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FASN Gene Polymorphism in Indigenous Cattle Breeds of Turkey*

Kemal OZTABAK, Feraye Esen GURSEL, Iraz AKIS, Atila ATES, Hasret YARDIBI, and Gulhan TURKAY

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The aim of this study was to determine the variants of the thioesterase (TE) β-ketoacyl reductase (KR) domains of the Fatty Acid Synthase (*FASN*) gene, in the East Anatolian Red (EAR) and South Anatolian Red (SAR) cattle breeds. It has been suggested that the *FASN* gene is effective on fatty acid composition of meat in cattle. In this study, the genotype and allele frequencies of g.17924 A>G, g.18440 G>A and g.16024 G>A, g.16039 T>C in TE and KR domains, respectively, were detected by using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) method. The g.18663 T>C polymorphism of the TE domain was determined by direct sequencing. The GG genotype of the g.17924 A>G polymorphism, which affects unsaturated fatty acid composition positively, has a high frequency in EAR and SAR breeds. The frequencies of the two haplotypes g.16024 G>A and g.16039 T>C in the KR domain were found to be significantly high in both breeds. These haplotypes also have positive effects on unsaturated fatty acid composition. The AA genotype of the g. 18440 G>A polymorphism, which is suggested to be absent in *Bos taurus* breeds, was detected in SAR and EAR breeds with frequencies close to those in *Bos indicus* breeds. In conclusion, we suggest that SAR and EAR cattle breeds have an advantage in terms of genotype and haplotype distribution of the polymorphisms in TE and KR domains of the *FASN* gene. Additionally g.18440 G>A polymorphism might be a potential marker for breed discrimination.

Key words: Indigenous Turkish cattle, *FASN*, SNP, TE domain, KR domain.

Kemal OZTABAK, Feraye Esen GURSEL, Iraz AKIS, Atila ATES, Hasret YARDIBI, Gulhan TURKAY, Istanbul University, Faculty of Veterinary Medicine, Department of Biochemistry, Avcilar, 34310 Istanbul, Turkey.
E-mail: hasret@istanbul.edu.tr

The fatty acid composition of milk and meat is an important factor affecting human health (MELE *et al.* 2007; KGWATALALA *et al.* 2009). According to the results of studies on human metabolism, it has been suggested that high levels of dietary saturated fatty acids (SFA) cause an increase in blood cholesterol level and tendency to atherosclerosis and cardiovascular disease (KROMHOUT *et al.* 2002). On the other hand, high levels of dietary monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decrease the risk of cardiovascular disease by decreasing total serum cholesterol and low density lipoprotein (LDL) levels (KRIS-ETHERTON *et al.* 1999; MENSINK *et al.* 2003). Previous studies were focused on the regulation of the fatty acid composition of ruminant diets in order to produce more nutritious and qualified milk and meat products (CHILLIARD *et al.* 2003; JENKINS & MCGUIRE 2006). In recent years researchers have tried to devise new genetic approaches to manipulate the fatty acid composition of livestock products (SOYEURT *et al.* 2006).

Fatty acid synthase (FASN) is a multifunctional enzyme complex that regulates de novo biosynthesis of long chain fatty acids. It has an important role in embryogenesis, whereas it is not essential for fatty acid synthesis in adulthood (CHIRALA *et al.* 2003). This cytosolic enzyme catalyses palmitic acid synthesis from asetyl coenzyme A and malonyl coenzyme A in the presence of NADPH (WAKIL *et al.* 1983). *FASN* is an important candidate gene affecting fat composition of milk and meat because of its role in de novo lipogenesis in mammals. The *FASN* gene has been identified in rat, hu-

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man, goose, chicken and cattle (AMY *et al.* 1992; JAYAKUMAR *et al.* 1995; KAMEDA & GOODRIDGE 1991; CHIRALA *et al.* 1997; ROY *et al.* 2006). The bovine *FASN* gene is 19770 bp long and consists of 42 exons and 41 introns (GenBank Acc. No: AF285607). The thioesterase (TE) domain within the FASN complex regulates the termination of fatty acid synthesis and rGWATALALAase of newly synthesized fatty acids. C14 acyl-ACP and C16 acyl-ACP are both substrates of the FASN TE domain. The hydrolysis rate of C14 acyl-ACP by FASN TE is slower than that of C16 acyl-ACP (LIN & SMITH 1978; PAZIRANDEH *et al.* 1989). The TE domain has an important role in determining the product chain length of FASN. Therefore, the TE domain of the *FASN* gene can be a candidate gene for variance in fatty acid composition and might help to produce healthier livestock products regarding fatty acid composition (ZHANG *et al*. 2008). The TE domain is the part between exons 39 to 41 of the *FASN* gene (ABE *et al*. 2009). Two single nucleotide polymorphisms (SNPs) affecting fatty acid composition, g.18663 (g – genomic sequence) T>C and g.17924 A>G, were identified in the TE domain (ZHANG *et al*. 2008; BHUIYAN *et al.* 2009). Furthermore a *Bos indicus* associated SNP, g.18440 G>A is also reported in the same region (BHUIYAN *et al*. 2009). Two SNPs existing in the *FASN* gene have been reported. These are g.16024A>G and g.16039T>C polymorphisms, which lead to amino acid changes (ROY *et al.* 2006).

KR and TE domains are very close to each other (MAIER *et al.* 2006). An amino acid substitution in the KR domain can affect the function of the TE domain by changing substrate binding to this region (ABE *et al*. 2009). It has been suggested that determination of the genotypes of g.16024A>G and g.16039T>C polymorphisms might be helpful in identifying the fatty acid composition of cattle products (ABE *et al*. 2009).

In this study, the variants of TE and KR domains of the *FASN* gene in East Anatolian Red (EAR) and South Anatolian Red (SAR) cattle breeds were determined. The aim of this study was to evaluate the potential of the *FASN* gene as a genetic marker, in order to produce healthier livestock products regarding fatty acid composition.

Material and Methods

DNA samples

In this study, DNA samples from SAR and EAR cattle were used. DNA samples were selected from a collection comprised of samples obtained by the standard ammonium acetate salt-out method (MILLER *et al*. 1988) from SAR and EAR cattle. SAR and EAR cattle were selected from small herds in different villages of South Anatolian Region and East Anatolian Region in Turkey, respectively. The animals were unrelated and had the phenotypic characteristics of their breed.

Genotyping

The primers and annealing temperatures used to amplify the regions including g.18663 T>C, g.17924 A>G and g.18440 G>A polymorphisms in the TE domain and g.16024 A>G and g.16039 T>C polymorphisms in the KR domain are shown in Table 1. The same PCR conditions were used for all the regions; initial denaturation at 94°C for 5 min, 1 min at 94°C, 1 min at the annealing temperature, 1 min at 72°C for 35 cycles and a final extension at 72°C for 10 min. Amplification was

Table 1

Oligonucleotide primers used in this study

SNP	Annealing Temperature	Sequence
TE domain		
g.18663 T>C	57°C	F: 5′-CGCTCACTGTCCTGTCCTAC-3′ R: 5′-GCTGTGAATAATACTAAGGATGGA-3′
g.17924 A>G	55.5°C	F: 5′-TCTTCACAGAGCTGACGGAC-3′ R: 5′-GGAGGAAGAGCTGTTGCAGT-3′
g.18440 G>A	62°C	F: 5′-TGCCACACGCGCCTCCAGA-3′ R: 5′-AGAGCCTGGCCACCTACTACATC-3′
KR domain		
g.16024 A>G and g.16039 T>C	57°C	F: 5′-CTACCAAGCCAGGCAGGTC-3′ R: 5′-GCCATTGTACTTGGGCTTGT-3′

performed in a volume of 25 μl containing 5 μl 10X PCR buffer, 100 mM dNTP, 10 pmol of each primer, 1.0 mM $MgCl_2$, 2U Taq polymerase and 50-100 ng genomic DNA.

The g.18663 T>C polymorphism in the TE domain was determined by direct sequencing of the amplified 373 bp fragment. The 624 bp fragment encompassing the g.17924 A>G polymorphism and 505 bp fragment containing the g.18440 G>A polymorphism, also in the TE domain, were digested with *Msc*I and *Hpy188*III, respectively. The amplified 335 bp fragment was digested with *Hha*I and *Nci*I enzymes to detect g.16024 A>G and g.16039 T>C polymorphisms in the KR domain, respectively. The restriction enzyme digestions were performed using 10 μl of PCR product mixed with 10 U of the enzyme and incubated at 37°C overnight.

Statistical analysis

Genotype and allele frequencies of g.18663 T>C, g.17924 A>G, g.18440 G>A polymorphisms in the TE domain and g.16024 A>G, g.16039 T>C polymorphisms in the KR domain were calculated by using the PopGene 32 software program (YEH *et al.* 2000). A chi-square test was also performed to check Hardy-Weinberg equilibrium at each locus by the same program. Results of the sequence analysis of the region including g.18663 T>C polymorphism in the TE domain were evaluated by using MEGA 4 (http://www.megasoftware.net/mega4/mega.html) (TAMURA *et al.* 2007). Haplotype frequencies of g.16024 A>G and g.16039 T>C polymorphisms were estimated using the Estimation Haplotype (EH) program (Jurg Ott, Rockefeller University).

Results

Genotypes of the polymorphisms in the TE domain

The direct sequencing results revealed two alleles, T and C, for the nucleotide polymorphism at position 18663 in the TE domain. The genotypes of g.17924 A>G polymorphism were determined by the PCR-RFLP method using the *Msc*I restriction enzyme, with the A allele representing 262, 195 and 167 bp fragments and the G allele representing 362 and 262 bp fragments of the 624 bp amplicon. The SNP g.18440 G>A was genotyped by using the *Hpy118*III restriction enzyme. Digestion of the 505 bp PCR product revealed two alleles. G allele was characterized by bands of 222, 98, 85, 83 and 17 bp. The A allele was characterized by bands of 175, 98, 85, 83, 47 and 17 bp.

Genotypes of the polymorphisms in the KR domain

The 336 bp PCR product was digested by *Hha*I and *Nci*I restriction enzymes in order to detect g.16024 A>G and g.16039 T>C polymorphisms, respectively. The A allele of g.16024 A>G represented an uncut band of 336 bp and the G allele represented two bands of 262 and 75 bp. PCR-RFLP detection of the g.16039 T>C polymorphism revealed two alleles. An uncut band of 336 bp indicated the T allele and two bands of 247 and 88 bp indicated the C allele.

Genotype and allele frequencies of the TE and KR domains of the *FASN* gene

The genotype and allele frequencies of the g.18663 T>C, g.17924 A>G, g.18440 G>A polymorphisms and g.16024 A>G, g.16039 T>C polymorphisms in TE and KR domains, respectively, are shown in Table 2.

The C allele frequency of the g.18663 T>C was found to be fairly high in SAR and EAR cattle, whereas the TT genotype had a very low frequency in both of the breeds. The frequency of the G allele was observed to be higher than that of the A allele and the frequency of the AA genotype was low in both Anatolian cattle breeds. Despite the higher G allele frequency of the SNP g.18440 and higher heterozygous AG genotype frequency in SAR cattle, the frequencies of A allele and AA genotype were higher in EAR cattle. The G allele of g.16024 A>G polymorphism in the KR domain had a higher frequency in both SAR and EAR cattle breeds. For the g.16039 T>C polymorphism in the KR domain, allele and genotype distribution characteristics were different in the two cattle breeds. While the T allele and TT genotype had higher frequencies in SAR cattle, frequencies of the C allele and CC genotype were observed to be higher in EAR cattle.

The genotype frequencies of the three polymorphisms in the TE domain were in Hardy-Weinberg equilibrium. The genotype frequencies of g.16024 A>G and g.16039 T>C polymorphisms were found to be in Hardy-Weinberg disequilibrium.

Haplotype frequencies in the KR domain

The g.16024 A>G and g.16039 T>C polymorphisms in the KR domain were nonsynonymous mutations which determine the nonconservative mutation of threonine (T) to alanine (A) and tryptophan (W) to arginine (R), respectively. Since these two SNPs were located close to each other and there was no evidence of a recombination event between them, alleles of these two mutations were signified by a haplotype, represented by the

Table 2

Allele and genotype frequencies of *FASN* gene g.18663 T>C, g.17924 A>G, g.16024 G>A, g.16039 T>C and g.18440 G>A polymorphisms in SAR and EAR cattle breeds

Polymorphism	Breed	n[1]	Allele frequency (%)		Genotype frequency (%)			
TE domain								
			C	T	CC	CT	TT	$(\chi^2)^2$
g.18663 T>C	SAR[3] EAR[4]	48 49	0.72 0.87	0.28 0.13	0.52 0.75	0.39 0.22	0.09 0.03	0.0480 Ns 0.0622 Ns
			A	G	AA	AG	GG	
g.17924 A>G	SAR EAR	49 42	0.32 0.19	0.68 0.81	0.06 0.06	0.51 0.25	0.43 0.69	1.4135 Ns 1.2751 Ns
			A	G	AA	AG	GG	
g.18440 G>A	SAR EAR	47 47	0.49 0.68	0.51 0.32	0.17 0.53	0.64 0.30	0.19 0.17	3.3365Ns 5.0083 Ns
KR domain								
			A	G	AA	AG	GG	
g.16024 G>A	SAR EAR	47 42	0.38 0.34	0.62 0.66	0.21 0.22	0.34 0.25	0.45 0.53	3.9754* 6.8736**
			C	T	CC	CT	TT	
g.16039 T>C	SAR EAR	47 42	0.36 0.58	0.64 0.42	0.24 0.50	0.22 0.17	0.54 0.33	13.3735 *** 11.0791***

[1]number of animals, [2]Hardy-Weinberg equilibrium; [3]South Anatolian Red, [4]East Anatolian Red
Ns: not significant, * P<0.05, **P<0.01, ***P<0.001.

amino acid codes of ABE *et al*. (2009). The observed haplotypes and their estimated frequencies are given in Table 3. There were six observed diplotypes in SAR and eight observed diplotypes in EAR cattle.

Discussion

The regulation of the fatty acid composition of meat is of great importance for human health (MELE *et al*. 2007). Livestock products might cause negative effects on human health, mainly cardiovascular diseases due to heir high SFA content (KROMHOUT *et al.* 2002). In recent years, genetic studies have focused on the manipulation of unsaturated fatty acid composition of livestock products which have healthier effects on human metabolism (TANIGUCHI *et al.* 2004; MELE *et al.* 2007; MOIOLI *et al.* 2007; SCHENNINK *et al.* 2008; KGWATALALA *et al.* 2009). The *FASN* gene is one of the most important genes affecting fatty acid composition of meat in animals (ZHANG *et al.* 2008; BHUIYAN *et al*. 2009).

ZHANG *et al*. (2008) studied the association between g.17924 A>G and g.18663 T>A polymorphisms in the *FASN* gene TE domain and beef fatty acid composition in Angus cattle. According to their results, g.17924 GG genotyped cattle had lower myristic acid (C14:0; P<0.0001), palmitic acid (C16:0; P<0.05), total SFA contents and higher oleic acid (C18:1; P<0.001) and total MUFA contents. The researchers suggested that the amino acid substitution in the TE domain as a consequence of the SNP g.17924 A>G may affect the structure of the substrate binding site and therefore change the activity of TE towards C14-acyl ACP. The substitution from threonine to alanine due to this polymorphism may result in less C14:0 content and higher C:16:0 to C14:0 ratio in animals with the 17924 GG genotype (alanine) than in animals with the 17924 AA genotype (threonine) (ZHANG *et al.* 2008).

The g.18663 T>C polymorphism is a silent mutation. The association between this polymorphism and fatty acid content may be a result of the strong linkage disequilibrium between g.18663 T>C and

Table 3

Genotype and haplotype frequencies of the g.16024 GA and g.16039 TC polymorphisms in SAR and EAR cattle breeds

Breed	n[1]	Genotype	Frequencies	Haplotype	Frequencies			
					Calculated[2]	Estimated[3]	Global χ^2	P-value
SAR[4]	47	TW/TW	0.26	TW	0.297784	0.512673	54.393137	<0.000001
		TW/AR	0.26	TR	0.297784	0.013643		
		AW/AW	0.19	AW	0.268006	0.053116		
		AR/AR	0.21	AR	0.205679	0.420568		
		TW/AW	0.06					
		TR/AR	0.02					
EAR[5]	42	TW/TW	0.17	TW	0.183673	0.304709	9.162996	<0.000001
		TW/AR	0.04	TR	0.244898	0.123863		
		AW/AW	0.13	AW	0.244898	0.123863		
		AR/AR	0.37	AR	0.326531	0.447566		
		TW/AW	0.04					
		TR/AR	0.13					
		TR/TW	0.08					
		TR/AW	0.08					

[1]number of animals
[2]calculated from allele frequencies under the assumption of no association
[3]estimated from the data, allowing for association, assuming Hardy-Weinberg equilibrium
[4] South Anatolian Red cattle
[5] East Anatolian Red cattle

g.17924 A>G. (ZHANG *et al.*) BHUIYAN *et al.* (2009) reported a significant association of the GG genotype of g.17924 A>G with C16:0 and C18:1 fatty acid content. GG genotype frequency was calculated as 13% in Angus cattle (ZHANG *et al.* 2008) and 73% in Hanwoo cattle (BHUIYAN *et al.* 2009). In this study, the GG genotype had the highest frequency in the EAR breed and also a high frequency in the SAR breed. These results show that these cattle breeds may have an advantage in terms of fatty acid content.

The g.18440 G>A polymorphism in the TE domain leads to a replacement of glutamic acid to lysine. It has been suggested that the A allele of g.18440 G>A is a *Bos indicus* associated allele. The frequency of the A allele was found to be 0.93 and 0.84 in Brahman and Red Chittagong cattle breeds, respectively. This allele was absent in taurine breeds such as Hanwoo, Angus, Hereford, Simmental and Shorthorn (BHUIYAN *et al.* 2009). This polymorphism may have a role in the difference of fatty acid content in taurine and indicine cattle and may be a marker for breed discrimination. In this study, the frequency of the A allele was 0.49 and 0.68 in SAR and EAR cattle, respectively. Previous studies on mitochondrial DNA, Y chromosome and autosomal genes point to autosomal gene flow from zebu cattle to Near Eastern cattle breeds (EDWARDS *et al.* 2007). The relatively higher frequencies of the A allele in SAR and EAR cattle might have come from *Bos indicus*. This is consistent with the results of previously conducted studies on *PRL* (OZTABAK *et al.* 2008), *GHRH-R* (EKEN *et al.* 2011), *IGF-1* (AKIS *et al.* 2010) and *DGAT1* (KAUPE *et al.* 2004) genes in SAR and EAR cattle breeds.

ABE *et al.* (2009) determined the haplotype distribution of the g.16024 G>A and g.16039 T>C polymorphisms in a F2 population of Japanese Black and Limousine cattle. TW haplotyped animals had higher C18:0 and C18:1 fatty acid content and MUFA/SFA ratio and lower C14:0, C14:1, C16:0 and C16:1 fatty acid content in both intermuscular and intramuscular fat. The researchers also determined frequencies of the TW haplotype in different cattle breeds. The highest

frequency was observed in the Japanese Black cattle breed with 0.67. Frequencies of 0.7, 0.02 and 0.07 were found in Holstein, Angus and Hereford breeds, respectively. In this study the highest frequencies in SAR cattle breed belonged to the TW haplotype with 0.42 and AR haplotype with 0.34. In the EAR cattle breed, AR and TW haplotypes had frequencies of 0.42 and 0.25, respectively. SAR cattle might be more advantageous in terms of the effects of this haplotype.

ABE *et al.* (2009) detected only two haplotypes, namely TW and AR. Therefore, the researchers suggested that there is no evidence of a recombination event between g.16024 G>A and g.16039 T>C polymorphisms. Contrary to these results, four haplotypes were observed in this study. A haplotype different from TW and AR was also found in a previous study. The TR haplotype can be observed in the bovine *FASN* sequence data (AF285607) (ROY *et al.* 2001). These results might be evidence for a recombination event between two mutations in some cattle populations. The Hardy-Weinberg disequilibrium of both of the g.16024 G>A and g.16039 T>C polymorphisms in KR domain might cause a change in the distribution of the TW haplotype in the future. The effect of the amino acid changes in the KR domain on the function of the TE domain might have an important role in fatty acid production (ABE *et al.* 2009). The deviation from Hardy-Weinberg equilibrium is highly significant for g.16039 T>C locus, which can be explained by heterozygote deficit. Decreasing population sizes and inbreeding in SAR and EAR cattle breeds may have caused this situation in this locus.

In conclusion, we suggest that both SAR and EAR cattle breeds have important advantages regarding the genotype and haplotype distributions of the g.18663 T>C, g.17924 A>G polymorphisms in the TE domain and g.16024 G>A, g.16039 T>C polymorphisms in the KR domain which affect fatty acid composition of meat products. It would be helpful to consider these data for selection studies in the future. The genotype distribution of the g.18440 G<A polymorphism in SAR and EAR cattle was found to be closer to the distribution of *Bos indicus* breeds and these results are consistent with previous studies on different autosomal genes. This polymorphism may be a marker for breed discrimination.

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