphisms found in the gene coding the *STAT5A* protein and the milk yield traits [2,23]. It is also reported that in cattle there is a relationship between fertilization and embryonal viability rates of another SNP determined on the exon 8 of the *STAT5A* gene [24]. In addition, there is a relationship between genotypes and meat yield traits in terms of a SNP on the exon 7 of cattle *STAT5A* gene [25]. Both the physiological processes it is involved in and the studies mentioned above, indicate that the *STAT5A* gene is associated with many yield traits in farm animals particularly in milk yield and milk composition.

In this study, *MYF-5* and *STAT5A* gene polymorphisms, which have potential to be used as a marker in the process of improving meat and milk yield in Anatolian water buffalo are investigated.

2. Material and methods

In this study, blood samples of Anatolian water buffalo (n = 120) were genotyped in terms of *MYF-5-Taq*I and *STAT5A-Ava*I polymorphisms. Blood samples of the animals used in the study were taken from the vena jugularis of the animals into EDTA tubes. DNA samples used for PCR were obtained by the phenol-chloroform-isoamyl alcohol extraction method [26].

The PCR reaction for the *MYF-5-Taq*I polymorphism was performed in the final volume of 20 µL by adding 2.5 mM $MgCl_2$, 50 µM dNTP mix, 0.2 µM GenBank: NW_005785620.1 accession numbered forward (5'-AGAGCAGCAGTTTTGACAGC-3 ') and reverse (5'-GCAATCCAAGCTGGATAAGG -3') primer set, 1.25 U of Taq DNA polymerase and 50 ng/µL DNA. PCR was performed at 95 °C 4 min after the denaturation stage, 34 cycles at 95 °C for 45 s, 64 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. The 512 bp products obtained at the end of PCR were digested with *Taq*I restriction enzyme to determine the genotypes of the individuals.

PCRprimersetofGenBank:NW_005784710.1acssesion number (forward: 5'- CTGCAGGCTGTTCTGAGAG-3' and reverse: 5'- TGGTACCAGGACTGTAGCACAT -3') was used in the PCR reaction for the *STAT5A-Ava*I polymorphism. The PCR was performed in the final volume of 20 µL by adding 2.5 mM $MgCl_2$, 50 µM dNTP mix, 0.2 µM forward and reverse primers, 1.25 U of Taq DNA polymerase and 50 ng/µL DNA. The 215 bp products obtained at the end of PCR were digested with *Ava*I restriction enzyme to determine the genotypes of the individuals.

Genotypic data obtained after PCR-RFLP for the *MYF-5* and *STAT5A* genes were analyzed on the free OEGE web page [27].

3. Results

PCR was performed for *MYF-5-Taq*I restriction enzyme digestion, and 3 different genotypes were observed. Digestion of PCR products for *MYF-5* gene, 3 bands (512, 396, and 116 bp) and 2 bands (396 and 116 bp) mean the genotypes AA, AG, and GG, respectively. The samples examined in present study only showed AG and GG genotypes (Figure 1). There is no individual with AA genotype (Figure 1).

The genotypes of GG (0.77) were found to be the most common genotypes in Anatolian water buffalo and individuals with AA genotype were not observed (Table).

The PCR was performed for *STAT5A* gene and obtained 215 bp amplicon. The amplified PCR product of *STAT5A* was digested with *AvaI* restriction enzyme. The digestion products of the *STAT5A* gene were separated by 2% agarose gel electrophoresis. The end of the restriction enzyme digestion observed 2 bands of 181 and 34 bp in CC genotype, 3 bands of 215, 181, and 34 bp in CT genotyped, the single band of 215 bp in the TT genotype. However, all of the samples were seen CC genotype (Figure 2).

4. Discussion

In the traditional improvement methods, in the improvement of the yield-related traits, studies are conducted without determining the genes related to these traits. However, if the generation interval is long, the trait occurs only in one sex and after a certain age, a slow genetic progress is achieved with traditional improvement methods [28]. For this reason, the genotypes of individuals should be determined in terms of yield-related genes in breeding studies. In addition, breeding of animals with genotype determined for candidate genes is thought to increase the success of breeding studies [29].

*MYF-5-Taq*I polymorphism was studied on different cattle breeds. However, in the literature review, no study was found related to the *MYF-5* gene in any water buffalo breed. Therefore, this study is the first one where *MYF-5-Taq*I polymorphism has been investigated in a water buffalo breed.

On the other hand, it is observed that there are also limited number of studies related to the *MYF-5* gene

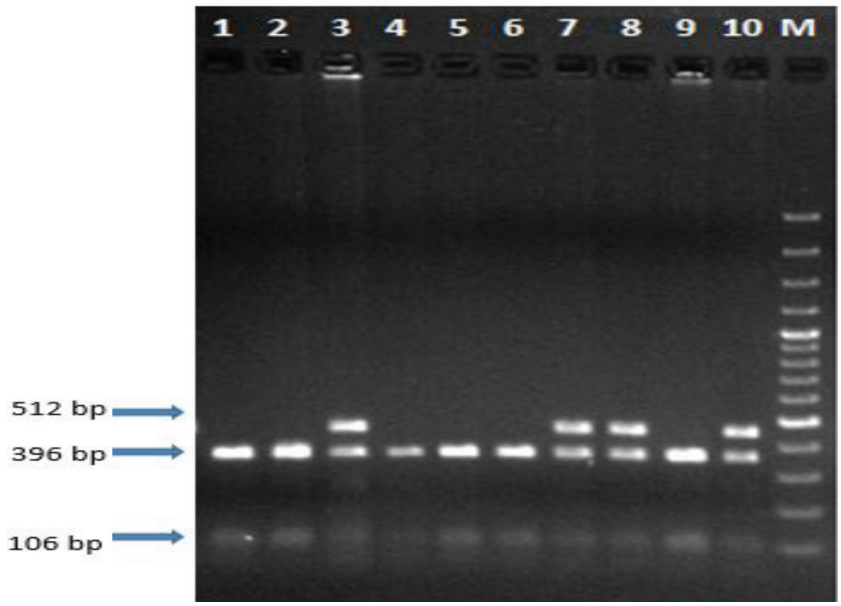

Figure 1. *MYF-5* gene PCR-RFLP result (M: 100 bp Ladder, 1, 2, 4, 5, 6, and 9 GG genotype, 3, 7, 8, and 10 AG genotype).

Table. Allele and genotype frequencies for *MYF-5* gene in Anatolian water buffalo.

n	Genotype frequency (n)			Allele frequency		χ2	P-value
	AA	AG	GG	A	G		
120	0.00 (0)	0.23 (28)	0.77 (92)	0.12	0.88	2.093	0.147[NS]

χ2: Chi-square: [NS:] Not significant

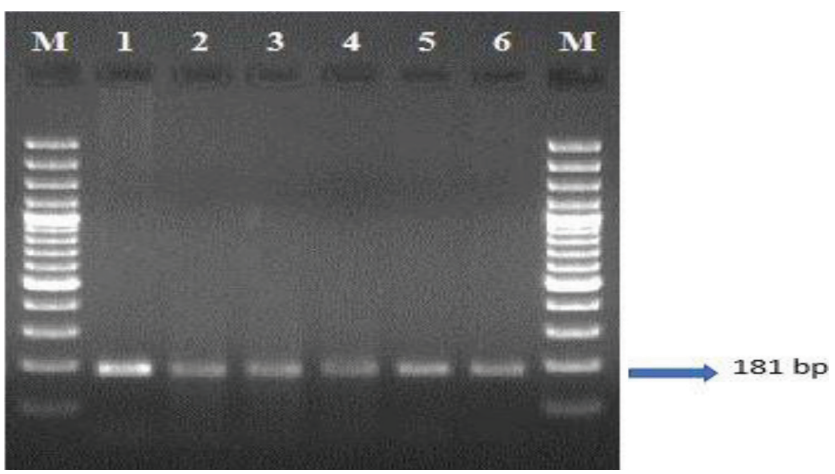

Figure 2. *STAT5A* gene PCR-RFLP result (M: 100 Ladder, 1-6 CC genotype).

polymorphism in different cattle breeds [30]. The A allele frequency (0.80, 0.80, and 0.86 respectively) was found to be the highest in the 3 breeds in the study where the *MYF-5* gene polymorphism was analyzed in Nanyang, Qinchuan, and Jiaxian red cattle breeds originating from *Bos taurus* raised in China. On the other hand, GG genotype was not found in cattle breeds. AA genotype was found to be at the highest frequency in 3 cattle breeds [31]. Similarly, in a study which addressed the MYF-5 gene polymorphism was reported in the Angus breed, which is a cattle breed of

European origin, and Hanwoo breed, which is a domestic Korean cattle breed. In this study it was reported that in the Hanwoo breed, the G allele frequency (0.74) was higher than the A allele frequency (0.26), GG genotype was the most common genotype (0.55), and the AA genotype was the least common (0.08) genotype seen in this breed. In Angus breed, the A allele frequency (0.51) was higher than G (0.49), AG genotype frequency (0.46) was found to be the highest in the 3 genotypes [14]. Similarly, in a study examining the *MYF-5* gene polymorphism in Charolais, a European-origin cattle breed grown in Hungary, it was revealed that the AG genotype frequency (0.48) was higher than the other 2 genotypes and AA genotype frequency (0.36) was higher than the GG genotype (0.16) [32]. In the study which analyzed the *MYF-5-Taq*I polymorphism in Simmental, Holstein, Brown Swiss and native cattle, and Eastern Anatolia Red cattle breeds raised in Turkey, it was found that the AG genotype frequency in the Simental, Holstein, Brown Swiss and Eastern Anatolia Red breeds having the three genotypes AG was higher than the other 2 genotypes and the G allele frequency was higher than the A allele frequency in all of the breeds [30].

In this study analyzing the *MYF-5-Taq*I polymorphism in Anatolian water buffalo, AA genotype was not found and GG genotype frequency was found to be higher than the other genotypes (Table 1). The reason of this may be related to the breeding purpose of the Anatolian water buffalo. Because, in a study that examined 7different cattle breeds (Hanwoo, Angus, Simmental, Hereford, Shorthorn, Brahman, and Red Chittagong) originating from *Bos taurus* and *Bos indicus*, it was observed that the animals with AA genotype had higher live weight at the rate of 3.52% compared to the GG genotypes and 3.70% compared to the AG genotypes. In addition, it was observed that carcass yield of the individuals with the AA genotype was better than the other 2 genotypes [16]. In another study conducted by Çınar et al. [33]. Holstein calves with the AA genotype were found to have higher birth weight than the other genotypes. Since water buffalo is a livestock raised for milk production in Turkey, as a result of the breeding strategies to increase milk yield, it can be considered that the AA genotype frequency has significantly decreases in Anatolian water buffalo. According to the data of the Water Buffalo Breeders Association of Turkey, there are 80,456 breeding water buffalo and 8108 bulls as of 2017 [34]. In this case, it is thought to cause a decrease in the AA genotype frequency in the population. However, in the chi-square analysis, it was observed that the Anatolian water buffalo population was genetically stable in terms of the *MYF-5-Taq*I polymorphism. Therefore, it is thought that AA genotype frequency is very low in Anatolian buffalo and it is a race feature.

In the Anatolian water buffalo samples analyzed in this study focusing on the *STAT5A-Ava*I polymorphism in Anatolian water buffalo, it was seen that there was only CC genotype. Since there was no literature regarding the analysis of this polymorphism, it was not possible to comment on the *STAT5A-Ava*I polymorphism in different buffalo breeds. However, it was thought that it could be compared with the results obtained in different cattle breeds due to the high rate of homology of the water buffalo genome with the cattle genome from many gene aspects [35,36]. On the other hand, it was thought that these genes showed homology in cattle and water buffalo because of obtaining the reported-sized PCR products in cattle at the end of the conducted PCR process and cleaving the obtained PCR products with the reported restriction enzymes in cattle. The results obtained for the SNPs screened for these genes are consistent with the cattle study results in the literature.

In a study carried out on Simental breed cattle, it was reported that the CC genotype was the most common (0.77) and there was no TT genotype [37]. In a study which examined the meat yield traits of *STAT5A-Ava*I polymorphism in the cattle breeds of European origin (Red Angus, Charolaise, Limousine, and Hereford), it was reported that the CC genotype was the most common and TT genotype was not found in the analyzed cattle breeds [24]. Similarly, it was reported that there was no TT genotype in the Polish native black-white cattle and the most common genotype was the CC genotype [38,39]. A previous study examined the *STAT5A-Ava*I polymorphism in Turkey native cattle breeds and reported that C allele frequency was high in the analyzed cattle breeds [39].

The relationships between *STAT5A-Ava*I polymorphism and yield-related traits in different cattle breeds were analyzed. In one of these studies, it was suggested that the breeds having CC genotype were superior than the breeds with CT genotype in terms of their weight increase between 8 and15 months [38]. In another study carried out on the relationship between *STAT5A-Ava*I polymorphism and meat yield, it was revealed in a similar way that the breeds with CC genotype were superior than the breeds with CT genotype in terms of their live weight and carcass traits between the ages of 0–15 months [24]. In the studies conducted on the relationships between *STAT5A-Ava*I polymorphism and milk yield in cattle, it was put forward that *STAT5A* gene was related to C allele frequency and milk yield, and *STAT5A* gene could be related to the marker supported selection for genetic improvement of milk production in cattle [19, 40].

In a study carried out on river buffalo raised in Italy, the mutation found on exons 8 and 9 of the *STAT5A* gene and causing a C > T change was related to milk protein rate with the difference of the *STAT5A-Ava*I polymorphism

analyzed in this study [41]. For this reason, it is believed that in the process of increasing the yield in different water buffalo breeds including the Anatolian water buffalo, which is an important livestock both in our country and the world, the planning of the studies where the relationships between yield traits and *MYF-5-Taq*I polymorphism and different polymorphisms found in the *STAT5A* gene will be able to be used in the improvement of this breed.

With this study, the genotypic status of the Anatolian water buffalo specific to Turkey and having an economic importance in terms of *STAT5A* and *MYF-5* gene polymorphism was put forward for the first time. As a result of this study, it was determined that the Anatolian water buffalo were monomorphic in terms of the *STAT5A-Ava*I polymorphism and polymorphic in terms of the *MYF-5-Taq*I polymorphism. It was though that this situation could be a breed trait. As a result, comprehensive studies are necessary for analyzing the relationships between important yield traits and these genes thought to be markers for different yield traits in cattle breeds.

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