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**RESEARCH ARTICLE**

# Leptin Gene Polymorphisms in Native Turkish Cattle Breeds [1]

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## Summary

The aim of the study was to determine leptin gene polymorphisms in South Anatolian Red (SAR), East Anatolian Red (EAR) and Turkish Grey Cattle. In the study unrelated 40 SAR, 40 EAR and 40 Turkish Grey cattle were used. Target sites in leptin gene exon 2, exon 3 and intron 2 were amplified by polymerase chain reaction (PCR). The single nucleotide polymorphism (SNP) consisting site in exon 2, exon 3 and intron 2 were determined as a result of digestion with Kpn2I, HphI and Sau3AI restriction enzymes, respectively. The highest T allele frequency related with production traits for Kpn2I polymorphism was found for SAR cattle. For HphI polymorphism, T allele frequencies were detected clearly predominant. Within each breed for Sau3AI polymorphism B and C allele frequencies that effect production traits were found to be dramatically lower than A allele frequency. As a result we can suggest that there was no clearly difference that can create any advantage in terms of leptin gene SNPs among the three native Turkish cattle breeds.

**Keywords:** *Turkish cattle breeds, Leptin gene, Single nucleotide polymorphis*

# Türkiye'deki Yerli Sığır Irklarında Leptin Geni Polimorfizmleri

## Özet

Bu çalışmada Doğu Anadolu Kırmızısı (DAK), Güney Anadolu Kırmızısı (GAK) ve Boz ırk sığırlarda leptin geni polimorfizmlerinin belirlenmesi amaç edinilmiştir. Çalışmada birbiri ile yakınlığı olmayan 40 adet GAK, 40 adet DAK ve 40 adet Boz ırk sığır kullanılmıştır. Genomik DNA örneklerinin elde edilmesinin ardından leptin geni ekson 2, ekson 3 ve intron 2'deki hedef bölgeler polimeraz zincir reaksiyonu (PCR) ile çoğaltılmıştır. Ekson 2'deki tek nükleotid polimorfizmi (SNP) içeren bölge Kpn2I, ekson 3'te SNP içeren bölge HphI ve intron 2'de SNP içeren bölge Sau3AI restriksiyon enzimleri ile sindirilmesi sonucu belirlenmiştir. Kpn2I polimorfizmi için verim özellikleriyle ilişkili T allel frekansı en yüksek GAK ırkı sığırlarda bulunmuştur. Her üç sığır ırkında da HphI polimorfizmi için T allel frekansı oldukça yüksek bulunmuştur. Her ırk içinde Sau3AI polimorfizmi için verim özelliklerini etkileyen B ve C allel frekansları, A allel frekansından dikkat çekici şekilde düşük olduğu bulunmuştur. Sonuç olarak istatistiki açıdan üç sığır ırkı arasında leptin geni SNP'leri açısından avantaj doğurabilecek önemli bir farklılığın olmadığını ileri sürebiliriz.

**Anahtar sözcükler:** *Yerli sığır ırkları, Leptin geni, Tek nükleotid polimorfizmi*

## INTRODUCTION

Mammalian leptin is a 14-16 kDa protein produced by obese gene [1]. Leptin is proposed to be effective on ruminants in terms of feed intake, energy metabolism, reproduction and immune functions [2,3]. The leptin gene was identified in 1994 by positional cloning, and its mutation was found to underlie the phenotype of extreme obesity and infertility in *obese* (*ob/ob*) mice [1]. Linderson et al.[4] reported a Quantitative Trait Loci (QTL) for milk production traits on chromosome 4 in a region where the leptin gene and serum amylase-1 gene are

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located. The leptin gene itself is considered a potential QTL, influencing different production traits in cattle, for example meat quality [5-8], milk yield and content [9,10], reproduction [11] and feed intake [7,12,13]. Leptin gene consists of three exons, separated by two introns with coding regions in exon 2 and exon 3 [14].

Buchanan et al.[5] reported that in exon 2, position 305 there is a single nucleotide polymorphism (SNP) formed as a result of C/T substitution digestible with *Kpn2*I restriction enzyme and this substitution results in coding of arginine instead of cysteine. After that in the same exon at position 252, another SNP formed by A/T substitution, which can be digested by *Cla*I restriction enzyme, was determined by Lagonigro et al. [13]. This substitution causes tyrosine coding instead of phenylalanine. The existence of a C/T substitution that causes coding alanine instead of valine in exon 3, was indicated with *Hph*I restriction enzyme by Heageman et al.[15]. Lagonigro et al.[13], on the other hand, found a SNP in which valine instead of alanine was coded as a result of C/T substitution at position 130. The SNP digestible with *Sau3A*I restriction enzyme and located in intron 2 was reported for the first time by Pomp et al.[16].

The aim of the study was to determine the genotype and allele frequencies of leptin gene that affects many production traits in South Anatolian Red (SAR), East Anatolian Red (EAR) and Turkish Grey (TG) cattle.

## MATERIAL and METHODS

### *Animals*

In the study 40 SAR, 40 EAR and 40 Turkish Grey cattle were used. SAR cattle were supplied from southeastern of Turkey, EAR cattle from eastern of Turkey and the Turkish Grey cattle were supplied from Marmara Animal Breeding Research Institute, Bandırma. The blood samples were taken into 2 ml sterilized tubes with EDTA.

The study had an approval from the Ethical Committee of Faculty of Veterinary, Istanbul University. 2005/14, 12.01.2005.

### *DNA Extraction and RFLP Analyses*

The genomic DNA extraction from the whole blood samples was obtained using the standard salt-out method [17]. After that target sites were amplified with polymerase chain reaction (PCR). The PCR was carried out in a final volume of 25 µl containing 1 U Tsq DNA polymerase (Takara, Biotechnology Co, Ltd, Japan), 2-2.5 µl 10XPCR buffer (100 mM KCl, 20 mM Tris HCl (pH 8.0), 0.1 mM EDTA, 0.5 mM PMSF, 1 mM DTT, 50% glycerol), 2-2,5 mM $MgSO_4$, 50-100ng genomic DNA, 100 µM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol of each primer. The primer sequence used for the *LEPKpn2*I polymorphism: Forward 5´ATGCGCTGTGGACCCCTGTATC 3´ and Reverse 5´TGGTGTCATCCTGGACCTTCC 3´ (5). The thermal cycling program was as follows: 94°C for 2 min; 35 cycles of 94°C for 45 s, 59°C for 45 s, 72°C for 1 min. and a final extension at 72°C for 5 min. The Primer sequence used for the *LEPSauA*I polymorphism: Forward: 5´GTCACCAGGATCAATGACAT 3´and Reverse: 5´AGCCCAGGAATGAAGTCCAA 3´ [16]. The thermal cycling program was as follows: 94°C for 5 min; 40 cycles of 94°C for 1 s, 57°C for 30 s, 72°C for 1 min. and a final extension at 72°C for 10 min. In exon 3 amplification of the target site for *Hph*I polymorphism had been troublesome. As a result while using the forward primer proposed by Haegeman et al.[15] the reverse primer was redesigned. The primer sequence used for the *LEPHph*I polymorphism: Forward 5´GGGAAGGGCAGAAAGATAG3´´and Reverse 5´CCAAGCTCTCCAAGCTCTC 3´. The thermal cycling program was as follows: 94°C for 2 min; 35 cycles of 94°C for 30 s, 57°C for 1 min, 72°C for 30 s and a final extension at 72°C for 15 min. 2% agarose gel used for amplification of the target site for each sample was also checked. The 94 bp site amplified in exon 2 was digested for two and a half h in 55°C with 2 U *Kpn2*I (Fermantas Life Sciences, Canada) restriction enzyme. The 458 bp site amplified in exon 3 was digested for 16 h in 37°C with 5 U *Hph*I (Fermantas Life Sciences, Canada) restriction enzyme and the 1820 bp site amplified in intron 2 was digested for 16 h in 37°C with 5 U *Sau*AI (*Mbo*I, Takara, Biotechnology Co, Ltd, Japan) restriction enzyme. Restriction fragments were separated in 2% agorose gel in TEB buffer with ethidium bromide and visualized under ultraviolet light.

### *Statistical Analyses*

Direct counting was used to estimate genotype and allele frequencies of leptin gene variants. The chi-square tests ($\chi^2$) was used to check whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software [18].

## RESULTS

### *Detection of PCR-RFLP Polymorphisms*

As a result of digestion of 94bp site in leptin gene exon 2 with *Kpn2*I restriction enzyme CC (75 and 19 bp), CT (94, 75 and 19 bp) and TT (94 bp, uncut product) genotypes were determined. As a result of digestion of the 458 bp site in leptin gene Exon 3 with *Hph*I restriction enzyme 3 genotypes, namely CC (undigested, 458 bp), CT (458, 311,147 bp) and TT (311, 147bp) were determined.

Digestion of 1820 bp intron 2 site resulted in genotypes AA (730, 690 and 400 bp), AB (730, 690, 400, 310, 90 bp) and BB (730, 690,310 and 90 bp) and a rare AC polymorphism resulting from an additional restriction site (the 690 bp fragment was digested into 470 and 220 bp) genotypes were determined.

### *Distribution of Leptin Gene Genotypes and Allele Frequencies for Kpn2I, HphI ve Sau3AI polymorphisms*

Genotypes and allele frequency distributions of *Kpn2*I, *Sau3A*I and *Hph*I polymorphisms for SAR, EAR and Turkish Grey cattle are listed in *Table 1*. In terms of *Kpn2*I polymorphism the homozygote genotype frequencies expected for each of the three cattle breeds, are significantly higher than those observed ($P < 0.05$). The highest T allele frequency was found for Southern Anatolian Red cattle and the lowest was found for the Turkish Grey cattle. For all the three breeds no significant difference was detected between the expected and the observed genotype frequencies *Sau3A*I polymorphism. For all the three breeds B and C allele frequencies were determined to be much lower than the A allele frequency. Among all the three breeds the highest B allele frequency belongs to the Turkish grey cattle while the highest C allele frequency belongs to the SAR cattle. For the SAR and EAR breed cattle the expected heterozygote genotype frequency of *Hph*I polymorphism was significantly higher than observed ($P<0.05$); whereas, there was no significant difference between the expected and the observed genotype frequencies of the Turkish grey cattle. Among the three cattle breeds the T allele frequency was the highest in SAR cattle. For all the breeds T allele frequency was very dominant over C allele frequency.

## DISCUSSION

Leptin gene were identified in 1996 in cattle and many of studies conducted on leptin gene and its receptors revealed that leptin has an important effect on fertility and body condition [19]. In leptin gene five SNPs that affect many production traits in cattle were determined [5,13,16]. Buchanan et al.[5] reported that in cattle *Kpn2*I polymorphism in leptin gene Exon 2 is related with levels of carcass fat and feed intake of the cattle that have T genotype were higher than those of the C genotype cattle. In another study, Buchanan et al.[6], put forward that milk yield and somatic cell score of the cattle with T genotype were higher than those of the C genotype cattle but this polymorphism had no connection with the fat and protein of milk content. Nkrumah et al.[7] stated that serum leptin concentrations of the cattle of TT and CT genotypes were higher than those of the cattle that are CC genotype. Schenkel et al. [8] reported that the SNP located in exon 2 was effective on the size of body fat mass. The highest T allele frequency for the *Kpn2*I polymorphism was found as 57.50% in the SAR breed cattle in the study. Buchanan et al.[7] determined T allele frequencies for Holstein cattle as 46%, for Brown Swiss as 45%, for Angus as 42%, for Simmental as 68%, for Jersey as 53%, for Guernsey as 6% and for Canadienne cattle as 11%.

The *Hph*I polymorphism located in leptin gene exon 3 was first reported by Haegeman et al.[15]. Madeja et al.[10], reported T genotype cattle had higher milk yield with higher fat and protein content in Polish black and white cattle. They also suggested that the *Hph* I restriction site resulting in a change from alnine to valine is located at

**Table 1.** *Distribution of genotype and allele frequencies of LEPKpn2I, LEPSau3AI and LEPHphI polymorphisms in native Turkish cattle breeds*
**Tablo 1.** *Türkiye'deki yerli sığır ırklarında LEPKpn2I, LEPSauAI ve LEPHphI polimorfizmlerinin genotip ve allel frekansı dağılımları*

| Locus | Breed | n | Allel Frequency | | | Genotype Frequency | | | | χ2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 40 | T | C | | TT | CT | CC | | |
| LEP (Kpn2I) | SAR | 40 | 0.58 | 0.42 | | 0.25 | 0.65 | 0.10 | | 4.0318* |
| | EAR | 40 | 0.51 | 0.49 | | 0.18 | 0.67 | 0.15 | | 4.5757* |
| | TG | 40 | 0.45 | 0.55 | | 0.10 | 0.70 | 0.20 | | 6.4519* |
| | | 40 | A | B | C | AA | AB | BB | AC | |
| LEP (Sau3AI) | SAR | 40 | 0.72 | 0.19 | 0.09 | 0.48 | 0.32 | 0.03 | 0.17 | 2.8858Ns |
| | EAR | 40 | 0.71 | 0.26 | 0.03 | 0.45 | 0.48 | 0.02 | 0.05 | 3.0111Ns |
| | TG | 40 | 0.68 | 0.30 | 0.02 | 0.37 | 0.55 | 0.03 | 0.05 | 5.1366Ns |
| | | 40 | A | B | | AA | AB | BB | | |
| LEP (HphI) | SAR | 40 | 0.89 | 0.11 | | 0.83 | 0.12 | 0.05 | | 6.4886* |
| | EAR | 40 | 0.86 | 0.14 | | 0.78 | 0.17 | 0.05 | | 2.2066Ns |
| | TG | 40 | 0.89 | 0.11 | | 0.80 | 0.17 | 0.03 | | 0.8060Ns |

*χ2, test of Hardy-Weinberg equilibrium; SAR, South Anatolian Red cattle; EAR, East Anatolian Red cattle; TG, Turkish Grey cattle; * P< 0.05; Ns, not significant*

the conserved region of the β helix of the leptin protein. Because alanine and valine have similar nonpolar aliphatic R-groups, the substitution should not affect the protein structure or binding to its receptor. This polymorphism should not, therefore, directly influence production traits, but may be linked with other unknown milk production QTL in vicinity. Liefers et al.[12], on the other hand, reported that *Hph*I polymorphism had no certain effect on productivity properties. In this study, the SAR, EAR and Turkish Grey cattle the T allele frequency was determined to be much higher than the C allele frequency. The relation between *Hph*I polymorphism and production traits has not been clearly revealed yet.

The SNP located in intron 2 was first revealed by Pomp et al.[16]. Liefers et al.[12] proposed that the polymorphism in this site was related to milk yield and feed intake and reported that despite the fact that B genotype cattle had higher milk yield and feed intake compared to the other genotypes, a negative energy balance is not formed. Zwierzchowski et al.[20] reported that the milk protein content of the AC genotype cattle was higher than that of the other genotypes. Madeja et al.[10] reported that the AC genotype Polish black and white cattle milk had higher fat and protein content. In this study the B and C allele frequencies for the breeds were found to be much lower than the A allele frequency. The Turkish Grey cattle were found to have 67.50% A allele frequency which is the lowest. Pomp et al.[16] reported that A allele frequency was 70% in Limousin breed cattle, 79% in Simmental breed cattle, 71% in Holstein breed cattle, 73% in Angus breed cattle, 50% in Hereford breed cattle and 60% in Brangus breed cattle.

As conclusion, it can be suggested that T allele frequency for *Kpn2*I polymorphism and B and C allele frequencies for *Sau3A*I polymorphism, which are effective on production traits of the three breeds, were found nearly on the equal level with each other. We could not find any remarkable difference that can create an advantage to one of the three breeds in terms of SNPs of leptin gene. Besides, we can suggest that in the forthcoming studies, searching for different QTLs that affect production traits of SAR, EAR and Turkish Grey cattle could be useful for determining their genetic structure.

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