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The Genetic Characterization of *DGAT1* Gene in Donkey Populations Reared in Thrace Region of Turkey

Fulya ÖZDIL[a]

[a]*Namık Kemal University, Faculty of Agriculture, Department of Agricultural Biotechnology, 59030 Tekirdağ, TURKEY*

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Corresponding Author: Fulya ÖZDIL, E-mail: fozdil@nku.edu.tr, Tel: +90 (282) 250 2233
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AUTHORS ORCID ID
(Fulya ÖZDIL: 0000-0002-5298-6997)

ABSTRACT
AcylCoA: diacylglycerol acyltransferase (*DGAT1*) gene has a considerable effect on milk content and yield in cattle with a substitution of lysine by alanine in the exon 8 of the gene. Moreover there are many other researches comprising the *DGAT1* gene on different farm animals, such as buffalo, sheep and goat but there is no information about the *DGAT1* gene in donkeys. In this study, the polymorphism of *DGAT1* gene in donkey populations reared in Thrace region of Turkey has been investigated by restriction fragment length polymorphism (RFLP) via *Eae*I (*Cfr*I) restriction enzyme. *Eae*I restriction site was found in cattle breeds which resulted after K232A substitution, Lysine (AAG) to Alanine (GCG) variant but this restriction site was not found in donkey populations. A novel single-nucleotide polymorphism (G→A substitution) in the *DGAT1* gene at position 10,435 lacks this restriction site which results only Alanine variant (GCA) instead of Lysine variant. This novel single-nucleotide polymorphism in the *DGAT1* gene was found in the studied donkey breeds.

Keywords: *DGAT1* Gene; RFLP; *Equus asinus*; Donkey; Turkey

1. Introduction

Triglycerides are mainly synthesized from diglycerides that *DGAT1* is an enzyme that catalyzes a crucial role in mammalian triglyceride synthesis. *DGAT1* enzyme acts an important role in lipogenesis pathway in many tissues (Cases et al 1998) so the *DGAT1* gene, encoding this enyzme has been found relevant in milk production. In many of the studies, associations between *DGAT1* gene polymorphism and milk composition and production traits have been investigated (Spelman et al 2002; Weller et al 2002; Thaller et al 2003; Gautier et al 2007). Fat is an important component of mammalian milk. *DGAT1* gene is found as a potential candidate gene for milk fat yield in cattle (Schennink et al 2007). Moreover *DGAT1* gene, is also a candidate gene, because it has been found at the centromere region of the bovine 14th chromosome and includes 17 exons of variable sizes encoding a 489 amino acid protein that spans a quantitative trait locus (QTL) for milk production traits (Coppieters et al 1998; Grisart et al 2002). In the 8th exon of *DGAT1* gene (10.433th and 10.434th bp), two single-nucleotide polymorphisms (SNPs) have been reported and generated to QTL (quantitative trait loci) variation. These polymorphisms caused the substitution of lysine to alanine (K232A) and consulted to considerably affect the milk fat composition in cattle (Coppieters et al 1998; Winter et al 2002; Grisart et al 2002; 2004; Kaupe et al 2004). In *DGAT1* gene, Alanine variant (A allele) and Lysine variant (K allele) were related with high milk yield and high milk fat yield in cattle, respectively (Coppieters et al 1998; Winter et al 2002; Grisart et al 2004).

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In many countries, donkey breeds which were used as pack animals in rural areas have become extinct or critically endangered. In the last years, donkey populations have declined dramatically in Turkey. All over the World, donkeys which are under threat of extinction, have to be characterized both morphologically and genetically in order to constitute conservation strategies. For the last years, donkey milk has been used as curative, reformative, nutritive substance as well as cosmetics. Due to its rich content, the scientific interest to donkey milk has been increased recently. Cow's milk allergy is an important problem in infants and many researches show exciting findings on equid (horse and donkey) milk tolerability (Salimei et al 2004). So the candidate gene, *DGAT1*, which is found in association with milk production traits, should be investigated in donkeys in order to identify the gene regulation of donkey milk genetic parameters. So the aim of this study was to search the *DGAT1* gene in donkey populations reared in Thrace region of Turkey and introduce the variation in this gene region. Also the RFLP characterization of *DGAT1* gene in donkeys was conducted for the first time in Turkey.

2. Material and Methods

In this study, 61 blood samples were collected from Thrace region of Turkey, Kırklareli Province. 41 samples were collected from a donkey farm in Koruköy Village and 10 samples each were collected from Üsküp and Kuzulu Villages. Blood samples were collected from the vena jugulars of the donkeys and used for the DNA extraction.

All DNA isolations were done according to phenol chloroform extraction method with slight modifications (Sambrook et al 1989). PCR reactions and cycling conditions were carried out as reported in Kaupe et al (2004) in a 25 µL volume using 50 ng of genomic DNA

437 bp of *DGAT1* gene were amplified by Polymerase Chain Reaction (PCR) using the primers given in Kaupe et al (2004). To check whether the allelic variation that were reported in cattle, (10.433^{th}-10.434^{th} bp of the *DGAT1* gene, Genbank Accession no. JF894305) was also found in donkeys; *DGAT1* gene region amplification was digested with *Eae*I restriction enzyme (NEW England Biolabs Inc). The digested fragments were separated using 2% agarose gels, stained with SYBRSafe DNA gel stain (Thermo Fisher Scientific) and visualized with Vilber Lourmat gel imaging system.

The *DGAT1* genes of two samples were sequenced on an Applied Biosystems 3500XL Genetic Analyzer System (Applied Biosystems, USA) in order to verify the sequence variations of the *Eae*I restriction site.

3. Results and Discussion

The 437 bp of PCR products (including the primers) were amplified and digested with *Eae*I restriction enzyme. In all of the studied DNA samples uncut single band of 437 bp were obtained (Figure 1).

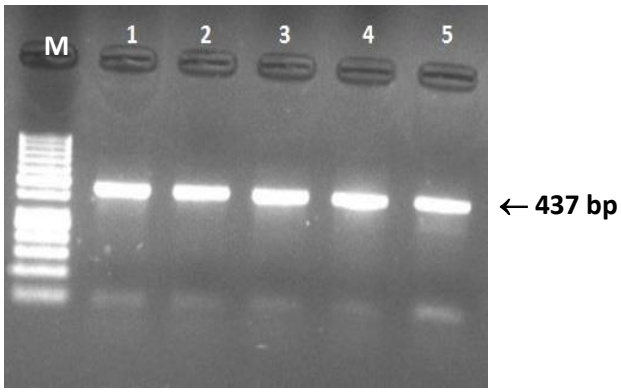

Figure 1- Undigested PCR products of *DGAT1* gene (437 bp) via *Eae*I restriction enzyme in donkeys. M: 50 bp DNA ladder (Invitrogen 10416014)

The sequencing of this region revealed polymorphisms when compared to cattle and buffalo *DGAT1* gene sequences. In the 8th exon of the gene, 10.433th-10.434th bp of the *DGAT1* gene, two SNPs were reported in cattle which revealed two different alleles, Lysine (A A G) and Alanine (G C G) alleles (Table 1). A→G and A→C base substitutions revealed *Eae*I recognition site and Alanine allele was obtained. Genbank records of these alleles are given in Table 1. The DNA sequence of this gene region in donkey revealed again Alanine allele, A→G and A→C base substitutions at position 10,433 and 10,434 were again determined but also a novel polymorphism at 10.435th of the *DGAT1* gene, G→A transition which also revealed Alanine (GCA) allele was obtained but this substitution resulted the loss of *Eae*I restriction site. As a result, no *Eae*I restriction in *DGAT1* gene was obtained in donkey populations.

Table 1- Allelic variation of *Eae*I restriction site in *DGAT1* gene in cattle, buffalo and donkeys

Nucleotide positions (Genbank no: AJ318490)	*10433*	*10434*	*10435*	*10436*	*10437*	*10438*	*10439*	*Allele*	*Genbank accession no*	*EaeI restriction*
Cattle	G	C	G	G	C	C	A	Alanine (A)	EU348567	+
Cattle	A	A	G	G	C	C	A	Lysine (K)	EU077528	-
Buffalo	A	A	G	G	C	C	A	Lysine (K)	JQ627609	-
Donkey	G	C	A	G	C	C	A	Alanine (A)	NW_014638167	-

*Eae*I (*Cfr*I) restriction Site: (T/C)GGCC(A/G) 10434 to 10439

4. Conclusions

Around the middle of the 20th century, as a consequence of industrialization in agriculture and the spreading several highly selected breeds, many animal populations have become extinct or are declining and endangered. In many countries, donkey breeds which were used as pack animals in rural areas have become extinct or critically endangered. In the last years, donkey populations have declined dramatically in Turkey (Anonymous 2018). So both morphological and genetic studies have to be conducted on Turkish native donkey breeds.

In this study, we used PCR-RFLP method by *Eae*I restriction enzyme of *DGAT1* gene to introduce the genetic polymorphism in donkey populations from Thrace region of Turkey. The 437 bp of *DGAT1* gene were amplified and digested with *Eae*I restriction enzyme and no restriction site was obtained in Turkish donkey populations as well as Anatolian buffalo populations (Özdil & İlhan 2012).

The sequencing of this region revealed polymorphisms when compared to cattle and buffalo *DGAT1* gene sequences. In the 8th exon of the gene, 10.433th-10.434th bp of the *DGAT1* gene, two SNPs were reported in cattle which revealed two different alleles, Lysine and Alanine alleles (Table 1). The DNA sequence of this gene segment in donkeys revealed Alanine allele, G and C at position 10.433 and 10.434, respectively, but also produced a novel polymorphism at position 10.435, G→A which also revealed Alanine allele but without *Eae*I digestion.

In many of the studies, *DGAT1* gene is indicated as a functional candidate gene that has a substitution of lysine by alanine (K232A) allele generating a fundamental effect on milk fat composition and yield (Coppieters et al 1998; Smith et al 2000; Winter et al 2002; Grisart et al 2002; 2004). In *DGAT1* gene while Lysine (AAG) variant (K allele) was related with high fat percentage of milk, Alanine variant (A allele) of this gene was related with high milk yield (Winter et al 2002; Grisart et al 2002; 2004). Also K allele reported to be the wild type allele (Coppieters et al 1998; Grisart et al 2002; Kaupe et al 2004). In this study only Alanine allele which is responsible for high milk yield in cattle, is found in donkeys. Also a novel polymorphism (G →A) at position 10.435 bp of the *DGAT1* gene is reported in donkey populations. This study provides an insight to donkey genetics and indirect evidence that all of the Thrace donkey populations in Turkey have fixed allele with respect to *DGAT1* Alanine allele.

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