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Araştırma Makalesi ***Research Article***

Determination of *Stearoyl-Coenzyme A Desaturase 1 Gene* Variants in South Anatolian Red and East Anatolian Red Cattle

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East Anatolian Red, South Anatolian Red, SCD1gene, indigenous cattle breeds, sequence analysis

Abstract

Fat composition in ruminant's milk is one of the factors that can affect human health in positive or adverse ways. Optimizing ruminant feed to achieve ideal fatty acid composition in milk has been an ongoing area of research in recent years, without satisfactory results to date. It has been argued that in addition to changes in feed, genetic information can also be utilized to improve milk fatty acid composition. The aim of the study is to investigate the incidence of stearoyl-CoA-desaturase 1 (SCD) gene variants, which are claimed to affect fat content and quality of milk in Turkish native cattle breeds. Fifty South Anatolian Red (SAR) and 50 East Anatolian Red (EAR) cattle were used in the study. The 5th exon of SCD gene was amplified using polymerase chain reaction (PCR) and the PCR products were subjected to sequencing analysis. Among the samples sequenced polymorphism at three nucleotide positions have been observed on the 5th exon of the SCD gene, namely A702G, T762C and C878T. Of these three, the polymorphic position C878T was utilized to determine peptide variants of A (293Ala) or the V (293 Val) of individual samples. Frequency of A variant and AA genotype in SAR and EAR cattle breeds was 0.91 and 0.77 as well as 0.43 and 0.29, respectively. In particular the SAR exhibits a very low frequency of the V allele, believed to have been an ancestral allele. In both samples, 2 individuals were identified to have the VV genotype. The results suggested that high frequency of A allele and AA genotype which confers great advantage on milk composition and meat fatty acid composition was present in SAR and EAR cattle breeds

Özet

Güney Anadolu Kırmızısı ve Doğu Anadolu Kırmızısı Irkı Sığırlarda *Stearoil-Koenzim A Desatüraz 1* Geni Varyantlarının Belirlenmesi

Ruminantlarda sütteki yağ içeriği insan sağlığını pozitif veya negatif olarak etkileyen faktörlerdendir. Süt yağ asidi içeriğinin en ideal şekle kavuşturulması amacıyla ruminantların yem içeriğinin değiştirilmesi üzerine son yıllarda çalışmalar yapılmış olmasına karşın tatmin edici sonuçlar elde edilememiştir. Süt yağ asidi kompozisyonunun geliştirilmesinde yem değişikliklerinin yanı sıra genetik bilginin de kullanılmasının yaralı olabileceği ileri sürülmüştür. Çalışmamızda Güney Anadolu Kırmızısı (GAK) ve Doğu Anadolu Kırmızısı (DAK) ırkı sığırlarda süt yağ içeriğini ve kalitesini etkilediği ileri sürülen Stearoil-KoA-desatüraz 1 (SCD1) geni varyantlarının dağılımının belirlenmesi amaçlanmıştır. Bu amaçla çalışmada 50'şer baş GAK ve DAK ırkı sığır kullanılmıştır. Genomik DNA örneklerinde SCD1 geni polimeraz zincir reaksiyonu (PZR) ile çoğaltılmıştır. Daha sonra, PCR örnekleri dizin analizine tabi tutulmuştur. Dizin analizi yapılan örneklerde öncelikle SCD1 geni 5. ekzonda yer alan 3 adet tek nükleotid polimorfizmi (SNP), sırasıyla, A702G, T762C ve C878T, tespit edilmiştir. Bunlardan özellikle C878T SNP sonucuna bakılarak bireyin A (293Ala) ve V (293 Val) protein varyantından hangisi olduğu belirlenmiştir. Çalışmamızda hem GAK hem de DAK ırkı sığırlarda A varyantı ve AA genotipi sırasıyla 0,91, 0,71 ve 0,43, 0,29 olarak belirlenmiştir. Özellikle GAK ırkı sığırlarda atasal bir allel olduğu ileri sürülen V allel frekansı oldukça düşüktür. Her iki sığır ırkında da VV genotipe sahip 2 adet birey tespit edilmiştir. GAK ve DAK ırkı sığırlara yüksek düzeyde AA genotipine ve A alleline sahip olmaları süt ve et yağ içeriği açısından büyük bir avantaj sağlamaktadır.

Introduction

The fatty acid composition of ruminant's milk is one of the important factors for human health (Kgwatalala et al., 2009; Mele et al., 2007). Average bovine milk contains 70% saturated (SFA), 25% monounsaturated (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). The intake of high level SFA in milk increases plasma cholesterol concentrations and, this

can lead to an increase of the risk of atherosclerosis, cancer, obesity and coronary heart disease in humans (Kromhout et al., 2002). Stearoyl-CoA desaturase (SCD) (EC 1.14.99.5) is an important enzyme affecting milk fatty acid composition. It is located in endoplasmic reticulum and catalyses the conversion of saturated fatty acids into unsaturated fatty acids (Corl et al., 2001). The *SCD1* is associated with leptin-signaling pathway and it is a primary candidate gene to change the proportion of saturated to unsaturated fatty acids in milk (Islas-Trejo et al., 2002). Bovine *SCD1* is localized on *Bos taurus* autosomal chromosome (BTA) 26 and contains 17,088 bp. Its open reading frame includes 1,080 nucleotides and codes for 359 amino acids (Medrano et al., 1999). Eight single nucleotide polymorphisms (SNPs) have been identified at this gene. Three of them were found in exon 5 and 5 SNPs were found in 3'untranslated region of *SCD1*. Three SNPs (A702G, T762C and C878T) are in linkage disequilibrium and only the C878T SNP has two protein variants, A (293Ala) and V (293Val). The amino acid substitution changes the catalytic activities of SCD enzyme (Taniguchi et al., 2004). Previous studies estimated that significant association between Ala293Val *SCD1* genotype and the fatty acid composition of intramuscular fat and milk fat. They reported that genotyping of this region would be a useful tool for selection of favourable beef carcasses and milk (Kgwatalala et al., 2009; Mele et al., 2007; Moioli et al., 2007; Schennik et al., 2008; Taniguchi et al., 2004).

The objective of this study was to determine the variants of the Ala293Val polymorphism of *SCD1* which is claimed to be a candidate gene for affecting fatty acid composition, in South Anatolian Red (SAR) and East Anatolian Red (EAR) cattle.

Materials and Methods

Sample Collection and DNA Extraction

In the study, 50 SAR and 50 EAR cattle were used. SAR cattle were supplied from the south eastern region of Turkey, EAR cattle came from the eastern region of Turkey. The blood samples were taken in 2 ml sterilized tubes with EDTA. The genomic DNA was extracted from whole blood samples by using the standard salt-out method (Miller et al., 1985).

PCR Amplification and Sequencing

The PCR for *SCD1* polymorphisms was carried out in a final volume of 25µl containing 1 U Taq DNA polymerase (Fermantas Life Sciences, Canada), 2-2.5 µl 10XPCR buffer (750 mM Tris-HCl (pH 8.0), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 1.5mM $MgCl_2$, 50-100 ng genomic DNA, 100 µM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol each primer. The primer sequences used for the *SCD1* polymorphism: Forward 5´-CAGTCCTTGCTCCACCACTT-3´ and Reverse 5´-AGCATTTGTGGCTTGCTCTT-3´ (GenBank no. AY241932.1). The thermal cycling program was as follows: 94°C for 5 min.; 35 cycles of 94°C for 1 min., 56°C for 1 min., 72°C for 1 min. and a final extension at 72°C for 5 min. 725 bp PCR products were run through 2% agarose gel to check the results of amplification. Sequencing was performed by using an ABI-3100 sequencer (PE Biosystems, Germany) and the BigDyeTM terminator cycle sequencing kit, after the purification of the PCR products. Forward primer was used to sequence the PCR products.

Genotyping and Statistical Analysis

Nucleotide sequences of 725 bp region in *SCD1* of 100 animals were aligned for detection of A702G, T762C and C878T SNPs by using the MEGA 4 software program (http://www.megasoftware.net/m_con_select.html) (Kumar et al., 2004). The *SCD1* variants were determined by the valine and alanine substitution on the 293rd residue (C878T). Direct counting was used to estimate genotype and allele frequencies of Ala293Val polymorphism of *SCD1*. The chi-square test (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software (Yeh et al., 2000). Haplotypes were determined by the nucleotides in the A702G, T762C and C878T SNPs. Direct counting was used to estimate haplotype frequencies.

Results

The genotype and allele frequencies of Ala293Val polymorphism of *SCD1* in SAR and EAR breeds are given in Table 1. The frequency of A allele was found to be extremely higher than the frequency of V allele in SAR and EAR cattle breeds. AA genotype is the most observed genotype in both cattle breeds. For SAR cattle breed, the expected heterozygote (AV) genotype frequency of Ala293Val polymorphism was found to be significantly higher than observed frequency ($P<0.05$). SAR cattle population was not found in Hardy-Weinberg equilibrium; whereas, there was no significant difference between the expected and the observed genotype frequencies in EAR cattle. EAR cattle were found in Hardy-Weinberg equilibrium.

Haplotype frequencies are presented in Figure 1. Three different haplotypes (GTC, GCT and ATC) of *SCD1* were found in both cattle breeds. The most frequent haplotypes were GTC in SAR and ATC in EAR cattle breeds.

Discussion

Mele et al. (2007) suggested that AA genotyped Italian Holstein cattle have higher levels of *cis*-9 C18:1, total monounsaturated fatty acids and C14:1/C14 ratio in milk than VV genotyped cattle have. But, they could

not find any significant relationship with *SCD1* genotype and *cis*-9, *trans*-11C18:2 content of milk. Schennink et al. (2008) found that V allele of *SCD1* is associated with lower C10, C12 and C14 indices and with higher C16, C18 and CLA indices in Dutch Holstein Friesian cattle. Kgwatalala et al. (2009) reported that A allele of *SCD1* is related to higher C10, C12 and C14 indices and V allele is found to be associated with higher C10:1, C12:1 and C14:1 indices. They also suggested that A allele is also positively associated with increased 305-d milk and protein yields. Despite these results, Mocciotta et al. (2008) reported that VV genotyped cows produce more milk and protein compared to AA genotyped cows. The results of these studies suggested that Ala293Val polymorphism of *SCD1* is related to milk fat and protein content but, the effect of *SCD1*variants on milk production traits is controversial. Taniguchi et al. (2004) observed a correlation between the *SCD1*gene variant A and the high MUFA percentage and the low melting point of intramuscular fat in Japanese Black cattle. These results suggest that Ala293Val polymorphism of *SCD1* could be used as a marker for the improvement of fatty acid composition in milk and beef products of cattle.

Table 1. Distribution of Ala293Val polymorphism of *SCD1* genotypes and allele frequencies in South Anatolian Red and East Anatolian Red cattle.
Tablo 1. Güney Anadolu ve Doğu Anadolu Kırmızısı sığır ırklarında SCD1 geni Ala293Val polimorfizmi genotip ve allel dağılımı.

Breed	n[1]	Genotypes						Allele Frequencies (%)		
		AA		AV		VV				
		Ob[2]	Ex[3]	Ob	Ex	Ob	Ex	A	V	χ^2
SAR[4]	50	43	41,36	5	8,27	2	0,36	0,91	0,09	8,7230*
EAR[5]	50	29	29,55	19	17,88	2	2,558	0,77	0,23	0,2002

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,
*: P<0.05

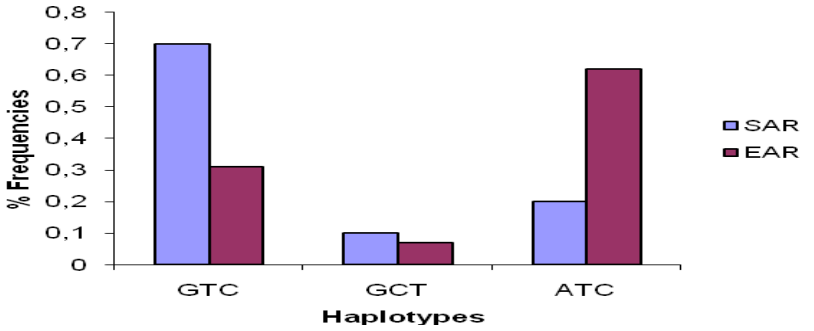

Figure 1. Haplotype frequencies of *SCD1* gene in South Anatolian Red and East Anatolian Red cattle.
Şekil 1. Güney Anadolu ve Doğu Anadolu Kırmızısı sığır ırklarında SCD1 geni haplotip frekansları.

Frequencies of the A allele and AA genotype were found to be higher in both Turkish cattle breeds. These frequencies are higher in SAR cattle breed. Only two individuals with VV genotype were noted in each cattle breed. Although, the effect of *SCD1* variants on fatty acid composition of milk and carcass is controversial, most of the studies positively emphasize the effect of A allele and AA genotype on fatty acid composition (Kgwatalala et al., 2009; Mele et al., 2007; Schennink et al., 2008). The high frequencies of A allele and AA genotype in SAR and EAR breeds may be an advantage for fatty acid composition. In SAR cattle breed, this might change in a negative way in the future, because the expected frequency of AA homozygote genotype was found to be statistically lower than the observed frequency (P<0.05). V allele frequencies were observed between 0.27-0.58 in different cattle breeds, which have been analysed until today (Kgwatalala et al., 2009; Macciota et al., 2008; Matsuhashi et al., 2011; Mele et al., 2007; Ohsaki et al., 2009; Schennink et al., 2008; Taniguchi et al., 2004). In this study, we found the frequency of V allele as 0.09 in SAR and 0.23 in EAR. Previous studies on mitochondrial DNA, Y chromosome and autosomal genes indicate an autosomal gene flow

from zebu cattle to Near Eastern cattle breeds (Akis and Oztabak, 2013; Edwards et al., 2007). The reason of the lower frequency of V allele might originate from this gene flow.

Milanesi et al. (2008) observed five haplotypes (ATC, GTC, ACT, GTC and ACC) in 11 different Italian cattle breeds. The most frequent haplotypes were ATC and GTC in all of the breeds. They also reported that the GTC haplotype is only present in the indigenous beef breeds (Chianina, Marchigiana, Romagnola, Podolica and Piedmontese). Moreover, only two haplotypes (ATC and GCT) were found in Japanese Black and Italian Holstein cattle breeds (Taniguchi et al., 2004). In the present study, only GTC, GCT and ATC haplotypes were found in both Turkish cattle breeds. Haplotype varieties are very limited in SAR and EAR cattle breeds according to Italian cattle breeds. The frequencies of GTC haplotype in SAR and ATC haplotype in EAR were found to be higher than the frequencies of other haplotypes. This finding leads us to suggest that *SCD1*gene haplotypes can vary widely between breeds and further studies should be conducted on linkage analysis between the haplotype and production traits.

Conclusion

The frequencies of the A allele and AA genotype that influence production traits were found to be higher in both Turkish cattle breeds. This may be advantageous for improving milk production traits. The haplotypes with the highest frequencies differ in SAR and EAR breeds. We recommend that further studies should be conducted on genotypes and haplotypes of Ala293Val polymorphism of *SCD1*gene in native Turkish cattle breeds using linkage analysis.

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