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DGAT1-exon8 polymorphism in Anatolian buffalo ☆

Fulya Özdil *, Fatma İlhan

Faculty of Agriculture, Department of Animal Science, Division of Biometry and Genetics, Selçuk University, Konya 42075, Turkey

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ABSTRACT

Previous studies have reported that a non-conservative substitution of lysine by alanine (K232A) in the 8th exon of acyl CoA:diacylglycerol acyltransferase (*DGAT1*) gene in cattle has a major effect on milk composition and yield. But yet little research has been utilized in this gene segment in buffalo. In this study the genetic differentiation of three indigenous Anatolian buffalo populations has been investigated in the 8th exon of *DGAT1* gene. Fourteen out of the 24 restriction enzymes have a recognition site on the *DGAT1* gene segment, generating a total of 26 restriction sites. *Alu*I, *Hinc*II and *Hph*I restriction enzymes out of these 14 enzymes were found to detect polymorphism. K232A substitution found in cattle breeds with *Cfr*I restriction enzyme is not a diagnostic site in Anatolian buffalo. Only lysine variant (K allele) is found in all of the Anatolian buffalo tested. In this study, four different haplotypes were obtained and sequencing of this gene revealed three polymorphic nucleotide substitutions in the 8th exon of the buffalo *DGAT1* gene. C→T, A→G and C→G base substitutions at positions 43, 154 and 373, respectively, each generated two different fragment patterns in Anatolian buffalo. Type 1 haplotype was the most common haplotype, found in 73.2% of the samples. Along with the polymorphic substitution sites in *DGAT1* gene, this study provides evidence that all the Anatolian buffalo have fixed allele with respect to *DGAT1* K allele reported to be responsible for high milk fat yield. Our study presents the first comprehensive sequencing analysis of Anatolian buffalo and it is the first time that sequencing data from *DGAT1* gene segment have been obtained at the population level.
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1. Introduction

Most of the world's buffalo live in Asia, Egypt, southern and south-eastern Europe and Mediterranean countries. Buffalo play an important role in the rural economy of developing countries. In addition to the utility of these animals for milk and meat production, their adaptation to natural conditions and tolerance to diseases makes them economically advantageous (Aytekin et al., 2011; Mishra et al., 2007; Yilmaz et al., 2011).

Water buffalo have been raised in Turkey for more than 1000 years. The Turkish water buffalo, commonly known as the Anatolian water buffalo is a part of Mediterranean type (Yilmaz et al., 2011). This category is classified as "River" type, which all buffaloes of Europe and countries of the Near East belong to (Gargani et al., 2009; Yilmaz et al., 2011). The Anatolian water buffalo population has declined dramatically over the last decades. The total population according to Turkish Statistical Institute, TUIK (2010) is 84,726 heads. The buffalo population is decreasing year after year in Turkey and this trend is also affecting the production of buffalo milk and meat, which were 35,487 and approximately 1000 t, respectively, in 2010 (TUIK, 2010).

☆ The department where the study was conducted: Department of Animal Science, Division of Biometry and Genetics, Selçuk University, Konya 42075, Turkey.

* Corresponding author. Tel.: +90 332 2232810; fax: +90 332 2410108.
E-mail address: fulyaozdil@selcuk.edu.tr (F. Özdil).

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Acyl coenzyme A:diacylglycerol acyltransferase 1 (*DGAT1*) is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis. *DGAT1* catalyzes the addition of fatty acyl CoA to 1, 2, diacylglycerol to yield CoA plus triglycerol and is important in lipogenesis in many tissues (Cases et al., 1998). In cattle, *DGAT1* gene became a strong functional candidate gene for milk fat percentage after the description of the lactation deficiency in female mice (Smith et al., 2000) lacking both copies of *DGAT1* gene which can result deficient triglyceride synthesis in mammary gland. Furthermore *DGAT1* is also a positional candidate in cattle, because the gene is located within a region on centromeric end of the bovine chromosome 14 that contains a quantitative trait locus (QTL) for this trait (Bennewitz et al., 2004; Coppieters et al., 1998; Grisart et al., 2002). Two single-nucleotide polymorphisms (SNPs) in the 8th exon of *DGAT1* at positions 10,433 and 10,434 lead to QTL (quantitative trait loci) variation that cause the substitution of lysine with alanine (K232A) and this substitution was considered to significantly affect the milk fat content in cattle (Coppieters et al., 1998; Grisart et al., 2002, 2004; Winter et al., 2002). While Lysine variant (K allele) of *DGAT1* gene was associated with high milk fat yield, Alanine variant (A allele) of *DGAT1* gene was associated with high milk yield (Coppieters et al., 1998; Grisart et al., 2004; Winter et al., 2002).

Since buffalo yield higher milk fat than *Bos indicus* or *Bos taurus* cattle, the characterization of *DGAT1* in Anatolian buffalo was found necessary. Buffalo genetic resources are depleting and the loss of some genotypes may jeopardize the continuation of the species (Aytekin et al., 2011; Gargani et al., 2009; Mishra et al., 2007; Soysal et al., 2005; Yilmaz et al., 2011). For this reason, the Anatolian Water Buffalo are involved in a protection program of gene resources with the declaration of guidelines for subsidizing animal farming (Prescript no: 2006/9) by the Ministry of Agriculture and Rural Affairs in Turkey. So it is highly important to characterize the molecular structure of Anatolian buffalo in Turkey. In the last decade, many molecular studies have been conducted using microsatellites (Gargani et al., 2009; Soysal et al., 2007), ISSR markers (Aytekin et al., 2011) and PRNP gene promoter (Öztabak et al., 2009) in Anatolian buffalo. *DGAT1* polymorphism was identified in some of the indigenous cattle breeds in Turkey (Kaupe et al., 2002, 2004; Kepenek, 2007), but not in indigenous Anatolian buffalo. So the purpose of the present research was to identify the *DGAT1* exon8 polymorphism in indigenous Anatolian Water Buffalo and to find out whether these results could be useful for developing conservation strategies for the Anatolian buffalo.

2. Material and methods

2.1. Sampling

In this study, a total of 41 blood samples were collected from three local Anatolian Water Buffalo herds including 13 from Afyon, 14 each from Konya and Sivas provinces in Turkey. Blood samples were collected from the vena jugularis of each buffalo into 4-mL tubes containing EDTA and stored at −20 °C till the DNA extraction.

2.2. DNA preparation, PCR and RFLP test

Isolation of DNA included the salting-out procedure (Miller et al., 1988). PCR reactions were performed in a 25 μl volume using 50 ng of genomic DNA, 1 × PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 pM of each primer, and 0.5 U of Taq DNA polymerase (Fermentas Life Sciences, Lithuania). Primer sequences were: forward 5′-GCACCATCCTCTTCCTCAAG-3′ and reverse 5′-GGAAGCGCTTTCGGATG-3′. The PCR profile included 15 min at 95 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C; and a final 10 min extension at 72 °C (Kaupe et al., 2004). To detect allelic variation at nucleotide positions 10,433 and 10,434 of the *DGAT1* gene (Genbank Accession no. JF894305), 10 μl of amplified DNA was firstly digested with 1 U of *Cfr*I restriction enzyme (MBI Fermentas GmbH, Germany) for 3 h at 37 °C. Moreover the amplified segment was subsequently screened for polymorphism with the following 24 restriction endonucleases: *Alu*I, *Ava*I, *Ava*II, *Bam*HI, *Bgl*II, *Bsa*I, *Bsp*1286I, *Bst*UI, *Cfr*I, *Dra*I, *Eco*RI, *Hha*I, *Hin*cII, *Hin*fI, *Hph*I, *Nco*I, *Pst*I, *Sac*I, *Sau*3AI, *Sau*96I, *Ssp*I, *Sty*I, *Vsp*I and *Xho*I. The digested fragments were separated electrophoretically on 2% or 3% agarose gels in 1 × TBE buffer, stained with ethidium bromide and photographed with Vilber Lourmat gel imaging system. DNA fragment sizes were determined by using Digital Image analysis software (Vilber Lourmat Deutschland GmbH Eberhardzell, Germany). To detect the nucleotide substitutions of RFLP composite genotypes, the *DGAT1* gene was sequenced on an ABI Prism 310 automated sequencer (Applied Biosystems, Foster City, CA, USA) using standard protocols in order to verify the sequence variations. Sequences were aligned with the computer program Clustal X (Thompson et al., 1997).

2.3. Data analysis

Composite genotypes (haplotypes) for each individual were defined from all the restriction patterns of *DGAT1* gene. All the data were analyzed using various programmes contained in the Restriction Enzyme Analysis Package, REAP (McElroy et al., 1991).

While Neighbor-Joining, Maximum Parsimony and Maximum Likelihood methods all have difficulties in resolving relationships among closely related haplotypes, statistical parsimony allows display of genealogical relationships among sequences with a limited number of mutations. The results of statistical parsimony among four haplotypes were represented using a network calculated with TCS version 1.21 software (Clement et al., 2000).

3. Results and discussion

3.1. RFLP results

Fourteen out of the 24 restriction enzymes used to screen the three local buffalo which have a recognition site on the *DGAT1* gene segment and these enzymes were used for routine screening of the breeds, generating a total of 26 restriction sites. Three out of these 14 restriction enzymes were found to detect polymorphism. Typing

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