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Indel mutation of the ADD1/SREBP-1c gene in the South Anatolian Red and East Anatolian Red cattle breeds

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Abstract: The present study was designed to determine genotypes of adipocyte determination and differentiation-dependent factor 1 (ADD1)/sterol regulatory element-binding protein-1c (SREBP1c) in South Anatolian Red (SAR) and East Anatolian Red (EAR) cattle. Fifty cattle from the SAR breed and 43 cattle from the EAR breed were used. Intron 7 of the ADD1/SREBP1c gene containing an 84 bp indel mutation was amplified by polymerase chain reaction (PCR). The number of SS genotyped individuals with the 84 bp deletion associated with fatty acid composition and growth traits were very low in both breeds. The frequency of the LL genotype with the 84 bp insertion was significantly higher. The 2 cattle populations were in Hardy–Weinberg equilibrium. The SS genotype was observed in the SAR and EAR breeds. As a result, we can conclude that determination of ADD1/SREBP1c genotype distribution in a great number of cattle breeds of *Bos taurus* and *Bos indicus* would allow us to observe more SS genotypes.

Key words: Native Turkish cattle, PCR, indel mutation, ADD1/SREBP1c gene

1. Introduction

The amount and distribution of fat, for example monounsaturated fatty acids (oleic acid) and conjugated linoleic acid, in beef and dairy cattle are factors affecting human health (1,2).

Previous studies were conducted on manipulation of diet to regulate the fatty acid composition of milk and meat and to influence the nutritional quality of dairy and beef products (3,4).

In recent years, molecular techniques have become very important for identification of the genetic background of fat composition. Current methods also include the improvement of genetic status for modifying milk and meat fat to a favorable composition (5). Studies for optimizing fat composition are mainly focused on fatty acid profile (1,2,6–8).

Bovine milk consists of 70% saturated fatty acids (SFAs), 25% monounsaturated fatty acids (MUFAs), and 5% polyunsaturated fatty acids (PUFAs) (9). Bovine meat contains 80% SFAs and 20% various types of fatty acids like palmitic, stearic, and oleic acids (10).

Sterol regulatory element binding proteins (SREBPs) are transcription factors that regulate the energy balance by activating glycolysis, lipogenesis, and adipogenesis (11). These transcription factors have an important role in the control of 30 genes affecting the synthesis of fatty acids, triacylglycerol, and glycerophospholipids (12,13). SREBPs are members of the helix-loop-helix/leucine-zipper family of transcription factors (11,14).

In the case of sterol privation in the cell, SREBP transcription factors are released from endoplasmic reticulum to bind to sterol regulatory elements (SREs) or E-boxes in the promoter region of target genes (13,15,16).

SREBP transcription factors play an important role in the regulation of stearoyl-CoA desaturase (Scd-1) gene and fatty acid synthase (FASN) gene transcription (15,17). It has been reported that both the Scd-1 and FASN genes affect the fatty acid composition of milk, meat, and adipose tissue (1,18).

The SREBP family has 3 major isoforms, called SREBP-1a, SREBP-1c, and SREBP-2. In humans and mice, SREBP-1a and SREBP-1c isoforms are encoded by 2 different regions of 1 gene (12). SREBP-2 is encoded by a separate gene (19).

SREBP-1c is identified as adipocyte determination and differentiation-dependent factor 1 (ADD1) (20). The human ADD1/SREBP-1c gene and SREBP-2 are located on chromosome 17 (12) and chromosome 22 (21), respectively. The bovine ADD1/SREBP-1c gene is located on chromosome 19; it consists of 21 exons and codes for a polypeptide of 1183 amino acids (22).

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The bovine ADD1/SREBP-1c gene has an 84 bp indel mutation, which leads to several gene variants in cattle (22–24). There is a high homology between amino acid sequences of bovine and human SREBP-1: 82.9% in SREBP-1a and 71.9% in SREBP-1c (23).

The aim of the present study was to determine the variants of the ADD1/SREBP-1c gene associated with fatty acid composition among South Anatolian Red (SAR) and East Anatolian Red (EAR) cattle.

2. Materials and methods

DNA samples of 50 SAR and 43 EAR cattle were selected from DNA collections obtained during previous research projects. SAR breed cattle were selected from South Anatolia (Diyarbakır and Hatay), whereas EAR cattle were selected from East Anatolia (Kars). The animals were not relatives and had phenotypic characteristics of their breed.

The PCR for ADD1/SREBP1c was carried out in a final volume of 25 µL containing 1 U of Taq DNA polymerase (Fermantas Life Sciences, Canada), 2–2.5 µL of 10X PCR buffer (750 mM Tris-HCl (pH 8.0), 200 mM $(NH4)_2SO_4$, 0.1% Tween 20), 1.5 mM $MgCl_2$, 50–100 ng of genomic DNA, 100 µM dNTP (Takara, Biotechnology Co, Ltd, Japan), and 10 pmol of each primer. Primers used to amplify 778 bp product included the whole intron 7 and part of the exon 7 and exon 8 regions of the bovine ADD1/SREBP1c gene (GenBank accession number: NC_007317.3): F: 5'-CCA CAA CGC CAT CGA GAA ACG CTA C -3' and R: 5'- TTC TGG TTG CTG TGC TGA AGG AAG CGG -3' (22). Amplification conditions were 95 °C for 7 min, 35 cycles of 95 °C for 1 min, 58.5 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were loaded into 2% agarose gel with ethidium bromide. After electrophoresis at 110 V for 30 min the products were visualized with a UV transilluminator. The individuals were genotyped according to the length of the PCR products.

Direct counting was used to estimate genotype and allele frequencies of ADD1/SREBP1c variants. The chi-square test (χ^2) was used to check whether the populations were in Hardy–Weinberg equilibrium using PopGene32 software (25).

3. Results

The indel polymorphism in intron 7 of the ADD1/SREBP1c gene had 3 genotypes: LL (long type) with 84 bp insertion revealed 778 bp fragment, SS (small type) with 84 bp deletion revealed 694 bp fragment, and heterozygote LS genotype revealed both the 778 and 694 bp fragments (Figure).

The genotype and allele frequencies of the indel mutation in the bovine ADD1/SREBP1c gene of SAR and EAR breeds are given in the Table. The frequency of the L allele was much higher than the frequency of the S allele in both breeds. No significant differences were found between observed and expected genotypes. Both of the populations were in Hardy–Weinberg equilibrium.

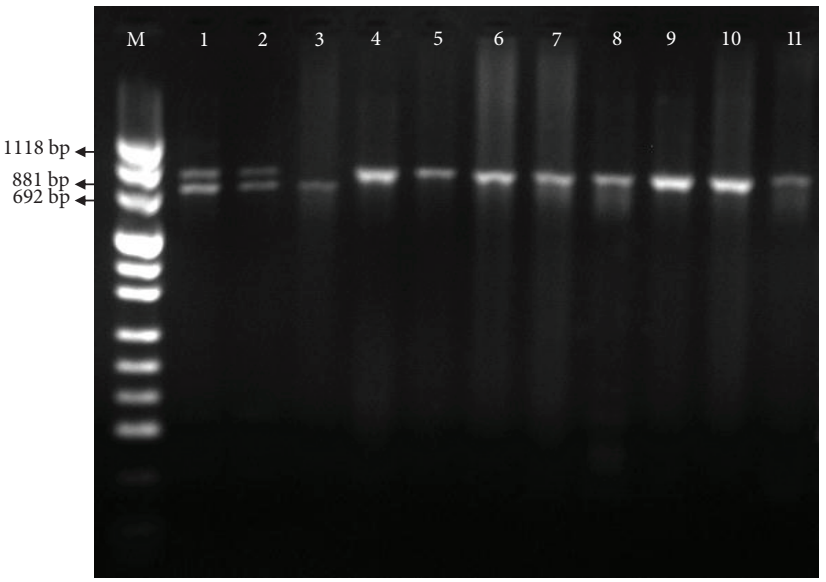

Figure. Indel mutation of bovine *ADD1/SREBP-1c.* M: Marker (pUC Mix marker, 8; Fermantas Life Sciences, Canada); Lane 1: LS genotype characterized by the presence of 778 and 694 bp fragments; Lane 2: LS; Lane 3: SS genotype characterized by the presence of 694 bp fragment; Lane 4: LL genotype characterized by the presence of 778 bp; Lane 5: LL; Lane 6: LL; Lane 7: LL; Lane 8: LL; Lane 9: LL; Lane 10: LL; Lane 11: LL.

Table. The distribution of *ADD1/SREBP-1c* genotypes and allele frequencies in South Anatolian Red and East Anatolian Red cattle.

		Genotypes						Allele frequencies (%)		
		LL		LS		SS		L	S	χ^2
Breed	No. of animals[1]	Ob[2]	Ex[3]	Ob	Ex	Ob	Ex			
SAR[4]	50	40	38.56	7	9.86	2	0.56	88.78	11.22	4.5073 Ns
EAR[5]	43	36	35.32	6	7.34	1	0.32	90.70	9.30	1.6228 Ns

χ^2: test of Hardy–Weinberg equilibrium, [1]Number of animals, [2]observed, [3]expected, [4]South Anatolian Red cattle, [5] East Anatolian Red cattle, Ns: not significant

4. Discussion

Epidemiologic, clinical, and animal studies show that high levels of dietary SFAs can increase serum cholesterol, atherosclerosis, and coronary heart disease in humans (26). MUFAs and PUFAs in the diet can lead to a decrease in serum total cholesterol and low-density lipoprotein (LDL) levels and therefore reduce the risk of coronary heart disease (27,28).

The ADD1/SREBP1c gene has a major role in the regulation of stearoyl-CoA desaturase (Scd-1) and fatty acid synthase (FASN) gene transcriptions. Both of the Scd-1 and FASN genes are associated with the fatty acid composition of cattle's milk and meat (1,18). Hoashi et al. (23) reported that Japanese Black cattle with the SS genotype had 1.3% more MUFAs in intramuscular fat. The melting point of intramuscular fat was found to be 1.6 °C lower in the SS genotype compared to the LL genotype. They suggested that SREBP-1 genotypes may be associated with the physiological character of lipid tissue. Bhiyon et al. (24) found that unsaturated fatty acid proportion is higher in Hanwoo cattle with the SS genotype than in those with the other 2 genotypes. The results of a study conducted on indigenous Chinese cattle breeds showed that the LS genotype can reveal higher birth weight, body weight, and average daily gain compared to the LL genotype (22).

In our study, the number of LL genotypes was found to be higher in SAR and EAR cattle. The number of heterozygous individuals was very low. Two individuals of the SAR breed and one individual of the EAR breed with the SS genotype were observed. Hoashi et al. (23) calculated the frequency of the SS genotype as 11% and the LS genotype as 72% in Japanese Black cattle. Huang et al. (22) did not observe the SS genotype in Chinese indigenous breeds and the frequencies of heterozygous genotypes varied between 0% and 26%. In the Hanwoo cattle breed, the frequencies of SS and LS genotypes were found to be 8% and 40%, respectively (24).

We may suggest that an increase in S allele frequency of the SREBP-1 gene would affect the fatty acid composition of meat and milk in SAR and EAR cattle. Huang et al. (22) explained the absence of the SS genotype in Chinese breeds as a result of the decreasing trend in artificial selection, migration, and genetic drift function or the natural selection of SS genotyped animals.

Hoashi et al. (23) suggested that the S allele can be breed-specific and therefore the variation in S allele frequency should be investigated in different cattle breeds. The SS genotype was observed only in Hanwoo and Limousine breeds and was absent in Angus, Simmental, Brahman, and Red Chittagong breeds. The researchers claimed that the SS genotype may not be segregating widely in different cattle populations (24).

In our study, we observed SS genotyped animals in low numbers. We think that further investigations of the ADD1/SREBP1c SS genotype in high trait European breeds (*Bos taurus*) and zebu breeds (*Bos indicus*) are needed to explain the low numbers of SS genotyped animals observed in the present study. The results of previous studies on mitochondrial, Y-chromosomal, and autosomal DNA showed that there has been a gene flow from zebu to taurine cattle breeds in the Near East and that this gene flow is observed only in the autosomal DNA of Anatolian breeds (29). In the recent QTL analyses on SAR and EAR breeds *Bos indicus* alleles and genotypes have been observed in these taurine breeds (30).

In conclusion, determination of the ADD1/SREBP1c genotype distribution in a great number of cattle breeds of *Bos taurus* and *Bos indicus* would allow us to observe more SS genotypes.

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