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A determination of growth hormone receptor gene polymorphisms in East Anatolian Red cattle, South Anatolian Red cattle, and Turkish Grey cattle

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Abstract: The objective of this study was to determinate the genotype and allele distributions of growth hormone receptor gene polymorphisms in East Anatolian Red cattle (EAR), South Anatolian Red cattle (SAR), and Turkish Grey (TG) cattle. To this end, 50 EAR, 50 SAR, and 50 TG cattle were used in the study. DNA samples were isolated by using the standard ammonium acetate salt-out method. Target regions were amplified and digested by *Alu*I, *Acc*I, *Stu*I, *Nsi*I, and *Fnu4*HI restriction enzymes. The + allele frequency of growth hormone receptor (GHR)/*Alu*I polymorphism related with milk traits in TG cattle and the – allele frequencies of GHR/*Alu*I polymorphism related with meat traits in EAR and SAR cattle were found to be low. The + allele frequency of GHR/*Stu*I polymorphism affecting milk traits was found to be low in the EAR breed. The –/– genotype of GHR/*Nsi*I polymorphism was also low in all 3 of the breeds. The + allele frequency of GHR/*Fnu4*HI polymorphism related with meat traits turned out to be low in the SAR and EAR breeds. In conclusion, it can be said that some of the alleles of GHR polymorphisms that are favorable for trait qualities occur less frequently in SAR, EAR, and TG cattle breeds than they do in high-trait European breeds.

Key words: PCR-RFLP, GHR gene, SNP, East Anatolian Red cattle, South Anatolian Red cattle, Turkish Grey cattle

Doğu Anadolu Kırmızısı, Güney Anadolu Kırmızısı ve Boz ırk sığırlarda büyüme hormonu reseptör geni polimorfizmlerinin belirlenmesi

Özet: Bu çalışmada Türkiye'deki yerli sığır ırklarından Güney Anadolu Kırmızısı (GAK), Doğu Anadolu Kırmızısı (DAK) ve Boz ırk sığırlarda, büyüme hormonu reseptör (GHR) genindeki polimorfizmlerin genotip ve allel dağılımlarının belirlenmesi amaçlanmıştır. Her sığır ırkından 50'er adet bireyden kan alınarak standart amonyum asetatla çöktürme yöntemiyle DNA örnekleri izole edilmiştir. Hedef bölgeler polimeraz zincir reaksiyonuyla çoğaltılmış ve AluI, AccI, StuI, NsiI ve Fnu4HI restriksiyon enzimleri ile sindirilmiştir. Süt verimiyle ilişkili GHR/AluI polimorfizmi + allelinin frekansı Boz ırk sığırlarda, et verimiyle ilişkili GHR/AluI polimorfizmi – allelinin frekansı GAK ve DAK ırkı sığırlarda düşük bulunmuştur. Süt verimini etkileyen GHR/StuI polimorfizmi + allelinin frekansı DAK ırkında düşük, GHR/NsiI polimorfizmi –/– genotipi ise üç ırkta da düşük olarak tespit edilmiştir. Et verimiyle ilişkili GHR/Fnu4HI polimorfizmi + allelinin frekansı GAK ve DAK ırklarında düşük bulunmuştur. Sonuç olarak GHR genindeki verim özellikleri açısından etkili bazı allellerin yerli ırklarda yüksek verimli Avrupa ırklarına kıyasla daha düşük frekanslara sahip olduğu belirlenmiştir.

Anahtar sözcükler: PZR-RFLP, GHR geni, SNP, Doğu Anadolu Kırmızısı, Güney Anadolu Kırmızısı, Boz ırk

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Introduction

Hormones, growth factors, and other regulatory proteins are candidate markers for quantitative traits in livestock animals (1,2). The biological effects of growth hormone (GH) involve select tissues as well as the metabolism of carbohydrates, lipids, proteins, and minerals (3). GH also has a major role in mammogenesis and lactation (4-6).

GH affects the target cells through GH receptors (GHR). GHRs are members of the cytokine/hematopoietin superfamily of receptors (7) and contain a transmembrane domain that consists of 24 amino acids, an extracellular hormone-binding domain, and a long cytoplasmic domain (8). GH binds to GHR on target tissues in order to initiate its biological effect. After a ligand-induced dimerization of GHR, cascades in GH signaling get started. Mutations of GH and/or GHR genes can deactivate GH by inhibiting dimerization (9).

In cattle, the GHR gene is located on chromosome 20 (10). The bovine GHR gene has 9 exons (2-10) in the translated part and 9 exons (1A-1I) in the 5′-noncoding regulatory region. A long interspersed nuclear element (LINE-1) about 1206 bp long was found upstream from exon 1A (11,12). The 5′-regulatory region contains promoter elements, enhancers, repressors, and regions responsible for tissue-specific gene expression (13); polymorphisms in this region are therefore important for quantitative traits (14). Several polymorphic sequences have been identified in the 5′-regulatory region of the GHR gene (14,15). The adenine/thymine transition at position 1182, cytosine/thymine transitions at positions 892 and 232, adenine/guanine transition at position 154, and thymine/cytosine transition at position 1104 are mutations that affect the quality and production of milk and meat in cattle.

The objective of this study was to determine the allele and genotype frequencies of 5 polymorphisms in the 5′-regulatory region of the GHR gene in native Turkish cattle breeds. The mutations in question are associated with milk and meat production traits, and it is hoped that the present study will contribute to the scientific basis for the selection parameters of native breeds.

Material and methods

For the study, blood was collected from 50 South Anatolian Red (SAR) cattle, 50 East Anatolian Red (EAR) cattle, and 50 Turkish Grey (TG) cattle in herds located in southern Anatolia, eastern Anatolia, and the Marmara region of Turkey, respectively. The animals were not relatives and had phenotypic characteristics of their breed. We collected 2 mL of total blood into vacuum tubes containing EDTA. Genomic DNA samples were isolated using the standard salt-out method (16).

Animals were genotyped for 5 polymorphisms in the 5′-regulatory region of the GHR gene. *Polymerase chain reaction* (PCR) reaction was performed in a reaction volume of 25 µL using 1 U Taq DNA polymerase (Fermentas Life Sciences, Canada), 2-2.5 mL 10× PCR buffer, 1.5 mM $MgCl_2$, 50-100 ng genomic DNA, 100 mM dNTP (TaKaRa Biotechnology Co., Ltd., Japan), and 10 pmol of each primer for all of the fragments.

The primer sequences used for the 747-bp fragment containing a polymorphic *Alu*I restriction site at the 1182 position were 5'- TGC GTG CAC AGC AGC TCA ACC -3' and 5'- AGC AAC CCC ACT GCT GGG CAT -3', and for the 1119-bp fragment containing a polymorphic *Acc*I restriction site at the 232 position and *Stu*I site at the 892 position, they were 5'- ATG CCC AGC AGT GGG GTT GCT -3' and 5'- GGC AAA CAG TGC GGG GTT GGA -3'. For both of the regions, amplification was carried out at 94 °C for 10 min, with 35 cycles of 94 °C for 60 s, 66 °C for 80 s, and 72 °C for 2 min, and then a final extension at 72 °C for 5 min. The primer sequences used to amplify the 836-bp fragment containing a polymorphic *Fnu4*HI restriction site at the 1104 position were 5'- TGC GTG CAC AGC AGC TCA ACC -3' and 5'- AGC AAC CCC ACT GCT GGG CAT -3'. Amplification was carried out for 95 °C for 2 min, with 39 cycles of 95 °C for 30 s, 65 °C for 45 s, and 72 °C for 2 min, and then a final extension at 72 °C for 10 min.

The primers sequence used for the 836-bp fragment containing a polymorphic *Nsi*I restriction site at the 318 position were 5'- CTG GCG TAT GGT CTT TGT CA -3' and 5'- TGG TCT TGC TGC TTT CCT AT -3'. Amplification was carried out for 94 °C

for 5 min, with 35 cycles of 94 °C for 60 s, 58 °C for 60 s, and 72 °C for 60 s, and then a final extension at 72 °C for 10 min.

For RFLP analysis, 10 mL of the PCR products were digested overnight with 10 units of restriction enzyme at 37 °C. The digested DNA fragments were separated by electrophoresis in 2% agarose gel. Genotype and allele frequencies of each polymorphism were calculated by using the PopGene32 software program (17).

Results

The allele and genotype frequencies of 5 polymorphisms obtained for 3 different cattle breeds are shown in the Table.

Digestion of the 747-bp fragment of GHR gene with *Alu*I restriction enzyme revealed a polymorphism with 2 alleles. The cut allele (+) was characterized by bands of 602 bp and 145 bp. The uncut allele (–) was characterized by a single band of 747 bp. It was found that the + allele had a higher frequency in SAR and EAR breeds and a lower frequency in TG. The distribution of genotypes and alleles of GHR/*Alu*I polymorphism followed the Hardy-Weinberg equilibrium (HWE) in the EAR breed. Significant differences were found between expected and observed values in the SAR ($P < 0.001$) and TG ($P < 0.001$) breeds, and genotype frequencies were not consistent with the HWE.

Digestion of the 1119-bp fragment of GHR gene with *Acc*I restriction enzyme revealed a

Table. The distribution of GHR gene genotypes and allele frequencies in South Anatolian Red, East Anatolian Red and Turkish Grey cattle.

Locus	Breed	*n*	Allele frequency (%)		Genotype			
			+	–	+/+	+/–	–/–	(χ^2)[1]
	SAR[2]	49	0.54	0.46	7	39	3	17.1914***
*Alu*I	EAR[3]	50	0.56	0.16	14	28	8	0.7977 Ns
	TG[4]	44	0.36	0.64	15	2	27	36.8437***
			+	–	+/+	+/–	–/–	
	SAR	49	0.59	0.41	14	30	5	3.2398 Ns
*Acc*I	EAR	50	0.43	0.57	9	25	16	0.0049 Ns
	TG	44	0.39	0.61	6	22	16	0.0815 Ns
			+	–	+/+	+/–	–/–	
	SAR	49	0.59	0.41	15	27	7	0.7292 Ns
*Stu*I	EAR	50	0.43	0.57	3	17	30	0.1281 Ns
	TG	44	0.56	0.44	7	35	2	15.8639***
			+	–	+/+	+/–	–/–	
	SAR	49	0.22	0.78	2	18	29	0.0998 Ns
*Fnu4*HI	EAR	50	0.39	0.61	14	11	25	15.0330***
	TG	44	0.62	0.38	19	17	8	1.5478 Ns
			+	–	+/+	+/–	–/–	
	SAR	49	0.69	0.31	19	30	0	9.1659**
*Nsi*I	EAR	50	0.80	0.20	31	18	1	0.6758 Ns
	TG	44	0.74	0.26	23	19	2	0.5099

[1]Hardy-Weinberg equilibrium, [2]South Anatolian Red, [3]East Anatolian Red, [4]Turkish Grey; Ns: Not significant,

** $P < 0.01$, *** $P < 0.001$.

polymorphism with 2 alleles. The cut allele (+) was characterized by bands of 958 bp and 161 bp. The uncut allele (–) was characterized by a single band of 1119 bp. GHR/*Acc*I – allele frequency was found to be higher than + allele frequency in EAR and TG cattle. In SAR cattle, + allele frequency was higher than – allele frequency. The observed genotype frequencies were consistent with the HWE in all 3 breeds.

Digestion of the 1119-bp fragment of GHR gene with *Stu*I restriction enzyme resulted in a polymorphism with a cut allele (+) characterized by 824-bp and 295-bp bands and an uncut allele (–) characterized by a 1119-bp single band. In SAR and TG breeds, – allele frequencies of GHR/*Stu*I were lower than the + allele frequency and the results followed the HWE. In TG, however, genotype frequencies were not consistent with the HWE ($P < 0.001$).

After digestion of the 836-bp fragment of GHR gene with *Fnu4*HI restriction enzyme, a polymorphism with 2 alleles was detected. The cut allele (+) was characterized by bands of 763 bp and 73 bp. The uncut allele (–) was characterized by a single band of 836 bp. In the SAR and TG breeds, no significant differences were found between the observed and expected genotype frequencies. In the SAR and EAR breeds, – allele frequency was higher than the + allele, and the opposite was seen in TG. In the EAR breed, genotype frequencies were not consistent with the HWE.

The 318-bp fragment of GHR gene was digested with *Nsi*I restriction enzyme and a polymorphism with 2 alleles was detected. The cut allele (+) was characterized by bands of 166 bp. The uncut allele (–) was characterized by a single band of 318 bp. In all 3 of the breeds, the GHR/*Nsi*I + allele frequency was found to be higher than the – allele frequency. The results for the SAR breed did not follow the HWE ($P < 0.01$).

Discussion

Genetic studies on livestock animals are focused on the genes associated with economically important traits and health status. The main objective of molecular biological applications is to identify, map, and analyze the polymorphisms of genes that are involved in the main metabolic pathways affecting animal growth (18,19). Compared to many other countries, Turkey has a large animal population, but production traits per animal are very low. Besides the genotypes, environmental conditions and a lack of quality food also affect the low trait qualities (20). EAR, SAR, and TG cattle breeds are the most common breeds in Turkey, but there are limited studies on the quantitative trait loci (QTL) of these animals. In recent years, studies conducted on QTL affecting meat and milk traits in the cattle breeds of Turkey have shed some light on the genetic status of these breeds (21-25). This is particularly true of the genes involved in the somatotropic axis, which are very important for the quantitative traits because of their role in growth metabolism. In a study on GH polymorphisms in EAR and SAR cattle breeds, Yardibi et al. (25) found that the VV genotype of *Alu*I polymorphism and the –/– genotype of *Msp*I polymorphism were associated with a high percentage of milk fat. IGF and IGF-1R polymorphisms were found to be uncorrelated with milk and meat traits in EAR and SAR breeds (24). A study conducted on osteopontin, prolactin, and pit-1 polymorphisms in SAR and EAR cattle showed that these breeds are disadvantaged in terms of trait qualities when compared to European breeds (23).

The GHR gene has several polymorphisms. In the present study, polymorphisms at 5 different sites within the 5′-regulatory region of the bovine GHR gene were investigated. All of them were single-nucleotide polymorphisms (SNP). SNPs have gained popularity in genetic studies due to their high accuracy and reproducibility (26).

GHR/*Alu*I polymorphism is associated with milk production and composition (14,27). Aggrey et al. (14) suggested that the *Alu*I +/+ genotype is favorable for milk fat content in Holstein cattle. Maj et al. (27) found that the percentage of milk protein is higher in animals with the *Alu*I + allele. Despite earlier evidence that the + allele increases the fat percentage in Holstein cattle, Maj et al. (28) suggested that *Alu*I polymorphism does not affect milk traits in their study on Polish Black-White cattle. In the present study, TG cattle had the lowest + allele frequency among the 3 Turkish cattle breeds. EAR cattle are known to have a higher milk fat content than other Turkish breeds, and the + allele frequency associated with milk fat content was indeed found to be higher in

TG. Further studies should be conducted on linkage analysis. GHR/*Alu*I polymorphism is also associated with weight gain and carcass weight (29). It has been suggested that the +/– genotype is associated with a significantly faster growth rate and a higher daily weight gain between 13 and 15 months of life. The weight of the cold carcass and percentage of valuable cuts were highest for the individuals with –/– genotype. (15). In this study, the – allele frequency was found to be lower than the + allele frequency in SAR and EAR cattle breeds. In TG cattle, our results showed that in *Alu*I polymorphism, the – allele related to meat traits had a higher frequency. The +/– genotype, which has been associated with a higher growth rate (28), was determined to be the most common genotype in the SAR and EAR breeds. Out of 44 TG cattle, however, only 2 individuals with the +/– genotype were detected. The frequency of the –/– genotype, associated with cold carcass weight, was found to be very low in SAR and EAR cattle.

GHR/*Acc*I polymorphism has effects on milk production and quality. An association between the +/– genotype and gross energy, total solids, and fat percentage was observed in recent studies (27). Despite these results, Aggrey et al. (14) suggested that there is no evidence for an association between this polymorphism and dairy production traits. In studies conducted on European cattle breeds, + allele frequency was calculated at 0.58 in Holstein, 0.77 in Polish Black-White, 0.62 in Lithuanian Black, and 0.53 in Lithuanian Red (14,27,30). In our own study, we found the frequency of the + allele to be 0.59 in SAR, 0.43 in EAR, and 0.39 in TG cattle. EAR and TG cattle had lower + allele frequencies compared to the European cattle breeds. In a study on the relationship between GHR/*Acc*I polymorphism and meat production traits, researchers found a correlation between the +/+ genotype and valuable cuts and between the +/– genotype and daily weight gain (15). We observed 9 and 6 individuals with the +/+ genotype out of 50 EAR and 44 TG cattle, respectively. The +/– genotype associated with high daily weight gain was the most common in all 3 cattle breeds.

GHR/*Stu*I polymorphism is found to be associated with the fat and protein percentages of milk, but no association has been found with meat production (14). In Holstein cattle, the frequency of the + allele was calculated as 0.86. In the 3 native Turkish cattle breeds in this study, only EAR cattle had a lower + allele frequency (0.43) than – allele frequency. Maj et al. (27) observed a correlation between the –/– genotype and the fat, protein, and lactose percentages of milk. In a study on meat production, cattle with the –/– genotype had more valuable cuts, whereas the +/– genotype had lower feed intake (15). No –/– genotype was observed in the 49 SAR cattle examined in this study. Only 1 individual with this genotype was noted in the 50 EAR cattle, and 2 individuals were found out of 44 TG cattle. According to the results of Aggrey et al. (14), no *Stu*I (–/–) individual was detected in Holstein, Ayrshire, Boran, or N'Dama breeds. They suggested that the – allele is either very rare or deleterious. In the present study, 30 individuals with the –/– genotype were observed from the 50 studied EAR cattle. This finding leads us to suggest that the occurrence of the *Stu*I genotypes can vary widely between breeds.

The results of a study by Maj et al. (27) suggest that there is no correlation between GHR/*Fnu4*HI polymorphism and milk production traits. However, there is evidence for the association of the +/– genotype with carcass width and the +/+ genotype with growth rate and daily weight gain (15). Frequencies of the +/– genotype in the 3 cattle breeds were observed to be lower than those of other genotypes. The +/+ genotype, associated with a higher growth rate, was observed in only 2 individuals of the SAR breed. The GHR/*Fnu4*HI + allele frequency was found to be 0.83 in Aberdeen Angus, 0.63 in Charolais, 0.79 in Hereford, and 0.45 in Simmental cattle breeds. SAR and EAR cattle had 0.22 and 0.39 + allele frequencies, respectively.

In conclusion, it might be said that some of the alleles of GHR polymorphisms that are favorable for trait qualities have lower frequencies in SAR, EAR, and TG cattle in comparison to high-trait European breeds. We recommend that further studies be conducted on the GHR gene in native Turkish cattle breeds using linkage analysis.

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