



Identification of K232A mutant allele of DGAT1 Gene Encoding Diacylglycerol Acyltransferase Enzyme

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Summary: DGAT1 gene encodes Diacylglycerol acyltransferase enzyme that plays a key role in triacylglycerol (triglyceride) synthesis. DGAT1 is a quantitative trait locus (QTL) that has an effect on increasing milk fat content in the centromeric region of bovine chromosome 14. Lysine (K)→Alanin (A) mutation that resulted in AA genotypes decreases milk fat rate and increases milk yield. The substitution of lysine amino acid to alanin at position 232 was identified with *Cfr1* enzyme. The aim of this study was to identify both K232A mutation and the frequency of K and A alleles among Brown Swiss and Holstein cattle breeds which belong to Education Research and Application Farm of Istanbul University Veterinary Faculty. RFLP was used to distinguish KK, KA and AA genotypes. PCR products were screened by 2% agarose gel electrophoresis. K allele and A allele frequencies among 54 Holstein and 44 Brown Swiss cattle breeds were 0.287 and 0.713, 0.091 and 0.901 respectively. This research article constitutes the pre-study of frequency determination of DGAT1 K and A alleles in Turkish native cattle breeds.

Key Words: Brown Swiss, DGAT1, Diacylglycerol acyltransferase, Holstein, RFLP.

Diacylglycerol Acyltransferase Enzimini Kodlayan DGAT1 Genindeki K232A Mutant Allelinin Saptanması

Özet: DGAT1 geni triacylglycerol (trigliserid) sentezinde önemli bir rol oynayan Diacylglycerol acyltransferase enzimini kodlamaktadır. DGAT1 sığırlarda 14. kromozomunun sentromerik bölgesinde yer alan, sütteki yağ oranını arttırmasına neden olduğu bilinen bir kantitatif özellik lokusudur (QTL). 232. aminoasit bölgesinde bir lizin aminoasidinin alanine dönüştüğü nokta RFLP ile tespit edilmiştir. Bu çalışmanın amacı İstanbul Üniversitesi Veteriner Fakültesi Eğitim, Araştırma ve Uygulama Çiftliği'ne ait olan İsviçre Esmeri ve Holştayn sığır ırklarında K232A mutasyonu ile K ve A allellerinin frekansını saptamaktır. KK, KA ve AA genotiplerinin ayırt edilmesinde *Cfr1* enzimi kullanılmıştır. DNA bantları %2'lik agaroz jel elektroforezinde görüntülenmiştir. İncelenen Holştayn ve İsviçre Esmeri sığır ırklarında K ve A allellerinin frekansları sırasıyla 0.287 ve 0.713 ile 0.091 ve 0.901 olarak bulunmuştur. Bu araştırma makalesi Türkiye'deki diğer sığır ırklarında DGAT1 K ve A allellerinin frekanslarını saptamaya yönelik bir ön çalışma niteliği taşımaktadır.

Anahtar Sözcükler: DGAT1, Holştayn, İsviçre Esmeri, RFLP.

Introduction

Triglycerides are neutral lipids and their structure consists of a glycerol backbone and three long-chain fatty acids. Since triglycerides are the integral component of lipoprotein particles synthe-

sized by the liver and small intestine, skin sebum secreted by sebaceous glands and milk produced by mammary glands, they are essential for normal physiology (1). Triglycerides are the major source of energy for animals and plants (2, 11, 24).

Diacylglycerol acyltransferase enzyme has a central role in triacylglycerol (triglyceride) synthesis (3). DGAT1 is one of the two diacylglycerol acyltransferase enzymes (DGAT1/DGAT2) that catalyzes the final step in triglyceride synthesis by using diacylgly-

lycerol (DAG) and fatty acylCoAs as their substrates (1, 11, 23, 26). The reaction catalyzed by DGAT1 takes place primarily in the endoplasmic reticulum (1). DGAT1 has an important role in triacylglycerol synthesis and energy storage, it is also assumed to have a key role in intestinal fat absorption, lipoprotein assembly, and the regulation of plasma triacylglycerol concentrations, fat storage in adiposities, energy metabolism in muscle and mammalian oocyte production (3). Since its product is directly involved in triglyceride synthesis and its transcription took place both in the bovine mammary gland and in adipose tissue, DGAT1 has an important role both in milk fat content and in intramuscular fat deposition (21). As obesity was caused by imbalance between energy input - output and calories stored as triglycerides, inhibition of triglyceride synthesis (possible with inhibition of DGAT1 gene) may have a key role in the potential therapeutic strategy for human obesity (1, 6). DGAT1 can be a target gene for the treatment of obesity in humans (23).

Diacylglycerol acyltransferase 1 gene was emphasized as a strong candidate gene for milk fat percentage following a study with knock-out mice (DGAT1^{-/-}), which were deprived of DGAT1 gene that means of the lack of triglyceride synthesis in mammary gland (21, 23, 24, 26). Despite the fact that the mice were deprived of DGAT1 and were not able to produce milk, they were viable. These findings lightened the importance of the DGAT1 gene in milk fat synthesis and milk production (26).

Milk is an important agricultural product in the world. Over the last 200 years, milk fat has been assumed one of the most important energy sources in human nutrition. Dairy farmers profit mainly from milk yield, protein and fat content (16, 26).

Milk fat percentage is determined by the collective effect of multiple genes and environmental factors. The aim of the breeding is to concentrate on many of the positive gene variants as possible as in one animal to improve its genetic potential. There are some traits that express themselves in phenotype at reproductive age (i.e. litter size, egg yield), some expressed by animal only in one gender (i.e. milk yield, milk fat content), some of the traits that can not be observed when the animal is alive (i.e. meat yield, carcass quality) that determine their phenotype, the animal has to be sacrificed, and in some phenotypes that complicate due to long generation interspaces (i.e. milk yield quantitative trait of bulls), the selection is limited since it is based on phenotypic information (26). Significant improvements

have been made over the past decades through molecular genetics that have made possible the genotype identification of traits that are important in livestock production (7). Many studies in dairy cattle have shown that a quantitative trait loci (QTL) with major influence on milk production is located in the centromeric end of the chromosome 14 (19). The first research for QTL in cattle genome mapping experiments was based on the detection of QTL especially on economically important traits (20). DGAT1 gene is located in, chromosome 8 in human, chromosome 15 in mice, chromosome 14 in cattle and chromosome 4 in pig (14, 16, 26).

Recently, a lysine/alanin mutation in the exon VIII of DGAT1 gene has been identified to be related to with milk fat percentage and milk yield (3, 19, 21). The lysine variant of DGAT1 gene affects the milk fat content in cattle by binding of Acyl CoA more efficiently than alanin amino acid at position 232; therefore, K232A substitution decreases the activity of DGAT1 enzyme. The conservation of lysine allele among human, rat, pig, sheep and bison demonstrated its functional importance (9, 21).

K232A mutation caused an increase in fertility due to lower energy drain (12). Lysine variant is related to high milk fat content. Alanin variant has a negative effect on the acylCoA-binding capacity of DGAT1 and it is related to lower milk fat content (25). Since there is a negative correlation between milk fat percentage and milk yield, therefore DGA-T1A allele has been selected by breeders to increase the milk volume after the domestication of wild cattle (12).

Restriction Fragment Length Polymorphism (RFLP) method was used to identify K232A mutation in DGAT1 gene.

The aim of this study was to identify both K232A mutation and the frequency of K and A alleles among Brown Swiss and Holstein cattle breeds which belong to Education, Research and Application Farm of Istanbul University Veterinary Faculty. This study has been planned to identify K232A mutation in some Turkish cattle breeds.

Material and Method

This study was conducted based on the Istanbul University Animal Experiments Local Ethics Committee Approval (Approval No: 2014/44)

In this study, blood samples taken from 44Brown Swiss and 54 Holstein cattle, which belong to Ed-

ucation Research and Application Farm of Istanbul University Veterinary Faculty, were used as materials. Following PCR amplification *CfrI* enzyme was used to identify Lysine (K) and Alanin (A) alleles of the DGAT1 gene.

Isolation of DNA has been carried out with blood DNA isolation kit (BioRad). Simple PCR amplification was performed in a 26 µl total volume; 12.5µl 2X PCR Master Mix, 0.5 µM of each primer (forward primer 5'-GCACCATCCTCTTCCTCAAG-3' and reverse primer 5'-GGAGCGCTTTCGGATG-3') from NCBI database sequence AJ871176, 50 ng DNA sample (13). For optimization of PCR, 5% DMSO was added to enforce the equal amplification of two alleles and to get successful results. PCR amplification was carried out as following: 15 min at 95 °C, followed by 35 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min and final extension at 72°C 3 min. *CfrI* enzyme cleaved 411 bp alanin variant to 203 and 208 bp (6). DNA bands visualized by 2% agarose gel are stained with ethidium bromide.

Statistical analyses

The allelic and genotypic frequencies were calculated according to Cerit et al. (5) and Hardy-Weinberg equilibrium was tested in Genepop (17).

Results

The method in this study was based on lysine and alanin variants of DGAT1 gene. PCR products were successfully amplified after the edition of 5% DMSO. Wild type and mutant band patterns were obtained by 2% agarose gel electrophoresis. *CfrI* enzyme recognized and cleaved the alanin variant to 203 and 208 bp. Since *CfrI* enzyme was not able to recognize lysine allele, wild type allele could be observed as 411 bp sized single band. In our study KK, KA and AA alleles of DGAT1 gene were identified in 54 Holstein and 44 Brown Swiss cattles.

KK homozygous, AA homozygous and KA heterozygous individuals should have showed to be single, double and triple band patterns respectively. However, in agarose gel electrophoresis 203 and 208 bp band fragments were overlapped and could not be differentiated, thus A allele was observed as a single band as K allele. While 203 and 208 bp bands could not be distinguished by means of agarose gel electrophoresis, it is possible to differentiate K and A alleles on the gel as in Figure 1.

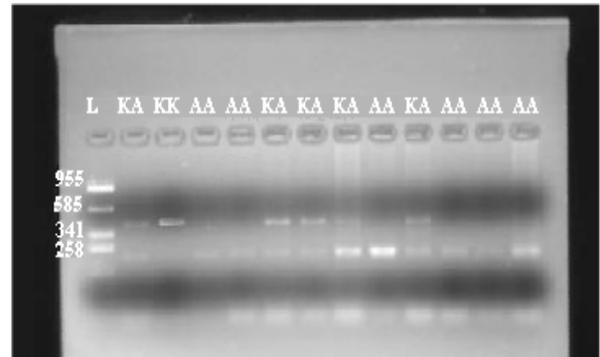


Figure 1. Band patterns of the KK, KA and AA alleles were screened on 2% agarose gel. [DNA ladder (L): pUC 19].

Genotypic and allelic frequencies observed and expected heterozygosity and Hardy-Weinberg Equilibrium (HWE) values were shown in Table 1. According to the values that were found out in this study, it can be said that K and A alleles are stable among these populations. Significantly different allele frequencies regarding KA gene were found between Holstein and Brown Swiss ($p<0.01$). The calculated X^2 values for the KA and DGAT1 genotypes indicate Hardy-Weinberg equilibrium in the population ($p=0.218$ and 0.011 for KA and DGAT1, respectively)

Breed	n	Genotyping Frequencies (%)						Allelic Frequencies (%)		Heterozgosity		HWE
		KK		KA		AA		K	A	Observed	Expected	P
		Observed	Expected	Observed	Expected	Observed	Expected					
Holstein	54	11.111 (n= 6)	4.449	35.185 (n= 19)	22.102	53.704 (n= 29)	27.449	0.287 ^a	0.713	0.352	0.402	0.302
Brown Swiss	44	4.545 (n= 2)	0.364	9.091 (n= 4)	7.273	86.364 (n= 38)	36.364	0.091 ^b	0.909	0.091	0.162	0.003

^{a,b} Within a column means without a common superscript differ letter ($p<0.01$)

Table 1. Genotypic and allelic frequency of DGAT1 gene K and A allele can be observed in Brown Swiss and Holstein cattle breeds which belong to Education Research and Application Farm of Istanbul University Veterinary Faculty.

Discussion

We prepared the PCR mixture and synthesised the primers as Kühn et al. (13) reported. In first PCR mixture, 5% DMSO was absent; therefore, there were no DNA bands observed. In second PCR mixture, 5% DMSO was added and it made the amplification and differentiation of the mutant and wild type alleles possible. Since simple primer couple and PCR master mix were used, there was less risk of contamination.

RFLP method was based on lysine and alanin variants of DGAT1 gene in this study. Wild type of DGAT1 gene carries ancestral lysine allele and it substitutes in mutant type (alanin allele) at the position of 232 in amino acid level (K232A). *CfrI* enzyme is able to recognize only the alanin (GCG) variant not the lysine (AAG) one. According to Grisart et al. (9), K232A substitution probably occurred after the divergence of the *Bos indicus* and *Bos taurus* lineages 20 000 years ago.

Milk fat yield has been one of the most important products in dairy breeding for many years; therefore, it was not a surprise that, it was observed more frequently than A allele because K allele had been selected positively.

Since BTA14 chromosome contains DGAT1 gene, it was chosen as a pilot region for the conducted investigation by Fisher et al. (8) who used selective pooling methodology. Schnabel et al. (18) reported that the most frequently identified QTL on BTA14 was DGAT1 gene.

Winter et al. (25) reported that increased and lowered milk fat content is strongly related to a lysine and an alanin at position 232 of DGAT1 gene respectively. Gristard et al. (10), described K232A mutation as a quantitative trait nucleotide which affects milk fat composition. As reported by Spelman et al. (19), K232A substitution increased milk protein from 2 to 3 kg and milk yield from 120 to 130 liter as average for both the Jersey and Holstein-Friesian breeds respectively. The average rate of substitution, the effect on milk fat, was 6 kg for Holstein-Friesians and 3 kg for Jerseys. In Israel Holstein population, the frequency of K allele increased the fat percentage by 8.9% in cows and 15.5% in bulls respectively (22). In this study, Holstein breed showed higher frequency for A allele (71%) than New Zealand Holstein (40%) and German Holstein (58%) populations (12, 19). This frequency is also higher than the ones found by Winter (26) in German Holstein dairy breed (66%). Brown Swiss breed showed a

lower frequency for A allele (91%) than German Brown (94%) and Brown Swiss (98%) dairy breeds reported by Winter (26). Differences among the allelic frequencies could be the result of the absence of an intensive indirect selection for milk production in the Brown Swiss and Holstein dairy populations used in this study.

Since K allele and A allele are the desired alleles for increasing milk fat and milk yield percentages respectively, K allele will be the preferred in Turkey for producing fatty milks which can be used in the production of cheese, butter, cream, etc. A allele will be preferred one for increasing the milk yield ratio and decreasing the milk fat percentage to produce low-fatty milk and low-fatty milk products for elderly people who need the treatment to lower the level of their cholesterol.

Since DGAT1 plays a key role in triglyceride metabolism, it should have an effect on intramuscular or subcutaneous fat deposition and backfat thickness in carcass. Thaller et al. (21) reported that DGAT1K allele has a positive effect on intramuscular fat deposition. However neither Moore et al. (15) nor Casas et al. (4) could identify its significant effect on backfat thickness which displays the carcass quality in beef and carcass traits such as ADG (average daily gain), hot carcass weight, marbling score, etc. Thus, the carcass quality and DGAT1K and A allele frequencies must be compared among beef cattle breeds of Turkey.

In addition, the classification of a wide range of cattle breeds can be made by DGAT1 analysis as Kaupe et al. (12) reported. This usage is also important for native cattle breeds of Turkey in their evolutionary analyses. Since the method that was used in this study is applicable in calves and bulls, DGAT1K and DGAT1A alleles can be identified without taking consideration age and sex. DGAT1 allele frequencies should be identified in native dairy cattle breeds of Turkey to increase the milk yield or milk fat depending on demands.

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