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RESEARCH ARTICLE

The determination of polymorphisms between 6-9 exons of the water buffalo STAT5A gene

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Manda STAT5A geninin 6-9 eksonları arasındaki polimorfizmlerin belirlenmesi

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Öz

Amaç: Bu çalışmanın amacı, Anadolu mandalarında, süt verimini ve süt bileşimini etkileyen STAT5A geninin 6. - 9. ekzonları arasındaki polimorfizmleri belirlemektir.

Gereç ve Yöntem: Araştırmanın materyalini, Afyonkarahisar bölgesinde yetişen 96 adet Anadolu mandasından alınan kan örnekleri oluşturmuştur.

Bulgular: Araştırmada, STAT5A geninin sekizinci ekzonunda g+12221 C>T polimorfizmi tespit edilmiştir.

Öneri: Sinyal transdüseri ve transkripsiyon aktivatörü 5A (STAT5A) geni, süt verimini ve bileşimini etkilediği için, süt hayvanı yetiştiriciliğinde oldukça önemlidir, ayrıca hücre içinde de birçok hayati görevi bulunmaktadır. Bu çalışmada bulunan 8. ekzondaki polimorfizm protein sentezinde ki amino asit dizilimde bir değişikliğe neden olmadığından sessiz bir mutasyon olarak nitelendirilmiştir. Islah çalışmalarının daha verimli yapılabilmesi için, memeli canlılarda süt verimi ve süt kompozisyonu üzerine etkili olan STAT5A geniyle ilgili çalışmaların çoğaltılması önem arz etmektedir.

Anahtar kelimeler: Anadolu Mandası, gen polimorfizmi, SNP, STAT5A

Abstract

Aim: The aim of this study was to determine the polymorphisms between 6^{th}-9^{th} exons of STAT5A gene which affects milk yield and milk composition in Anatolian buffaloes.

Materials and Methods: The material of the study was composed of the blood samples taken from 96 Anatolian buffaloes grown in Afyonkarahisar region.

Results: According to the results of the research, g+12221 C> T polymorphism was detected in the 8th exon of STAT5A gene.

Conclusion: The signal transducer and transcription activator 5A gene (signal transducers and activators of transcription 5A, STAT5A) is important in dairy animal breeding because it has many vital tasks within the cell as well in addition to affecting milk yield and composition. In this study, the polymorphism in the 8^{th} exon was described as a silent mutation since protein synthesis did not cause a change in amino acid sequence. In order to make the breeding studies more efficient, it is important to increase the studies on STAT5A gene, which affects milk yield and milk composition in mammals.

Keywords: Anatolian Water Buffaloes, gene polymorphism, SNP, STAT5A

Introduction

The water buffalo is an animal species that is highly tolerant to diseases and capable of adapting to different and difficult environmental conditions and from which humans have benefitted from in terms of various products such as milk, meat and leather for centuries. Efforts are made to extend the breeding of water buffalos in Turkey because of their ability to consume low quality affordable feed and convert it into animal products, milk which is low in cholesterol and rich in content and meat with a low fat content and suitability for dietary requirements (Şekerden et al 1999, Küçükkebabçı and Aslan 2002, Selvaggi et al 2009, Şahin et al 2013).

Environmental and genetic factors have an important influence in increasing yield on animal breeding. Many genes affect milk yield and composition (Dario and Selvaggi 2011). Among these genes, the signal transducer and transcription activator 5A (STAT5A) is important in buffalo breeding as it has many vital duties within the gene cell as well as an impact on milk yield and composition (Raven et al 2014). It has been discovered that signal transduction and transcription activators (STATs), known as mammalian gland factors, are an intracellular mediator of prolactin signal transduction and can activate the transcription of milk protein genes (Liu et al 2014, Paramitasari et al 2015). However, it is known that seven members of the mammalian STAT family transform vital signals for 50 ligands (Kisseleva et al 2002). STAT5 is promising for milk production and composition in milk-producing animals due to this important role.

STAT proteins function as silent (latent) cytoplasmic transcription factors that are activated with the signals they receive from cytokine, the growth factor or peptide receptors and transmit these signals to the nucleus (Ihle 1996, 2001). When they are activated, they are phosphorylated and then dimerized and steer toward the nucleus and bind to the promoter region of the target gene causing gene expression changes (Bromberg et al 1999, Yu and Jove 2004, Chaix et al 2011). Seven STAT proteins have been identified in mammalian cells, namely STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6 (Doğan and Güç 2004). Generally, STAT proteins, together with Janus kinases, play an important role in signal transduction to the cellular construct cytokines (Goldammer et al 1997). It is known that the most important STAT member activated by prolactin (PRL) is STAT5 and that two forms of STAT5 (STAT5A and STAT5B) convert PRL signalization (Frasor and Gibori 2003). In the conducted study, it has been determined that STAT5A was required for the formation of the udder epithelium (Bromberg et al 1999). Furthermore, STAT5A is a transcription factor that directs prolactin and growth hormone signals which are essential regulators of lactation and growth. Li et al (2018) completely sequenced of the STAT5A gene in buffalo (2502 bp) and the higher STAT5A gene expression was found in mammary glands. Decreased expression of the STAT5A gene significantly reduced expression of milk protein genes. Overexpression of STAT5A was resulted in significantly higher expression of milk protein genes (Li et al 2018).

This study was carried out to determine the polymorphisms of the STAT5A gene, which is known to affect growth, development, milk yield and composition, in the Anatolian Water Buffalo.

Material and Methods

Blood samples taken from the V. jugularis of 96 Anatolian Water buffalos within the scope of the Community Based Water Buffalo Improvement Project carried out in Afyonkarahisar province by the General Directorate of Agricultural Research and Policies of the Ministry of Food, Agriculture and Livestock, have been used in this study. The blood samples were stored in cryo tubes at -20 °C until DNA isolation. DNA isolation was carried out using a commercial kit (ThermoFisher Scientific K0722). Oligonucleotide primers (F1: 5'-TCCTCCAGCTCAGTTTGCCCA-3', R1: 5'-TCTGCCAGATGATCTCCGCCA-3', F2: 5'-TTGGAAGGCAGGGCATCTCTGC-3' and R2: 5'-CAGCGTACTTGCGGGTGTTCTC-3') were designed using the reference sequence (NW_005784710.1) of the STAT5A gene obtained from NCBI and the FastPCR Professional 6.1.2 package program (Kalendar et al. 2009). The manifestation of dimer and hairpin between the primers was checked with the same program.

DNA isolation was performed using spin column method. 10 µl Proteinase-K (20mg / ml) was placed in a 1.5 ml eppendorf tube. After than 200 µl blood samples and 200 µl extraction solution were added to the tubes. After centrifugation, 210 µl binding buffer was added. The lysates were transferred to spin column tubes. The tubes were centrifuged at 6,000 rpm for 1 minute, and the liquid portion under the collection tube was spilled. Then, 650 µl of washing solution-I was added to the spin columns and centrifuged at 6,000 rpm for 1 minute. The liquid portion under the collection tube was discarded. 500 µl of washing solution-II was added to the spin columns and centrifuged at 6,000 rpm for 1 minute. The liquid portion under the collection tube was discarded. 250 µl of washing solution-II was added to the spin columns and centrifuged at 14,000 rpm for 3 minute. The liquid portion under the collection tube was discarded. The spin column was transferred to new eppendorf tubes, and 200 µl of TE buffer (10mM Tris, 1 mM EDTA pH 8.0) was added and incubated for 5 min at room temperature. After the waiting period, the tubes were centrifuged for 1 min at 8,000 rpm and stored at -20 ° C for use.

PCR Analysis: 1×PCR buffer, 3.5 mM $MgCl_2$, 1 M betain, 0.2 nmol dNTP, 0.25 mM F1 and R1, 1 U Platinum Taq polymerase (Invitrogen, 10966034), 2 µl DNA (20 ng/µl) and ultra

distilled water to make a total volume of 20 µl per sample were used in the PCR with the F1 – R1 primers. The PCR device was programmed to operate 1 cycle in 2 min at 94 ° C; 45 seconds at 94 ° C, 30 seconds at 58 ° C, 35 cycles for 1 minute at 72 ° C and 10 minutes at 72 ° C.
1×PCR buffer, 2 mM MgCl2, 0.2 nmol dNTP, 0.25 mM F2 and R2, 1 U Platinum Taq polymerase, 2 µl DNA (20 ng/µl) was prepared with ultra-distilled water to give a total volume of 20 µl which was used in the PCR with F2 – R2 primers. The PCR device was programmed to operate 1 cycle in 2 min at 94 °C, 45 s at 94 °C, 30 s at 62 °C, 35 cycles in 1 min at 72 °C and for 10 min at 72 °C. PCR products were visualized using a Vilber Lourmat BIO-VISION gel imaging system using a 2% agarose gel.

Sequencing: After the PCR products were purified with ExoSAP-IT, sequenced PCR was carried out according to the kit protocol using BigDye Terminator v3.1 (Applied Biosystems, 4337455). The products obtained by sequenced PCR were purified with ethanol-EDTA precipitation and loaded into the ABI 3500 Genetic Analyzer device after adding 15 ml of Hi-Di formamide. The obtained data were edited by Sequencher 5.4.6 (Gene Codes Corporation) and aligned in the BioEdit Ver 7.2.0 program to identify polymorphic SNPs.

Statistical analysis: The GENETIX (4.05.02) computer program (Belkhir et al 1996) was used to calculate allele frequencies and heterozygosities in the STAT5A gene.

This study has been carried out with the permission of Afyon Kocatepe University Animal Experiments Local Ethics Committee (AKUHADYEK) no 49533702/122.

Results

The 1720 base length area between the 6th exon and the 9th exon of the STAT5A gene has been examined in this study. For this purpose, two different primer mixes were used. Primer mix– 1 (F1 – R1) and 1003 base pair (bp) and primer mix– 2 (F2 – R2) and 929 bp length area were amplified and visualized by agarose gel electrophoresis (Figure 1).

Three polymorphisms were detected in the STAT5A gene, one in the 6th intron of the gene, five in the 7th intron and three in the 8th intron of the gene in the performed analyses. Also, g+12221 C> T polymorphism was detected in the 8th exon. Sequence images of the SNPs found are shown Figure 2. The SNPs observed in the introns and exons, alleles, allele frequencies (X_i) observed (H_o) and expected (H_e) heterozygo-

Table 1. The allele frequencies and heterozygosity values of SNPs between the 6th to 9th exons of the STAT5A gene at the Anatolian Water Buffaloes

SNP	Allel	X_i	H_o	H_e	F_{IS}
11461	G	0.9792	0.0417	0.0408	-0.016
	A	0.0208			
11849	T	0.5104	0.4792	0.4998	0.046
	C	0.4896			
11893	G	0.4948	0.7604	0.4999	-0.517
	A	0.5052			
11923	G	0.4271	0.7500	0.4894	-0.529
	A	0.5729			
11983	G	0.6510	0.5938	0.4544	-0.302
	A	0.3490			
12004	C	0.1771	0.2708	0.2914	0.076
	A	0.8229			
12221	T	0.4688	0.0625	0.4980	0.876
	C	0.5313			
12356	G	0.9063	0.0833	0.1699	0.513
	A	0.0938			
12430	G	0.8802	0.1354	0.2109	0.362
	T	0.1198			
12582	G	0.7708	0.4583	0.3533	-0.293
	A	0.2292			

Abbreviations (SNP: Single Nucleotide Polymorphism, X_i: Allel frequency, H_0: Observed heterozygosity, H_e: Expected heterozygosity, F_{IS}: inbreeding coefficient)

sity and inbreeding coefficient (F_{IS}) values are given in Table 1. Chi-square analysis indicated that this population was not in the Hardy-Weinberg equilibrium (P <0.001).

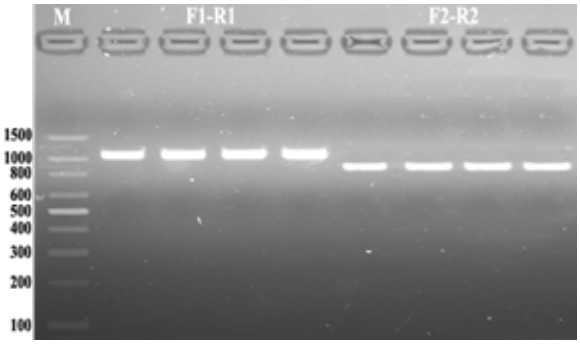

Figure 1. Agarose gel electrophoresis image of PCR products

Discussion

One polymorphism was detected in the 6th intron of the STAT5A gene, five were detected in the 7th intron and three polymorphisms were detected in the 8th intron in the analyses carried out on 96 Anatolian water buffalo calves raised in Afyonkarahisar region. Furthermore, g+12221C>T polymorphism was detected in the eighth exon. However, since the polymorphism protein synthesis in the 8th exon does not cause a change in the amino acid sequence it qualifies as a silent mutation. In a study carried out with Murrah breed water buffalos (Kale and Yadav 2012), STA5A gene was analyzed in terms of exon 7, intron 9, exon 14, intron 15 and 16. It has been reported that a significant impact of polymorphism detected in the 7th exon of Murrah breed on 305 days milk yield (5% level) has been observed. However, no polymorp-

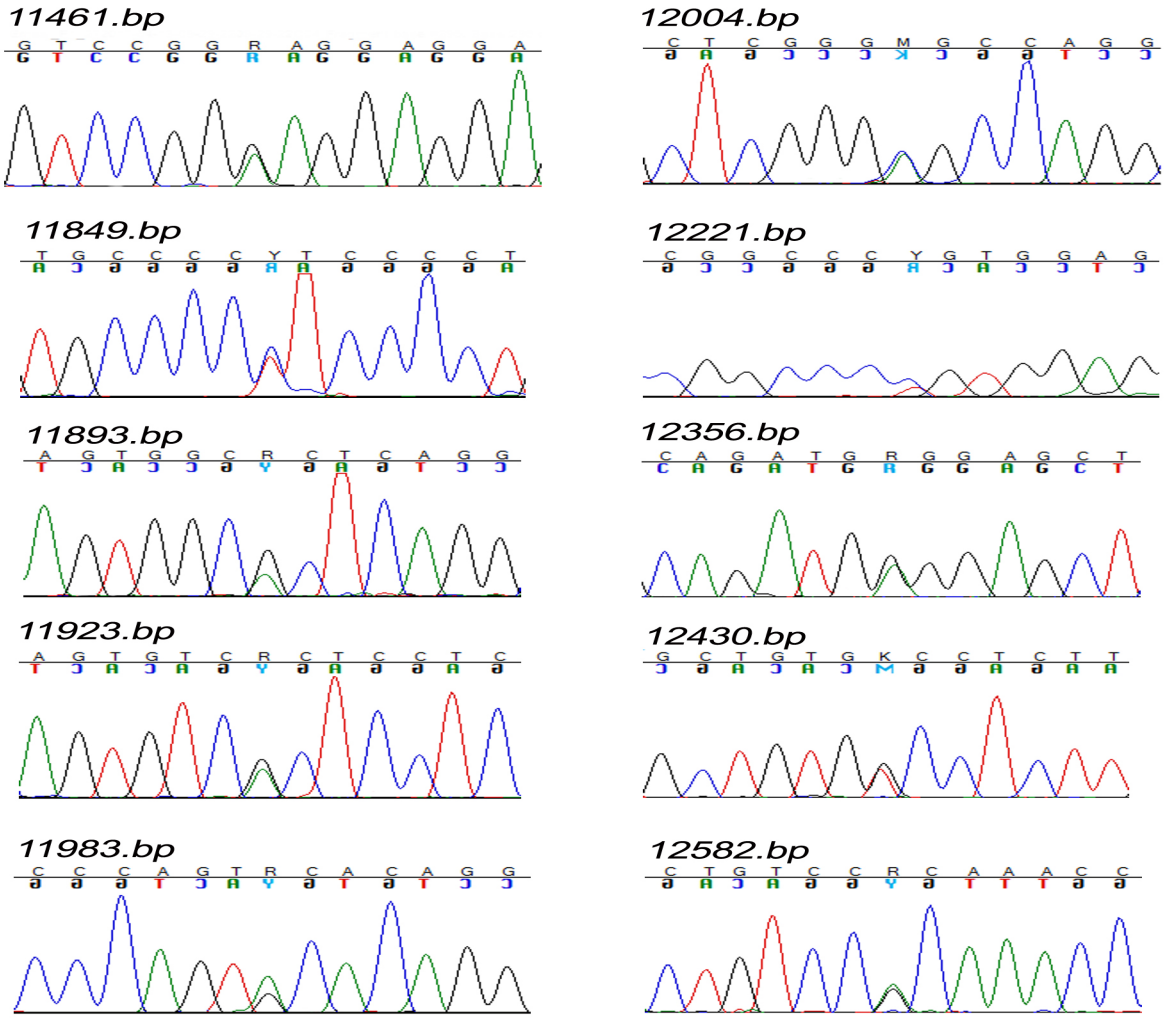

Figure 2. Sequence images of the SNPs

hism was determined in the 7th exon of the STAT5A gene in the Anatolian Water Buffalo.

TG repeats in the 12th intron of the STAT5A gene and a CCT deletion in the 15th intron have been found in cattle studies (Mccracken et al 1997, Flisikowski and Zwierzchoeski 2003). In a study conducted on Jersey cows, it was found that cows with AA and AG genotypes positioned at 9501 in the 9th intron 9 of STAT5A displayed higher protein content and GG genotyped cows had higher milk yield (Brym et al 2004). Flisikowski et al (2004) reported that STAT5A altered DNA binding properties of g+12743T>C polymorphism in exon 16 and caused some milk characteristics to change. Khatib et al (2009) found that the g.153137 G>C polymorphism found in exon 8 of STAT5A was significantly associated with embryo survival and fertilization rate compared to all examined SNPs. Selvaggi et al (2009) reported that CC genotype cows gave more milk protein and milk yield than the other genotypes due to the C → T polymorphism positioned at 6853 in the 7th exon of the STAT5A gene in Italian Brown cattle. Raven et al (2014) reported that the STAT5A gene in cattle had an impact on the percentage of fat in the milk composition, the amount of milk, the percentage and the amount of protein in their studies. He et al (2012) reported that mutations found at the A9501G point of the bovine STAT5A gene were associated with milk yield, milk protein yield and fat percentage in Chinese Holstein cows.

In the absence of STAT5A, negative effects such as a decline in udder alveolar development and milk release are observed. In addition, defects in the development of the mammary gland are observed in the absence of STAT5A (Ihle 2001, Hennighausen and Robinson 2008). As a consequence, milk yield decreases and the nutritional properties of the milk are compromised. Therefore, the STAT5A gene carries a candidate gene quality for quantitative qualities in livestock (Flisikowski et al 2004). In a study carried out with Murrah breed water buffalos, STA5A gene was analyzed in terms of exon 7, intron 9, exon 14, intron 15 and 16 (Kale and Yadav 2012). It has been reported that a significant impact of Polymorphism detected in the 7th exon of Murrah breed water buffalos on 305 days milk yield (5% level) has been observed.

As a result of statistical analysis, the negative outcome of some SNPs with F_{IS} value, which is a measure of the average deviation of the genotype frequencies observed for SNPs in the STAT5A gene from the Hardy-Weinberg ratios, may be due to the high frequency of homozygosities. These results can be an indication of the applied selection in population.

The STAT5A gene has very important duty. The effect on milk yield should not be ignored. In dairy animals, studies on this gene should be intensified in order to improve milk yield and content and breeding work.

Conclusion

In conclusion, there are very few genetic studies involving water buffalos. The genome contains millions of SNPs; many of these SNPs are intronic and have unknown the functional roles or benefits of introns. Therefore, it is necessary to further carry out more detailed studies for the STAT5A gene in water buffalo, which has been proven to be associated with udder development, milk yield and composition, fertility rate and embryonic life span in cattle.

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